Neuromodulation of cognitive-behavioral control

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Contents

Introduction

Without even realizing, human beings perform extraordinary feats on a daily basis. We navigate an increasingly complicated and demanding world by using our sophisticated ability to override habitual tendencies. Furthermore, we can meticulously plan, carry out and adapt actions in order to achieve goals that we set for ourselves. This capacity for goal-directed behavior—often considered a hallmark of human excellence over other animals—is commonly referred to as 'cognitive control' or 'executive function'. These are rather vague, typically synonymous concepts that serve more as an umbrella term for many different processes rather than referring to a single, unitary function. Decades of neuropsychological research have been devoted to understanding cognitive control and its component processes, the way it is implemented in the brain, and how we can alter—and possibly improve—its efficacy. These issues have driven the work included in this dissertation. In particular, the research presented in this dissertation concerns itself with the overarching questions of how chemical processes in the brain enable and affect cognitive control, and how we might non-invasively measure and manipulate these biological underpinnings of goal-directed behavior.

Cognitive control

When it comes to defining and operationalizing cognitive control, this dissertation has been inspired by two influential and certainly not mutually-exclusive theoretical frameworks. The first framework is represented in the seminal work by Miyake et al. (2000), who focused on identifying three major executive functions and determining their separability. They postulate that cognitive control consists of three major functions, including *inhibition* (i.e., the ability to withhold prepotent / dominant responses), *updating* (i.e., the ability to maintain and update working memory representations), and *shifting* (i.e., the ability to switch between goals or task-sets). A key finding by Miyake et al. is that these functions are (only) moderately correlated with each other,

implying that these are separable processes that might be sensitive to different manipulations. Consistent with this idea, cognitive training studies have shown that training one of these functions rarely produces transfer effects to the others. However, the moderate correlation of these functions also highlights that executive functions may have a common underpinning—which will be elaborated upon momentarily—and that their efficacy may rely on each other (see Diamond, 2013).

The second theoretical framework concerns itself less with specific cognitive functions and instead proposes that there are different cognitive control 'modes' or 'states' that determine the way in which the aforementioned functions might operate. In particular, control mode is thought to vary from (i) a more stable setting that supports maintenance of task goals and shields them from distraction, to (ii) a more flexible setting that promotes disengagement from and switching between goals (Cools & D'Esposito, 2011; Goschke, 2003; Hommel, 2015). Each control mode is advantageous in different situations, but also has its own notable disadvantages. Whereas a stable control mode allows for the pursuit of a particular goal, it also carries the risk of rendering one too rigid to adapt to a change in environmental demands. In contrast, a flexible control mode allows for efficient switching between goals or task-sets, but can render one distractible when this switching is not done selectively. As such, adaptive cognitive control requires a balance between the opposing demands of cognitive stability and flexibility, which is also known as the *cognitive control paradox* or the *paradox of the flexible mind*.

There is great compatibility between these two theoretical frameworks of cognitive control. For example, Miyake et al. (2000) report considerable individual differences in performance on tasks that tap into the three postulated executive functions, and these differences might stem from individual variability in cognitive control mode. That is, those with a more stable control mode would plausibly be better at the inhibition of responses triggered by distracting, task-irrelevant stimuli, whereas those with a flexible control mode would have an easier time updating their working memory representations and switching between goals or task-sets. This idea has been supported by a variety

of studies (for example, Colzato, Ozturk, & Hommel, 2012; Colzato, Sellaro, Samara, & Hommel, 2015; Colzato, Szapora, Lippelt, & Hommel, 2017; Fischer & Hommel, 2012; Fröber & Dreisbach, 2017). The question of *why* some individuals demonstrate superior inhibitory control or cognitive flexibility addresses another commonality between these theoretical frameworks and the shared biological underpinning of executive functions that was alluded to earlier: dopamine activity in the brain.

Dopamine

The neurotransmitter dopamine is thought to be a major determinant of individual differences in cognitive control mode and efficiency of the three major executive functions. Dopamine is commonly referred to as a neuro*modulator* because of its widespread, complex effects on neural activity (Nieoullon, 2002; Seamans & Yang, 2004). Rather than following a 'more is better' rule, the relationship between dopamine activity and cognitive performance typically follows a characteristic inverted-U-curved relationship (Cools & D'Esposito, 2011; Cools, 2006; Goldman-Rakic, Muly, & Williams, 2000). That is, a moderate level of dopaminergic activity is generally associated with optimal performance, whereas both lower and higher dopamine activity are associated with suboptimal performance.

Although dopamine is perhaps most well-known to the general public for its role in reward, the experience of pleasure, and addiction, its significance to cognitive control is difficult to overstate. To understand this significance, it is important to distinguish between two dopaminergic pathways in the brain that contribute differentially to cognitive control. These are (i) the mesocortical pathway that projects to cingulate and prefrontal cortices, and (ii) the nigrostriatal pathway that projects to the subcortical basal ganglia. In brief, dopaminergic activity in the former pathway is thought to support cognitive stability whereas activity in the latter pathway promotes cognitive flexibility (Cools & D'Esposito, 2011; Cools, 2006).

In more detail, within the prefrontal cortex (PFC) dopamine modulates cognitive control via two distinct receptor families: the D1-like and D2-like

receptors. As outlined in the dual-state theory of PFC function (Durstewitz & Seamans, 2008), dopaminergic stimulation of prefrontal D1-like receptors inhibits firing of neurons in a low, spontaneous firing state while enhancing the firing of neurons in a high, persistent activity state. This increases the cortical signal-to-noise ratio and facilitates the stability of mental representations maintained in PFC. On the other hand, activation of D2-like receptors leads to an overall decrease in inhibition of PFC neurons, which facilitates their spontaneous firing and thereby promotes flexible but also interference-prone representations (Robbins, 2005; Seamans, Gorelova, Durstewitz, & Yang, 2001; Seamans & Yang, 2004; Trantham-Davidson, Neely, Lavin, & Seamans, 2004). As such, dopamine in the PFC is assumed to impact the balance between a stable and flexible control mode through the ratio of D1 and D2-like receptor activation.

Within the basal ganglia, dopamine promotes flexible control via an input-gating mechanism that determines whether the PFC is open to new information. The *prefrontal-cortex basal-ganglia working memory model* (Frank, Loughry, & O'Reilly, 2001; Hazy, Frank, & O'Reilly, 2006; O'Reilly, 2006) proposes that phasic dopamine release in the basal ganglia opens a proverbial gate to the PFC, which renders cortical representations susceptible to updating and interference, whereas a lack of dopamine activity in basal ganglia keeps the gate closed and thereby shields cortical representations from distraction (see also Braver & Cohen, 2000). Notably, dopaminergic stimulation of D1-like receptors in the basal ganglia facilitates gating whereas D2-like receptors hinder it, and increased tonic dopamine level in the basal ganglia leads to preferential D1 over D2 stimulation (Hazy et al., 2006; O'Reilly & Frank, 2006; van Schouwenburg, Aarts, & Cools, 2010). Consequently, higher dopamine levels in the basal ganglia promote flexibility by facilitating access of information to PFC. At the same time, however, this also increases the risk that task-irrelevant information interferes with maintenance in PFC, thereby increasing not only flexibility but distractibility as well.

In sum, dopamine is of great importance to understanding cognitive control. Via regionally-specific effects in cortical and subcortical networks, it can bias cognitive processing to be either more stable or flexible, thereby impacting the efficacy of inhibition, updating and shifting. However, it would be remiss to imply that dopamine is the only neurotransmitter with relevance to cognitive-behavioral control. Other neurotransmitters are also known to play critical roles, such as noradrenalin (Robbins, 2005), serotonin (Cools, Roberts, & Robbins, 2008), and glutamate and GABA (de la Vega et al., 2014; Munakata et al., 2011). Accordingly, the final chapters of this dissertation shift the focus toward the latter two neurotransmitters, glutamate and GABA, and aim to investigate how manipulation of these neurotransmitter systems impacts control.

Glutamate and GABA

As the primary excitatory and inhibitory neurotransmitters, respectively, glutamate and GABA play an important role in the control over actions. In brief, it is thought that glutamate and GABA (and particularly the ratio between them) determine the level of intracortical inhibition, which in turn affects the ability to select one particular representation or action among alternatives (de la Vega et al., 2014; Munakata et al., 2011). This may affect common daily life situations, such as deciding which word to use in a sentence and making a decision when there is no clear best option.

In brief, higher levels of glutamate (and conversely, lower GABA levels) suppress the competition between representations in PFC, making it more likely that alternative, perhaps even task-irrelevant competitors become active. This can result in choosing an incorrect action, or slowing down the process of choosing the appropriate one. In contrast, greater inhibition (due to lower glutamate and/or higher GABA levels) has the opposite effect by suppressing activation of competing responses (de la Vega et al., 2014; Jocham, Hunt, Near, & Behrens, 2012). Several studies have confirmed this model of action selection within the brain, for example by showing that higher GABA concentration in certain regions predict faster (Dharmadhikari et al.,

2015) and more accurate (Haag et al., 2015) responses in the Simon task, a classical response-interference paradigm (Hommel, 2011).

In light of this model of action selection and inhibition in the brain, the final three chapters in this dissertation investigate how a presumed increase or decrease in neural inhibition affects response selection. This is examined by making use of the serial reaction time (SRT) paradigm (Abrahamse & Noordzij, 2011), in which one needs to carry out a sequence of button presses in rapid succession. This sequence can be either random, or contain an embedded second-order conditional (SOC) sequence. Whereas a random response sequence requires a strongly stimulus-based, reactive mode of control, in a SOC sequence it is possible to use knowledge about the previous two responses to anticipate which response is required next. As such, SOC sequences allow for a shift toward a more plan-based, proactive mode of control (Tubau, Hommel, & López-Moliner, 2007) that allows for increasingly faster and more accurate responses. As such, the SRT task allows us to investigate response selection, inhibition of non-target responses and the implicit formation of response sequence structures, each of which are assumed to be sensitive to a change in neural inhibition.

Overview

This dissertation can be divided into three overarching topics. The first section (Chapters 1-2) presents one literature review and one empirical study that focus on non-invasive markers of individual differences in dopamine function and whether it is possible to predict cognitive control performance based on these differences. The second section (Chapters 3-7) shifts away from this correlational approach and toward mild experimental manipulations of the dopaminergic system and their associated changes in cognitive control, as discussed in two literature reviews and two empirical studies. Lastly, the third section (Chapters 8-10) covers three empirical studies that used different methods of manipulating neural inhibition in order to examine the corresponding effects on action selection.

Chapter One presents a comprehensive review of literature that has used the spontaneous eye blink rate (EBR) as an indirect marker of dopaminergic activity. As covered in this chapter, there is a large body of literature showing a positive correlation between EBR and dopaminergic activity. In brief, pharmacological studies have shown that dopamine agonists and antagonists respectively increase and decrease EBR, and clinical populations with hypoactive dopamine activity exhibit lower EBR whereas those with hyperactive dopamine activity demonstrate higher EBR. Particularly interesting is the finding that EBR in healthy individuals can predict cognitive performance on a wide variety of experimental paradigms. Consistent with the idea that EBR is primarily associated with dopaminergic activity in the basal ganglia, higher EBR predicts greater cognitive flexibility as measured, for example, by task-switching and divergent thinking paradigms.

Considering the already extensive literature on EBR as marker of dopaminergic activity, **Chapter Two** presents a study that focuses on a different aspect of our eyes that may predict dopaminergic activity. Specifically, it appears that color vision, i.e., the ability to discriminate between colors, can predict individual differences in dopamine and associated cognitive functioning. This was investigated by assessing both color vision and performance on an action cascading (also referred to as multitasking or task-switching) paradigm. Action cascading refers to the ability to chain together and switch efficiently between different task goals. This can be done in a more serial, step-by-step manner in which the next task goal is activated only when the previous goal has fully finished, or in a more parallel, overlapping manner in which different task goals are activated simultaneously. Action cascading is known to be related to dopamine function, as a previous study demonstrated that individuals who are genetically predisposed to greater dopamine D2 receptor function (which is particularly prevalent in the basal ganglia) tend to process goals in a parallel manner. The results in Chapter Two demonstrate that, similarly, individuals with good color vision demonstrate performance that is consistent with a more parallel rather than serial processing mode. This

tentatively suggests that good color vision is particularly predictive of the dopamine D2 receptor and cognitive flexibility. A discussion of this interpretation, and an alternative perspective, will be elaborated upon in the Discussion section of this dissertation.

Although markers such as EBR and color vision can allow us to investigate presumed individual differences in dopamine function, this approach is correlational in nature and therefore does not confirm a causal role for dopamine in the reported findings. That is why the following chapters focus instead on a mild but effective method of manipulating dopaminergic activity. In **Chapter Three** a comprehensive review is presented regarding the cognitive-behavioral effects of administering the food supplement L-tyrosine, which is also the biochemical precursor of dopamine. Given that tyrosine can be converted into dopamine in the brain, many studies have investigated whether tyrosine supplementation can benefit cognitive processes that are modulated by dopamine. Indeed, tyrosine has been shown to enhance the three executive functions outlined by Miyake et al. (2000), that is inhibition (Colzato, Jongkees, Sellaro, van den Wildenberg, & Hommel, 2014), task-switching (Steenbergen, Sellaro, Hommel, & Colzato, 2015), and in particular working memory (Colzato, Jongkees, Sellaro, & Hommel, 2013; Jongkees, Sellaro, et al., 2017; Thomas, Lockwood, Singh, & Deuster, 1999). Notably, the effects of tyrosine appear to be reliable only when one is exposed to an external stressor such as heat, cold or noise, or an internal stressor such as high cognitive load. Therefore, tyrosine is proposed to be a 'depletion reverser', being effective only in circumstances where performance would normally be degraded by the depletion of cognitive resources, motivation, or dopamine levels.

Chapter Four serves as an extension of the previous chapter, by highlighting that the effects of tyrosine supplementation are likely to depend on individual differences in dopamine function. Indeed, it is often observed that the effect of dopaminergic manipulations are state-dependent and differ for those with low or high baseline dopamine levels. Typically, those with lower dopamine levels are shifted upward on the inverted-U-curve relating

dopamine and cognitive performance when provided with an increase in dopamine activity. In contrast, those with higher dopamine levels would subsequently shift downward to the right side of curve. For low and high baseline dopamine individuals this would respectively lead to an observed increase and decrease in cognitive performance relative to baseline[1]. That is why the brief review in Chapter Four proposes several possible markers of individual differences in dopamine function that might predict the efficacy of tyrosine supplementation. These markers include EBR and color vision as discussed in earlier chapters, as well as genetic markers of dopamine function in PFC or basal ganglia. A recent study has confirmed one of these hypotheses by demonstrating that tyrosine supplementation was most effective in individuals with a genetic predisposition toward lower dopamine activity in the basal ganglia (Colzato et al., 2016), who presumably have the most room for shifting upward on the curve relating dopamine activity and performance.

Chapter Five presents one of the empirical studies on tyrosine supplementation that is included in the review in Chapter Three. In particular, this chapter investigates the efficacy of tyrosine supplementation in enhancing inhibitory control, which is known to be dependent on dopamine activity. This is investigated by means of the stop-signal paradigm, in which participants must carry out a simple forced-choice reaction time task as fast as possible unless a stop signal indicates they should withhold their response. By varying the onset delay of the stop signal, it is then possible to estimate how much time someone needs to successfully inhibit their response. As expected, the results revealed that participants were faster in withholding their responses after tyrosine supplementation as compared to a placebo. In contrast, response execution remained unaffected, highlighting that tyrosine supplementation is only effective in enhancing performance on particularly demanding tasks.

In **Chapter Six** a different approach to dopaminergic manipulation is taken, by making use of transcranial direct current stimulation (tDCS). This is

[1] However, it should be noted that such a pattern of results can also be accounted for by regression to the mean (see Barnett, van der Pols, & Dobson, 2005). Future studies need to consider and address this alternative explanation, which is often not done in the existing literature.

a noninvasive method of brain stimulation that is known to alter cortical excitability and neural plasticity. It is thought that tDCS can affect dopamine not directly but indirectly by acting on GABA, which in turn has a modulatory influence on dopaminergic signaling. Although there are many studies showing that tDCS can affect cognitive performance, there is also considerable doubt about the reliability of its effects as results have varied across studies. This is likely related in part to methodological differences between studies, but it has been suggested that individual differences in dopamine can also contribute to variability in response to tDCS (Wiegand, Nieratschker, & Plewnia, 2016). There are some studies that support this notion. In light of the aforementioned inverted-U-curve relating dopamine activity and cognitive performance, previous studies indicate that applying excitatory (anodal) stimulation to individuals with already high dopaminergic signaling leads to an impairment in performance. Conversely, applying inhibitory (cathodal) stimulation to those with already low dopaminergic signaling also leads to impaired cognitive control. This pattern of results has been observed by distinguishing between individuals with a genetic predisposition toward higher or lower dopamine activity in the PFC. However, it is important to acknowledge that genetics studies can only offer correlation evidence and do not speak to the causal role of dopamine in the effects of tDCS. That is why the study presented in Chapter Six sought to take a more experimental approach by combining tDCS with tyrosine supplementation and assessing the effects on working memory, which is the most often investigated cognitive function in tDCS studies. As in the majority of previous studies, tDCS was applied to the dorsolateral PFC, which is a region important for cognitive control and in particular working memory. Consistent with the aforementioned findings on genetics, the results demonstrated that the combination of tyrosine and excitatory stimulation produced an impairment in working memory performance. This finding supports the notion that tDCS can affect dopamine in the brain and lead to a detrimental increase in dopaminergic signaling when combined with a manipulation that also augments dopamine activity.

In light of the evidence supporting a role for dopamine in the effects of tDCS, **Chapter Seven** investigated whether the pattern of results in the previous chapter can be mirrored by pre-existing individual differences in dopamine activity rather than an experimental manipulation thereof. If this were the case, then this and the previous chapter would have the important implications that (i) dopamine plays a role in the effects of tDCS and (ii) individual differences in dopamine activity might contribute to variability in the effects of tDCS. To investigate the latter hypothesis, this chapter presents a study using the same experimental set-up as in Chapter Six. Instead of a tyrosine manipulation, this time participants were genotyped for the COMT Val^{158}Met polymorphism, which determines the level of dopaminergic signaling in the PFC. Similar to the pattern of results observed in Chapter Six, it was hypothesized that applying excitatory stimulation to those with a predisposition toward higher dopamine activity would demonstrate worse working memory performance. Curiously enough, however, the study yielded only null-findings. That is, different COMT polymorphisms were not associated with different responses to the tDCS. Combined with the findings from the previous chapter, this implies that results from studies including pharmacological manipulation (e.g., tyrosine) should be generalized only with caution to findings of inter-individual differences (e.g., the COMT polymorphism). In this particular case, state (i.e., a manipulation of) and trait (i.e., baseline) differences in dopamine appear to exert different effects on tDCS

Chapter Eight marks the transition away from dopamine and toward the topic of neural inhibition and response selection. The next following chapters, each in their own way, investigate how a presumed increase or decrease in neural inhibition affects the ability to select the right response from several alternatives. In Chapter Eight the first study related to this topic is presented, which focused on the food supplement glutamine. Similar to how tyrosine is the precursor to dopamine, so is glutamine the precursor to glutamate and GABA. These are the main excitatory and inhibitory neurotransmitters, respectively, and therefore supplementation of glutamine

could potentially affect the level of neural inhibition. Despite glutamine being a popular supplement used often by bodybuilders, its cognitive-behavioral effects have been little examined so far. To investigate if and how glutamine affects response selection, participants were supplemented with glutamine or placebo and then performed the SRT task, which taps into both sensorimotor (i.e., stimulus-based) control and implicit sequential learning. The results revealed no effect of glutamine on motor learning, but those who received glutamine did demonstrate an overall increase in response errors, particularly when the task required them to switch responding from one hand to the other. This finding implies that glutamine enhanced the level of glutamate over GABA, thereby increasing cortical excitability and the response competition between different alternatives. This impairment appeared to be reliable only when switching between hands during the task, indicating that the increased cortical excitability allowed the laterality of the previous response to interfere with the current one. This is the first demonstration that glutamine can impair response selection via a presumed decrease in neural inhibition.

In **Chapter Nine** it was investigated whether the opposite can also be demonstrated. That is, whether an increase in neural inhibition could enhance response selection. Correlational evidence for this idea already exists, as studies have shown that individuals with greater GABA levels in striatal and thalamic regions are better at selecting a correct response from several competing options. In order to obtain causal evidence for this idea, the study in Chapter Nine made use of transcutaneous (through the skin) vagus nerve stimulation (tVNS), a non-invasive method of brain stimulation that can effectively enhance GABA level in the brain. This manipulation was again combined with the SRT task, to determine whether tVNS benefits response selection processes. Similar to the previous chapter, there were no differences in implicit sequence learning between those who received active (real) or sham (placebo) stimulation. However, as expected tVNS did enhance response selection. In particular, active tVNS eliminated a phenomenon similar to 'inhibition of return', in which participants are slower when the currently-required response is the same as the response on two trials earlier. In other

words, whereas those receiving sham tVNS did demonstrate this inhibition of return, also referred to as a reversal effect, those receiving active tVNS did not demonstrate such slowing of response speed. This finding converges on reports from previous studies that suggest tVNS, via a presumed enhancement of GABA, can be an effective tool to enhance cognitive-behavioral control.

Lastly, in **Chapter Ten** neural inhibition was manipulated with tDCS. However, whereas previously mentioned tDCS studies typically directly targeted PFC regions, in this chapter tDCS was instead applied to the cerebellum. This area is notable for the fact that it contains up to 80% of all neurons in the entire brain, and it is known to play an important role in the planning, initiation and coordination of movement. Few studies have yet investigated if cerebellar tDCS can affect response selection, but evidence in favor of this possibility comes from a study demonstrating that cerebellar tDCS can modulate a phenomenon called 'cerebello-brain inhibition' (CBI). This refers to the fact that the cerebellum exerts an inhibitory tone over the primary motor cortex, and this inhibition can be strengthened by excitatory and weakened by inhibitory stimulation of the cerebellum. This in turn can affect whether it is more difficult or easier to initiate movement. To investigate whether this modulation of CBI can indeed translate to a change in response selection ability, in Chapter Ten participants received either excitatory (anodal), inhibitory (cathodal) or sham (placebo) stimulation to the cerebellum while performing the SRT task. As in previous chapters, this manipulation did not appear to immediately affect implicit motor sequence learning, but the excitatory stimulation as compared to inhibitory and sham stimulation did impact response selection as evidenced by an overall increase in reaction time. This finding is consistent with the idea that excitatory stimulation of the cerebellum strengthens CBI and thereby hinders the ability to initiate movement. Notably, this study also included a 24 hour follow-up session without any tDCS, in order to investigate how stimulation during the task might have affected consolidation processes after the task had finished. This follow-up revealed that the pattern of results on the previous day persisted: those who had received excitatory stimulation still demonstrated increased

reaction times, but only when they were presented with two different response sequences in the same SRT block. Possibly, this indicates that excitatory stimulation of the cerebellum did affect how robustly participants acquired the motor sequence, which became apparent only when an untrained sequence on day two interfered with the trained sequence. These results are among the first to establish cerebellar tDCS as a potential tool for modulating response selection, and they suggest that its effects are mediated by a change in the inhibitory tone of the cerebellum over primary motor cortex.

Concluding this overview, the chapters in this dissertation provide further insight into if and how it is possible to measure individual differences in the neural chemistry underlying cognitive-behavioral control. Furthermore, it explores various methods for noninvasive manipulation of these biological underpinnings and provides evidence that some of these methods are promising tools for the purpose of cognitive-behavioral enhancement.

Chapter One

Spontaneous eye blink rate as predictor of dopamine-related cognitive function—a review

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Abstract

An extensive body of research suggests that the spontaneous eye blink rate (EBR) is a non-invasive indirect marker of central dopamine (DA) function, with higher EBR predicting higher DA function. In the present review we provide a comprehensive overview of this literature. We broadly divide the available research in studies that aim to disentangle the dopaminergic underpinnings of EBR, investigate its utility in diagnosis of DA-related disorders and responsivity to drug treatment, and, lastly, investigate EBR as predictor of individual differences in DA-related cognitive performance. We conclude that (i) EBR can reflect both DA receptor subtype D1 and D2 activity, although baseline EBR might be most strongly related to the latter, (ii) EBR can predict hypo- and hyperdopaminergic activity as well as normalization of this activity following treatment, and (iii) EBR can reliably predict individual differences in performance on many cognitive tasks, in particular those related to reward-driven behavior and cognitive flexibility. In sum, this review establishes EBR as a useful predictor of DA in a wide variety of contexts.

Introduction

Decades of research show the spontaneous eye blink rate (EBR) is closely associated with central dopamine (DA) function, particularly in the striatum. Specifically, EBR tends to correlate positively with DA activity at rest, illustrated by the fact that reduced and increased activity due to drugs or disorders is associated with low and high EBR, respectively. As a non-invasive and easily-accessible measure, EBR can serve as a reliable albeit non-distinctive method of assessing DA function in humans and might be preferable to invasive and expensive techniques such as positron emission tomography (PET). Indeed, ever since its relation to DA was postulated (Stevens, 1978b), EBR has become a popular method of investigating DA in a variety of contexts. For example, EBR has been used to evaluate effects of dopaminergic drugs on DA function, explore the role of DA in psychiatric disorders, and investigate the effects of individual differences in DA function on cognitive performance. In the present summary review, we provide a comprehensive overview of the literature on EBR as predictor of DA function, focusing on pharmacological studies of EBR, baseline EBR in atypical and healthy populations, and, lastly, whether EBR predicts cognitive performance thought to depend on DA. Lastly, we discuss the different methodologies for EBR assessment and provide recommendations for future research. We hope this review informs future studies of the applicability of EBR to a variety of paradigms and its utility in clarifying cognitive research findings by distinguishing results of low, intermediate, and high blinkers.

Dopamine and eye blink rate

To understand the relation between EBR and DA-driven cognition, and to allow theory-driven predictions to be made for results with EBR, it is necessary to first consider the role of DA in neurophysiology and how this translates to cognition. DA exerts widespread, non-linear modulatory influences on both prefrontal cortex and striatum, allowing it to affect a wide range of processes (Nieoullon, 2002; Seamans & Yang, 2004). One characteristic role of DA is that its phasic (stimulus-driven) release in striatum codes a reward prediction

error (Hollerman & Schultz, 1998; Schultz, Dayan, & Montague, 1997), with bursts indicating an outcome better than expected (i.e. a positive error) and dips and pauses indicating an outcome worse than expected (i.e. a negative error) (Maia & Frank, 2011). On the other hand, tonic (background) DA level enhances signal-to-noise ratio of neural activity by suppressing spontaneous firing in neurons with low membrane potentials but enhancing task-dependent firing in neurons with high membrane potentials (Frank, 2005; Hernández-López, Bargas, Surmeier, Reyes, & Galarraga, 1997).

When considering the effects of DA on cognition it is important to distinguish between two of its receptor subtypes that can serve opposite functions, D1 and D2, although more exist. D1 and D2 receptors in prefrontal cortex have been proposed to drive a 'closed' vs. 'open' processing state that facilitates robust online maintenance and flexible updating (gating) of cognitive representations, respectively (Durstewitz & Seamans, 2008). Particularly relevant for the present review, other models have highlighted a role for D1 and D2 in the basal ganglia, where these receptor systems interact to form a DA-modulated decision threshold for selecting responses and updating representations in the cortex (Bahuguna, Aertsen, & Kumar, 2015; Frank & O'Reilly, 2006; Maia & Frank, 2011). Specifically, a D1-rich direct pathway in the basal ganglia provides a 'Go' signal that facilitates updating of representations and selection of the response under consideration in the cortex, while a D2-rich indirect pathway provides a 'NoGo' signal that suppresses competing responses and representations. Importantly, whereas DA has excitatory effects on D1-driven Go signals, it is inhibitory on D2-driven NoGo signals (Maia & Frank, 2011). As such, higher levels of DA (e.g. due to positive prediction errors) lower the decision threshold and promote gating by facilitating D1-driven Go signals and inhibiting the D2-driven NoGo pathway, whereas at lower levels (e.g. due to negative prediction errors) it reduces inhibition of D2-driven NoGo signals and thus facilitates response suppression and stability of cortical representations.

These models of dopaminergic modulation of the stability and flexibility of cortical representations offer an explanation of why DA tends to

follow an inverted-u-shaped association with performance on tasks requiring cognitive control rather than following a more-is-better principle (Cools & D'Esposito, 2011). Cognitive control is popularly defined as achieving a balance between the opposing demands of stable maintenance of task goals in the face of distractors and their flexible updating when situational demands have changed (Cools & D'Esposito, 2011). This suggests that too high levels of DA can facilitate gating up to a point where it becomes dysfunctional, resulting in heightened distractibility and impaired response inhibition because the decision threshold is set too low. Conversely, too little DA might raise the threshold to a point of inducing inflexibility and perseveration. Hence, a moderate DA level is associated with an optimal compromise between stability and flexibility, although lower or higher DA, e.g. due to genotypic variation, may confer benefits in situations that require more of the former or latter (Cools & D'Esposito, 2011).

The association between DA, its receptor subtypes and cognitive functions allows us to form predictions on how EBR could predict DA-driven cognition and behavior. Although studies reviewed below suggest EBR can reflect both drug-induced D1 and D2 activity, there is evidence that resting EBR is more strongly related to the D2 receptor system (Groman et al., 2014), perhaps due to increased sensitivity of D2 receptors to DA as compared to D1 (Frank & O'Reilly, 2006). Given that D2 receptors are reportedly up to 11 times as prevalent in the striatum than frontal cortex (Camps, Cortés, Gueye, Probst, & Palacios, 1989) and D2 may have stronger effects on the decision threshold in basal ganglia than D1 (Bahuguna et al., 2015), it is possible EBR primarily relates to cognitive function via D2-driven modulation of the decision threshold in the basal ganglia. A higher EBR, indicative of higher DA activity, should then be related to increased inhibition of the basal ganglia NoGo pathway and a consequently reduced decision threshold and facilitated gating. Indeed, studies on EBR and cognitive flexibility reviewed below support the idea that a higher EBR is associated with increased flexibility, albeit at the potential cost of increased distractibility.

One question remaining is *why* EBR reflects DA activity. Although the neural circuitry through which DA modulates EBR remains open to further investigation, one prime candidate is the spinal trigeminal complex, which has been proposed to play a direct role in the spontaneous blink generator circuit (Kaminer, Powers, Horn, Hui, & Evinger, 2011; Kaminer, Thakur, & Evinger, 2015). Crucially, there is evidence that the basal ganglia, via the superior colliculus and nucleus raphe magnus, can modulate input to and excitability of the trigeminal complex, thus providing a pathway through which DA could affect the trigeminal complex and, in turn, blinking (Basso & Evinger, 1996; Basso, Powers, & Evinger, 1996; Basso, Strecker, & Evinger, 1993; Evinger et al., 1993; Evinger, Sibony, Manning, & Fiero, 1988; Gnadt et al., 1997; Harper, Labuszewski, & Lidsky, 1979; Kimura, 1973; Labuszewski & Lidsky, 1979; Napolitano, Bonuccelli, & Rossi, 1997; Schicatano, Peshori, Gopalaswamy, Sahay, & Evinger, 2000). In particular, Kaminer et al. (2011) proposed that DA inhibits the trigeminal complex, via effects on the nucleus raphe magnus, which results in increased spontaneous blinking, thus offering a potential account for the relation between DA and EBR.

Overview

The present review will be structured as follows. First, to provide insight in the dopaminergic underpinnings of the spontaneous EBR, we summarize studies examining the effects of dopaminergic manipulations on EBR in non-human primates, rats, and humans. Second, to illustrate EBR's relation to and utility in distinguishing between varying levels of baseline DA function, we review studies that measured EBR in different human populations such as individuals with neurological or psychiatric disorders or history of drug use, different age groups, and gender. Third, to demonstrate the applicability to and usefulness in a variety of paradigms of cognitive research, we provide an overview of studies relating EBR of healthy humans in rest to their performance on cognitive tasks. Lastly, we discuss the different methodologies used to assess EBR and offer recommendations for future research.

We performed an electronic search for articles using the PubMed and Web of Science databases, using the following search terms: (eye blink OR eye-blink OR eyeblink OR blink) AND rate. After selecting articles based on the title and abstract's relevance to DA function, we performed a forward and backward citation search for additional articles. We included only articles written in English.

Effects of dopaminergic manipulations on eye blink rate

In this section we review studies on the effects of dopaminergic manipulations on EBR in non-human primates, rats, and healthy humans. In Table 1 and 2 an overview of the following studies is provided, listing all drug and dose combinations, the associated EBR change, sample size, and methodology for EBR assessment. Note that many studies do not report the statistical significance of the change in EBR for every drug and dose combination. To avoid reporting inaccurate information, we list the EBR change as "not available" (NA) when the statistical significance for the given drug and dose combination is not explicitly reported in the text, tables, or figures. When a drug is reported to alter EBR but the significant dose was not specified, we report the EBR change as not available but include in parentheses the direction of effect suggested in-text. We revisit this issue in the discussion.

Table 1. Overview of dopaminergic single-drug studies in non-human primates, rats, and healthy humans.

Drug	Dose	EBR	*N*	Recording method	Condition	Study
Non-human primates						
Non-selective DA agonist						
Apomorphine	0.01 mg/kg	=	4	Observation	In cage	Kleven and Koek 1996
	0.02 mg/kg	=	4	Observation	In cage	Karson et al. 1981c
	0.02-0.12 mg/kg	=	4	Observation	In cage	Karson et al. 1981b
	0.03 mg/kg	=	4	Video	Primary gaze	Kotani et al. 2016
	0.04 mg/kg	↑	4	Observation	In cage	Kleven and Koek 1996
	0.1-0.2 mg/kg	↑	5	Observation	In cage	Lawrence and Redmond Jr. 1991
	0.1-1.0 mg/kg	↑	4	Video	Primary gaze	Kotani et al. 2016
	0.15 mg/kg	↓	3	Video	Video watching	Baker et al. 2002
	0.16 mg/kg	↑	4	Observation	In cage	Kleven and Koek 1996
	0.18-0.24 mg/kg	↑	4	Observation	In cage	Karson et al. 1981b
	0.25 mg/kg	↑	8	Observation	In cage	Casey et al. 1980
	0.36 mg/kg	↑	4	Observation	In cage	Karson et al. 1981c
	0.45 mg/kg	↑	4	Observation	In cage	Karson et al. 1981c
Cocaine	0.16 mg/kg	=	4	Observation	In cage	Kleven and Koek 1996
	0.63-2.5 mg/kg	↓	4	Observation	In cage	Kleven and Koek 1996
d-amphetamine	0.01-0.16 mg/kg	=	4	Observation	In cage	Kleven and Koek 1996
GBR 12935	0.16-2.5 mg/kg	=	4	Observation	In cage	Kleven and Koek 1996
Methamphetamine	0.03-3.2 mg/kg	NA (↑)	4	Video	In chamber	Jutkiewicz and Bergman 2004
Methylphenidate	0.16-2.5 mg/kg	NA (↓)	4	Observation	In cage	Kleven and Koek 1996
PD 128907	0.01-0.04 mg/kg	=	3-4	Observation	In cage	Kleven and Koek 1996
	0.16 mg/kg	↑	3-4	Observation	In cage	Kleven and Koek 1996
	0.3-1.0 mg/kg	↓	4	Video	Primary gaze	Kotani et al. 2016
Non-selective DA antagonist						
MPTP	0.63-1.25 mg/kg	↓	12	Observation	In cage	Mavridis et al. 1991
	2.0 mg/kg	↓	11	Observation	In cage	Lawrence and Redmond Jr. 1991
D1 agonist						
A77636	0.005-0.5 mg/kg	NA (↑)	10	Video	In chamber	Groman et al. 2014
Dihydrexidine	0.3-2.0 mg/kg	↑	5	Observation	In cage	Elsworth et al. 1991
R-6Br-APB	0.001-0.3 mg/kg	NA (↑)	7	Video	In chamber	Jutkiewicz and Bergman 2004
SKF 38393	3.0-17.8 mg/kg	=	7	Video	In chamber	Jutkiewicz and Bergman 2004
	30.0 mg/kg	NA (↓)	7	Video	In chamber	Jutkiewicz and Bergman 2004
SKF 75670	0.16-0.63 mg/kg	↑	3-4	Observation	In cage	Kleven and Koek 1996
	2.5 mg/kg	=	2	Observation	In cage	Kleven and Koek 1996
SKF 77434	0.03-1.0 mg/kg	NA	4	Video	In chamber	Jutkiewicz and Bergman 2004
	3.0-10.0 mg/kg	NA (↑)	4	Video	In chamber	Jutkiewicz and Bergman 2004
	17.8 mg/kg	=	4	Video	In chamber	Jutkiewicz and Bergman 2004
SKF 81297	0.03-3.0 mg/kg	NA (↑)	7	Video	In chamber	Jutkiewicz and Bergman 2004
	0.16 mg/kg	=	3-4	Observation	In cage	Kleven and Koek 1996
	0.63-2.5 mg/kg	↑	3-4	Observation	In cage	Kleven and Koek 1996
	0.03-1.0 mg/kg	NA (↑)	9	Video	In chamber	Jutkiewicz and Bergman 2004

SKF 82958	0.1-0.3 mg/kg	↑	4	Video	Primary gaze	Kotani et al. 2016
	0.16 mg/kg	=	3-4	Observation	In cage	Kleven and Koek 1996
	0.63-2.5 mg/kg	↑	3-4	Observation	In cage	Kleven and Koek 1996
SKF 83959	0.001-3.0 mg/kg	NA (↑)	7	Video	In chamber	Jutkiewicz and Bergman 2004
D1 antagonist						
SCH 23390	0.01 mg/kg	=	5	Observation	In cage	Elsworth et al. 1991
	0.05-0.3 mg/kg	↓	4	Observation	NA	Lawrence et al. 1991
	0.3 mg/kg	↓	5	Observation	In cage	Elsworth et al. 1991
	1.0-10.0 mg/kg	=	2	Observation	NA	Lawrence et al. 1991
SCH 39166	0.01-0.1 mg/kg	NA (↓)	4	Video	In chamber	Jutkiewicz and Bergman 2004
	0.03-0.1 mg/kg	=	4	Video	Primary gaze	Kotani et al. 2016
D2 agonist						
3-PPP	0.16 mg/kg	=	3-4	Observation	In cage	Kleven and Koek 1996
	0.63-2.5 mg/kg	↑	3-4	Observation	In cage	Kleven and Koek 1996
Bromocriptine	NA	↑	4	Observation	In cage	Karson et al. 1981c
Naxagolide	0.0025 mg/kg	=	3-4	Observation	In cage	Kleven and Koek 1996
	0.01-0.04 mg/kg	↑	3-4	Observation	In cage	Kleven and Koek 1996
PHNO	0.001-0.01 mg/kg	↑	5	Observation	In cage	Elsworth et al. 1991
	0.001-0.01 mg/kg	↑	5	Observation	In cage	Lawrence and Redmond Jr. 1991
	0.001-0.03 mg/kg	NA (↑)	4	Video	In chamber	Jutkiewicz and Bergman 2004
	0.001-0.1 mg/kg	↓	4	Video	Primary gaze	Kotani et al. 2016
	0.005-0.5 mg/kg	NA (↑)	10	Video	In viewing chamber	Groman et al. 2014
PPHT	0.01 mg/kg	=	3-4	Observation	In cage	Kleven and Koek 1996
	0.04-0.16 mg/kg	↑	3-4	Observation	In cage	Kleven and Koek 1996
Quinelorane	0.003-0.1 mg/kg	NA (↑)	3	Video	In chamber	Jutkiewicz and Bergman 2004
Quinpirole	0.01-0.16 mg/kg	↑	3-4	Observation	In cage	Kleven and Koek 1996
Roxindole	0.01 mg/kg	=	3-4	Observation	In cage	Kleven and Koek 1996
	0.04-0.16 mg/kg	↑	3-4	Observation	In cage	Kleven and Koek 1996
SDZ 208912	0.16-0.63 mg/kg	=	3-4	Observation	In cage	Kleven and Koek 1996
	2.5 mg/kg	=	2	Observation	In cage	Kleven and Koek 1996
Terguride	0.01-2.5 mg/kg	=	3-4	Observation	In cage	Kleven and Koek 1996
D2 antagonist						
Haloperidol	0.03 mg/kg	=	4	Video	Primary gaze	Kotani et al. 2016
	1.0 mg/kg	↓	5	Observation	In cage	Lawrence and Redmond Jr. 1991
Remoxipride	1.0 mg/kg	=	5	Observation	In cage	Elsworth et al. 1991
Sulpiride	100.0 mg/kg	↓	5	Observation	In cage	Lawrence and Redmond Jr. 1991
D4 agonist						
A 412997	0.1-0.3 mg/kg	=	4	Video	Primary gaze	Kotani et al. 2016
PD 168077	1.0-3.0 mg/kg	=	4	Video	Primary gaze	Kotani et al. 2016

Rats						
Non-selective DA agonist						
Apomorphine	1.0 mg/kg	↑	3	EMG	In cage	Kaminer et al. 2011
Methamphetamine	0.3-10.0 mg/kg	=	5-7	Video	Primary gaze	Desai et al. 2007
	10.0 mg/kg	=	2	Video	Primary gaze	Desai et al. 2007
D1 agonist						
Fenoldopam	0.01-1.0 mg/kg	=	5-7	Video	Primary gaze	Desai et al. 2007
R-6Br-APB	0.001-0.03 mg/kg	=	5-7	Video	Primary gaze	Desai et al. 2007
	0.1 mg/kg	↑	5-7	Video	Primary gaze	Desai et al. 2007
	0.3 mg/kg	=	5-7	Video	Primary gaze	Desai et al. 2007
	1.0-3.0 mg/kg	↑	5-7	Video	Primary gaze	Desai et al. 2007
SKF 82958	0.01-0.03 mg/kg	=	5-7	Video	Primary gaze	Desai et al. 2007
	0.1-0.3 mg/kg	↑	5-7	Video	Primary gaze	Desai et al. 2007
	1.0-10.0 mg/kg	=	5-7	Video	Primary gaze	Desai et al. 2007
SKF 83959, MCL 202	0.01-1.0 mg/kg	=	5-7	Video	Primary gaze	Desai et al. 2007
SKF 83959, MCL 204, 207	0.01-3.0 mg/kg	=	5-7	Video	Primary gaze	Desai et al. 2007
SKF 83959, MCL 206	0.03-1.0 mg/kg	=	5-7	Video	Primary gaze	Desai et al. 2007
SKF 83959, MCL 209	0.03-0.3 mg/kg	=	5-7	Video	Primary gaze	Desai et al. 2007
	1.0-3.0 mg/kg	↑	5-7	Video	Primary gaze	Desai et al. 2007
D1 antagonist						
SCH 23390	0.01 mg/kg	=	5-7	Video	Primary gaze	Desai et al. 2007
	0.03 mg/kg	↓	5-7	Video	Primary gaze	Desai et al. 2007
	0.1 mg/kg	=	5-7	Video	Primary gaze	Desai et al. 2007
	0.3 mg/kg	↓	5-7	Video	Primary gaze	Desai et al. 2007
	1.0 mg/kg	=	5-7	Video	Primary gaze	Desai et al. 2007
D2 agonist						
PHNO	0.0003-0.03 mg/kg	=	5-7	Video	Primary gaze	Desai et al. 2007
D2 antagonist						
Haloperidol	0.1 mg/kg	↓	3	EMG	In cage	Kaminer et al 2011
Healthy adult humans						
Non-selective DA agonist						
Apomorphine	0.0005-0.002 mg/kg	↑	8	Video	NA	Blin et al. 1990
d-amphetamine	0.25 mg/kg	↑	11	Observation	Interview	Strakowski et al. 1996
		↑	11	Observation	Interview	Strakowski et al. 1998
L-dopa	250 mg	=	39	EOG	Primary gaze	Mohr et al. 2005
Venlafaxine	12.5-25 mg	=	16	EOG	Auditory oddball task	Semlitsch et al. 1993
	50 mg	↑	16	EOG	Auditory oddball task	Semlitsch et al. 1993

D2 agonist						
Bromocriptine	0.04-0.05 mg/kg	=	11	Video	Primary gaze	Depue et al. 1994
	2.5 mg	=	12	Video	Silence	Ebert et al. 1996
Cabergoline	1.25 mg	Low blinkers: ↑	27	EOG	Rest	Cavanagh et al. 2014
		High blinkers: ↓	27	EOG	Rest	Cavanagh et al. 2014
Lisuride	0.2 mg	=	12	EOG	Primary gaze	Van der Post et al. 2004
D2 antagonist						
Sulpiride	400 mg	=	12	EOG	Primary gaze	Van der Post et al. 2004

↓, decreased at $p < .05$; ↑, increased at $p < .05$; =, no difference; DA, dopamine; EBR, eye blink rate; EMG, electromyography; NA; not available
Arrows between brackets indicate direction of effect reported in text without reporting specific EBR values and corresponding significance levels

Table 2. Overview of dopaminergic drug interaction studies in non-human primates and rats.

Main drug	Main drug dose	Pretreatment drug	Pretreatment drug dose	EBR	*N*	Recording method	Condition	Study
Non-human primates								
Non-selective DA agonist								
Apomorphine	0.1 mg/kg	(D2-) Haloperidol	1.0 mg/kg	Decrease is prevented	4	Observation	In cage	Lawrence and Redmond Jr. 1991
	0.3 mg/kg	(D1-) SCH 39166	0.1 mg/kg	Increase is prevented	4	Video	Primary gaze	Kotani et al. 2016
		(D2-) Haloperidol	0.03 mg/kg	Increase is unaffected	4	Video	Primary gaze	Kotani et al. 2016
	0.36 mg/kg	(D2-) Haloperidol	1.0 mg/kg	Increase is prevented	4	Observation	In cage	Karson et al. 1981c
	0.45 mg/kg	(D2-) Sulpiride	10.0 mg/kg	Increase is smaller	4	Observation	In cage	Karson et al. 1981c
D1 agonist			20.0 mg/kg	Increase is prevented	4	Observation	In cage	Karson et al. 1981c
Dihydrexine	0.3 mg/kg	(D1-) SCH 23390	0.01 mg/kg	Increase is prevented	5	Observation	In cage	Elsworth et al. 1991
		(D2-) Remoxipride	1.0 mg/kg	Increase is unaffected	5	Observation	In cage	Elsworth et al. 1991
R-6-BR-APB	0.01-3.0 mg/kg	(D1+) SKF 83959	0.1-3.0 mg/kg	Increase is smaller	3	Video	In chamber	Jutkiewicz and Bergman 2004
	0.03-10.0 mg/kg	(D1-) SCH 39166	0.1-1.0 mg/kg	Increase is smaller	4	Video	In chamber	Jutkiewicz and Bergman 2004
SKF 81297	2.5 mg/kg	(DA+) Cocaine	0.16-0.25 mg/kg	Increase is unaffected	3	Observation	In cage	Kleven and Koek 1996
SKF 82958	0.03-0.1 mg/kg	(D2+) PHNO	0.003 mg/kg	Increase is larger	3	Video	In chamber	Jutkiewicz and Bergman 2004
	0.03-3.0 mg/kg	(D2-) Haloperidol	0.01-0.1 mg/kg	Increase is unaffected	4	Video	In chamber	Jutkiewicz and Bergman 2004
	0.3-1.0 mg/kg	(D2+) PHNO	0.003 mg/kg	Increase is smaller	3	Video	In chamber	Jutkiewicz and Bergman 2004
D2 agonist								
PHNO	0.001 mg/kg	(D1-) SCH 23390	0.01 mg/kg	Increase is unaffected	5	Observation	In cage	Elsworth et al. 1991
		(D2-) Remoxipride	1.0 mg/kg	Increase is prevented	5	Observation	In cage	Elsworth et al. 1991
		(D2-) Sulpiride	100.0 mg/kg	Increase is prevented	5	Observation	In cage	Lawrence and Redmond Jr. 1991
Rats								
D1 agonist								
SKF 82958	0.003-0.3 mg/kg	(D1+) SKF 83959, MCL 204, 207	1.0 mg/kg	Increase is unaffected	5-6	Video	Primary gaze	Desai et al. 2007
	0.01-1.0 mg/kg	(D1+) SKF 83959, MCL 206	1.0 mg/kg	Increase is smaller	5-6	Video	Primary gaze	Desai et al. 2007
	0.03-1.0 mg/kg	(D1-) SCH 23390	0.03 mg/kg	Increase is smaller	5-6	Video	Primary gaze	Desai et al. 2007

+, agonist; -, antagonist; DA, dopamine; NA; not available

Animal studies

Early studies investigating the role of DA in EBR showed administration of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which destroys DA synthesizing cells in the substantia nigra, markedly reduced blink rate in monkeys (Lawrence and Redmond Jr., 1991; Mavridis et al., 1991). In contrast, in monkeys and rats the administration of apomorphine, a non-selective DA agonist, induced transient increases in EBR that lasted approximately one hour (Casey et al., 1980; Kaminer et al., 2011; Karson et al., 1981b, 1981c; Kleven and Koek, 1996; Kotani et al., 2016; Lawrence and Redmond Jr., 1991). One study reported EBR in monkeys was reduced 90 minutes after administration of apomorphine (Baker, Radmanesh, & Abell, 2002), suggesting a potential biphasic effect on DA activity not reported elsewhere. Although these studies support a link between EBR and DA, the non-selective nature of MPTP and apomorphine's effect on DA receptors does not reveal whether particular receptor subtypes might play different roles in EBR. One study demonstrated apomorphine-induced increases in EBR are completely blocked by a selective D1 but not a D2 antagonist (Kotani et al., 2016) whereas another study showed the D2 antagonist sulpiride could block an apomorphine-induced increase in EBR (Karson, Staub, et al., 1981b). As the nullfinding by Kotani et al. is possibly explained by a too low dose of D2 antagonist (haloperidol, 0.03 mg/kg) in comparison with Karson et al.'s higher dose of the same drug (1.0 mg/kg), it seems like apomorphine-induced changes in EBR are mediated both by D1 and D2 receptors.

To disentangle the contributions of D1 and D2 to EBR, studies have employed agonists that more selectively target either subtype and, in doing so, have shown both can affect EBR. D1 agonists increase EBR in monkeys and rats (Desai, Neumeyer, Bergman, & Paronis, 2007; Elsworth et al., 1991; Groman et al., 2014; Jutkiewicz & Bergman, 2004; Kotani et al., 2016) and this increase is negated by pretreatment with D1 antagonists (Elsworth et al., 1991; Jutkiewicz & Bergman, 2004). Treatment with only D1 antagonists can decrease EBR (Desai et al., 2007; Jutkiewicz & Bergman, 2004; Lawrence, Redmond, Elsworth, Taylor, & Roth, 1991). Likewise, D2 agonists have been

shown to increase EBR, although only in monkeys (Elsworth et al., 1991; Groman et al., 2014; Jutkiewicz and Bergman, 2004; Karson et al., 1981c; Lawrence and Redmond Jr., 1991), and this increase can be reversed by pretreatment with D2 antagonists (Elsworth et al., 1991; Kaminer et al., 2011). As with D1, treatment with only D2 antagonists can decrease EBR (Kaminer et al., 2011; Lawrence and Redmond Jr., 1991). One study further demonstrated the stimulating effect of the D2 agonist (+)-4-propyl-9-hydroxynapthoxazine (PHNO) on EBR was moderated by D2 receptor availability in the striatum, with the drug having stronger effects on EBR with increasing availability (Groman et al., 2014). No such relationship was found for the D1 agonist A 77636 and D1 receptor availability. Notably, baseline EBR was positively related to D2 but not D1 receptor availability, raising the possibility baseline EBR is primarily D2-driven while D1-mediated influences are restricted to pharmacological conditions. This is potentially explained by the fact D2 receptors are more sensitive to DA than D1 (Frank & O'Reilly, 2006).

Moreover, there is evidence suggesting the effects of D1 and D2 receptors on EBR are at least partly independent. This was first demonstrated by Elsworth et al. (1991), who administered monkeys either a D1 or D2 agonist, with or without a D1 or D2 antagonist. They found the D1 agonist produced a dose-dependent increase in EBR and this could be blocked only by the D1 and not the D2 antagonist. Conversely, the D2 agonist produced a dose-dependent increase in EBR that could be blocked only by the D2 and not the D1 antagonist. Similarly, Jutkiewicz and Bergman (2004) showed several D1 agonists produced significant increases in EBR that could be blocked by a D1 but not a D2 antagonist. Of particular interest is their additional finding that a D2 agonist can attenuate D1 agonist-induced increases in EBR, suggesting, although the two receptor subtypes can independently modulate EBR, they might also inhibit each other (Jutkiewicz & Bergman, 2004).

While most studies have focused on D1 and D2, others have also examined the effects of direct agonists targeting D3-4 receptors, as well as indirect agonists such as cocaine and amphetamine. Kotani et al. (2016) found

that in monkeys a D2/D3 agonist reduced EBR, proposed to be caused by increased drowsiness, while D4 agonists did not affect EBR. The indirect agonist amphetamine has been shown to increase EBR (Jutkiewicz & Bergman, 2004), although other studies found no effect (Desai et al., 2007; Kleven & Koek, 1996). Even a decrease in EBR following administration of the indirect agonists cocaine or methylphenidate was found (Kleven & Koek, 1996).

Overall, evidence from animal studies converges on the idea that pharmacological activation of D1 or D2 receptors modulates EBR and these receptors do so at least partly independent. Further, baseline EBR in monkeys was associated with D2 but not D1 receptor availability, suggesting D2 receptors in particular are linked to resting EBR. This is perhaps because the D2 receptor has been suggested to be far more sensitive to low DA levels than the D1 receptor (Frank & O'Reilly, 2006).

To conclude this section, it should be noted not all results have been unequivocal and this might be attributable to different drug doses across studies. The D2 agonist PHNO has also been found to decrease instead of increase EBR in monkeys (Kotani et al., 2016), proposed to be due to increased drowsiness, while the same drug did not affect rats (Desai et al., 2007). The former is surprising because the used dose was comparable to studies reporting increased EBR, but the nullfinding in rats may be due to a too low drug dose (0.0003-0.03 mg/kg). Also contradictory is Kotani et al. (2016) did not find lower EBR in monkeys following administration of only a D1 or D2 antagonist, but this might be because their doses were lower (0.01-0.1 mg/kg) compared to other studies that did report significant reductions. Indeed, Elsworth et al. (1991) found significantly reduced EBR after 0.3 mg/kg of the D1 antagonist SCH 23390 but not after 0.01 mg/kg. In sum, although results vary according to specific drugs and doses, the majority of animal drug studies indicate stimulation of either D1 or D2 receptors increases EBR, whereas blocking these receptors can reduce it.

Healthy human studies

Pharmacological studies investigating EBR in humans are less numerous than those conducted with animals but they reveal a similar albeit more complex picture than discussed so far. Consistent with previous studies, in healthy humans the non-selective DA agonist apomorphine increased EBR (Blin, Masson, Azulay, Fondarai, & Serratrice, 1990) and the indirect agonist amphetamine increased EBR as well (Strakowski, Sax, Setters, & Keck, 1996; Strakowski & Sax, 1998). Repeated doses of amphetamine induced sensitization effects, i.e. increases in EBR were larger for subsequent doses. On the other hand, administration of DA's precursor L-dopa did not affect EBR (Mohr, Sándor, Landis, Fathi, & Brugger, 2005). The antidepressant venlafaxine, which has DA reuptake-inhibiting effects, increased EBR at a dose of 50 mg but not 12.5 or 25 mg, although these findings may be confounded as placebo intake also resulted in increased EBR (Semlitsch, Anderer, Saletu, Binder, & Decker, 1993).

Studies investigating the effect of selective DA agonists in healthy humans are few and have all focused on D2 receptors, with mixed results. The D2 agonist bromocriptine did not affect EBR (Depue, Luciana, Arbisi, Collins, & Leon, 1994; Ebert et al., 1996) and, similarly, van der Post et al. (2004) did not find significantly altered EBR following either lisuride or sulpiride, D2 agonist and antagonist respectively. However, Cavanagh et al. (2014) showed administration of cabergoline, a D2 agonist, increased EBR in individuals with low blink rates at baseline but decreased EBR in those with high baseline blink rates. This indicates baseline EBR, and presumably the associated DA level, can modulate the effect of DA manipulations on blinking. This in turn suggests the previously mentioned nullfindings might be due to not considering baseline EBR of participants. Although an alternative explanation for these mixed findings might simply be related to different efficacies and doses of the respective drugs, as each used a different drug, the idea of modulation by baseline DA level fits the inverted-u-shaped relation DA typically has with cognitive-behavioral performance (Cools & D'Esposito, 2011).

In sum, drug studies in humans are mostly in line with the findings from animal studies, but they indicate drug-effects on EBR may not be linear and instead depend on baseline characteristics.

Baseline eye blink rate in human populations

Whereas the studies reviewed so far demonstrated pharmacological manipulations of DA can affect EBR, the following studies suggest endogenous differences, that is inter-individual variability in DA can also be of influence. For example, individuals with a history of neurological or psychiatric disorders or chronic/recreational drug use (hereafter referred to as 'atypical populations') can exhibit altered EBR. Indeed, Boutros and Hatch (1988) argued increased EBR might be a general marker of psychiatric illness, although as reviewed below severely decreased blink rates may be just as relevant. Individual differences are also found in healthy populations and might depend on factors such as age, gender, and certain lifestyle-practices. In the following sections we first focus on EBR in atypical populations thought to suffer from dysregulated DA activity, after which we examine factors of potential influence in healthy humans. In Tables 3 and 4 an overview of the following studies on atypical and healthy populations is provided, respectively.

Table 3. Overview of studies on EBR in atypical populations.

Population	EBR (relative)	EBR (mean atypical)	EBR (mean control)	*N* (atypical)	*N* (control)	Recording method	Condition	Study
Neurologic disorders								
Acute stroke	=	19.1	17.3	211	30	Observation	Conversation	Anagnostou et al. 2012
ALS (familial)	↓	6 (median)	13 (median)	11	42	NA	Watching video	Byrne et al. 2013
ALS (sporadic)	=	10.7 (median)	13 (median)	42	42	NA	Watching video	Byrne et al. 2013
Epilepsy (complex partial)	=	8.1	9.5	30	61	Video	Listening	Caplan et al. 1998
	↓	10.8	13.9	30	61	Video	Conversation	Caplan et al. 1998
	↓	14.6	19.7	30	61	Video	Verbal recall	Caplan et al. 1998
Epilepsy (myoclonic)	NA	8.3	NA	7	NA	Observation	NA	Schelkunov et al. 1986
Epilepsy (temporal)	NA	10.9	NA	19	NA	Observation	NA	Schelkunov et al. 1986
Generalized dystonia	=	23	24	9	82	Observation	Interview	Karson et al. 1984b
	↑	36.9	11.3	14	156	Observation	Primary gaze	Deuschl and Goddemeier 1998
Huntington's disease	NA	NA	NA	9	30	EOG	Primary gaze and/or movement	Valade et al. 1984
	=	36	24	10	82	Observation	Interview	Karson et al. 1984b
	NA	40	12 (median)	1	6	Video	Rest	Xing et al. 2008
Mild cognitive impairment	↑	27.6	20.2	36	33	EOG	Rest	Ladas et al. 2014
Multiple systems atrophy	↓	8.1	16.5	30	20	SMART	Primary gaze	Bologna et al. 2014
Parkinson's disease	↓	2.4	10.7	20	41	Video	Reading	Fitzpatrick et al. 2012
	=	4.7 (median)	9.5 (median)	10	14	Search coil	Watching video	Korošec et al. 2006
	↓	5.1	27.1	17	16	Video	Conversation	Kimber and Thompson 2000
	↓	5.8	11.3	51	156	Observation	Primary gaze	Deuschl and Goddemeier 1998
	↓	6.3	11.6	55	40	Observation	Rest	Aksoy et al. 2014
	↓	6.4	19.7	16	15	SMART	Primary gaze	Bologna et al. 2012
	↓	7.0	18.4	20	41	Video	Watching video	Fitzpatrick et al. 2012
	↓	7.3	10.6	30	338	Observation	Rest	Chen et al. 2003
	↓	7.4	21.9	10	10	SMART	Primary gaze	Agostino et al. 2008
	↓	8.0	19.1	13	11	Observation	Primary gaze	Agostino et al. 1987
	↓	8.5	12.4	4	5	Observation	Conversation	Reddy et al. 2013
	↓	12	16	34	24	Observation	Conversation	Karson et al. 1982b
	↓	12	24	25	82	Observation	Conversation	Karson et al. 1984b
	=	12.5	15.7	10	10	Video	Primary gaze	Golbe et al. 1989
	↓	12.7	21.7	56	34	Video	Watching video	Tamer et al. 2005
	=	14.8	9.1	10	10	Video	Horizontal versions	Golbe et al. 1989
	↓	17.1	24.8	30	31	NA	NA	Biousse et al. 2004
	↓	18.0	34.4	20	41	Video	Conversation	Fitzpatrick et al. 2012

	↑	20.4 (median)	9.5 (median)	6	14	Search coil	Watching video	Korošec et al. 2006
	=	24.3	21.9	6	10	SMART	Primary gaze	Agostino et al. 2008
	↑	32	16	21	24	Observation	Interview	Karson et al. 1982b
	↑	52.8	27.1	8	16	Video	Conversation	Kimber and Thompson 2000
Progressive supranuclear palsy	↓	1.9	12.4	7	5	Observation	Interview	Reddy et al. 2013
	↓	3.0	15.7	38	10	Video	Primary gaze	Golbe et al. 1989
	↓	4	24	5	82	Observation	Conversation	Karson et al. 1984b
	=	5.3	9.1	38	10	Video	Horizontal versions	Golbe et al. 1989
	↓	5.9	22.4	11	10	SMART	Primary gaze	Bologna et al. 2009
Schizophrenia	↑	NA	NA	13	13	EOG	SPEM	Klein et al. 1993
	↑	NA (summer)	NA (summer)	21	40	NA	NA	Karson et al. 1984a
	=	NA (winter)	NA (winter)	34	42	NA	NA	Karson et al. 1984a
	=	7.3 (median)	8 (median)	40	33	Observation	Counting	Chen et al. 1996
	↓	8.0	15.2	20	23	Video	Interview	Mackintosh et al. 1983
	NA	9.8	NA	5	NA	Observation	NA	Schelkunov et al. 1986
	↑	13 (median)	8.4 (median)	40	33	Observation	Listening music	Chen et al. 1996
	↑	20.3	11.2	10	12	Observation	Primary gaze	Helms and Godwin 1985
	↑	22	14	40	34	Observation	Interview	Adamson 1995
	↑	25	16	75	94	Observation	Listening music	Chan et al. 2010
	↑	28	22	27	36	Observation	Interview	Karson et al. 1983
	↑	30	NA	41	81	Observation	Interview	Kleinman et al. 1984
	NA	30+	NA	23	NA	EOG	NA	Stevens 1978a
	↑	31	23	44	54	Observation	Interview	Karson et al. 1981a
	↑	34.9	22.3	47	29	EOG	SPEM	Mackert et al. 1988
	↑	49.9	26.2	23	35	EOG	Rest	Swarztrauber and Fujikawa 1998
	NA	60+	NA	17	NA	EOG	NA	Stevens 1978b
	=	62.0	44.9	6	16	EOG	Cognitive tasks	Swarztrauber and Fujikawa 1998
Traumatic brain injury	↓	10.3	12.4	26	24	Video	NA	Konrad et al. 2003
Neurodevelopmental disorders								
AD(H)D	=	NA	NA	11 (on Mph)	12	EOG	Primary gaze	Groen et al. 2015
	=	NA	NA	13 (off Mph)	12	EOG	Primary gaze	Groen et al. 2015
	=	NA	NA	16 (off Mph)	18	EOG	Attention task	Groen et al. 2015
	=	NA	NA	16 (on Mph)	18	EOG	Attention task	Groen et al. 2015
	=	NA	NA	18	25	EOG	NA	Tantillo et al. 2002
	=	5.9	7.2	9	61	Observation	Counting	Daugherty et al. 1993
	↓	9.2	12.4	29	24	Video	NA	Konrad et al. 2003
	=	16.1	16.2	9	61	Observation	Interview	Daugherty et al. 1993

AD(H)D + conduct disorder	=	7.4	7.2	11	15	Observation	Counting	Daugherty et al. 1993
	=	18.9	16.2	11	15	Observation	Interview	Daugherty et al. 1993
Autism	↑	13	6	15	52	Observation	Interview	Goldberg et al. 1987
Conduct disorder	=	6.4	7.2	8	15	Observation	Counting	Daugherty et al. 1993
	=	15.6	16.2	8	15	Observation	Interview	Daugherty et al. 1993
Gilles de la Tourette syndrome	↑	NA	NA	19	21	Eyetracking	Cognitive task	Tharp et al. 2015
	↑	NA	NA	19	21	Eyetracking	Primary gaze	Tharp et al. 2015
	NA	12	NA	9	NA	Video	Calculating	Karson et al. 1985
	=	13	13	9	49	Video	Reading	Karson et al. 1985
	=	20	19	9	49	Video	Silence	Karson et al. 1985
	NA	26.6	NA	14	NA	Observation	NA	Schelkunov et al. 1986
	↑	35.1	14.1	9	10	Video	Rest	Tulen et al. 1999
	=	40.7	25.2	9	10	Video	Conversation	Tulen et al. 1999
	↑	44.0	16.7	9	10	Video	Watching video	Tulen et al. 1999
I(D)D	↓	8.9	19.6	25	19	Video	Primary gaze	Lee et al. 2010
I(D)D + stereotypy	↓	4.4	19.6	8	19	Video	Primary gaze	Lee et al. 2010
Mental retardation	↓	4	6	34	52	Observation	Interview	Goldberg et al. 1987
	↓	7.0	16.0	15	7	Observation	NA	Roebel and MacLean, Jr. 2007
Stereotypy	↓	6.0	14.7	10 (men)	10 (men)	Observation	Facing mirror	Maclean, Jr. et al. 1985
	=	10.4	6.3	10 (women)	10 (women)	Observation	Facing mirror	Maclean, Jr. et al. 1985
Psychiatric disorders								
Anorexia nervosa	↑	20	11	20	16	EOG	Primary gaze	Barbato et al. 2006
Anxiety withdrawal disorder	=	6.3	7.2	12	15	Observation	Counting	Daugherty et al. 1993
	=	14.7	16.2	12	15	Observation	Interview	Daugherty et al. 1993
Major depression	=	2.3	5.1	12	12	Video	Reading	Ebert et al. 1996
	=	20.1	19.6	12	12	Video	Listening	Ebert et al. 1996
	=	23.8	18.4	12	12	Video	Silence	Ebert et al. 1996
	↑	25.9	15.2	28	23	Video	Interview	Mackintosh et al. 1983
	=	30.5	27.4	12	12	Video	Calculating	Ebert et al. 1996
Major depression (psychotic)	↑	NA	NA	59	30	EOG	Rest	Giedke and Heimann 1987
	=	8.6	11.2	8	12	Observation	Primary gaze	Helms and Godwin 1985
Panic disorder	↑	NA	NA	11	16	Video	Rest	Kojima et al. 2002
	↑	NA	NA	11	16	Video	Watching video	Kojima et al. 2002
Psychosis	↑	16	10	13	35	Observation	NA	Karson et al. 1986
	NA	27.1	NA	38	NA	Observation	Interview	Ostow and Ostow 1945
	NA	104	NA	1	NA	NA	Interview	Lovestone 1992
Seasonal affective disorder	=	15	15	19	18	EOG	Primary gaze	Barbato et al. 1993

	↑	20.3 (winter)	10 (winter)	17	9	EOG	Silence	Depue et al. 1990
	↑	22.3 (summer)	8.3 (summer)	11	5	EOG	Silence	Depue et al. 1990
	↑	23.1	10.4	4	4	EOG	Silence	Depue et al. 1988
Miscellaneous disorders								
Fragile X syndrome	=	8.4	7.1	6	6	Video	Intelligence test	Roberts et al. 2005
	↑	12.7	6.9	6	6	Video	Watching video	Roberts et al. 2005
Graves' orbitopathy	=	17.6	19.8	10	10	Search coil	Watching video	Garcia et al. 2011
Iron-deficienct anemia	↓	4.0	5.3	19	42	Video	Watching bubbles	Lozoff et al. 2010
Prader-Willi syndrome	NA	18.7	NA	16	NA	Video	Watching video	Holsen and Thompson 2004
Wilson's disease	NA	32	NA	1	NA	NA	NA	Verma et al. 2012
Drug use								
Alcohol abuse	=	NA	NA	11	15	Video	NA	Upadhyaya et al. 2003
Cannabis	↓	10.2	17.5	25	25	EOG	Primary gaze	Kowal et al. 2011
Cocaine (recreational)	↓	9.3	17.1	12	12	EOG	Primary gaze	Colzato et al. 2008b

↓, decreased at $p < .05$; ↑, increased at $p < .05$; =, no difference; AD(H)D, attention deficit (hyperactivity) disorder; ALS, amyotrophic lateral sclerosis; EBR, eye blink rate; EOG, electrooculography; I(D)D, intellectual (and developmental) disorder; Mph, methylphenidate; NA, not available; SMART, SMART analyzer motion system; SPEM, smooth pursuit eye movement

Atypical populations

One of the first disorders to be associated with altered EBR is Parkinson's disease (PD; Hall, 1945), a condition characterized by severe progressive loss of dopaminergic neurons in the striatum (Dauer & Przedborski, 2003). Consistent with a hypodopaminergic state, PD or related features are associated with reduced EBR (Agostino et al., 2008; Agostino, Berardelli, Cruccu, Stocchi, & Manfredi, 1987; Aksoy, Ortak, Kurt, Cevik, & Cevik, 2014; Biousse et al., 2004; Bologna et al., 2014; Bologna, Fasano, Modugno, Fabbrini, & Berardelli, 2012; Deuschl & Goddemeier, 1998; Fitzpatrick, Hohl, Silburn, O'Gorman, & Broadley, 2012; Karson, Burns, LeWitt, Foster, & Newman, 1984; Karson, LeWitt, Calne, & Wyatt, 1982; Kimber & Thompson, 2000; Korošec, Zidar, Reits, Evinger, & Vanderwerf, 2006; Reddy, Patel, Hodge, & Leavitt, 2013; Tamer, Melek, Duman, & Öksüz, 2005), although three studies found only a nonsignificant decrease (Chen, Chiang, Hsu, & Liu, 2003; Golbe, Davis, & Lepore, 1989; Korošec et al., 2006). In line with the progressive nature of PD, some reported EBR was more strongly reduced with increasing disease severity or duration (Aksoy et al., 2014; Karson, Burns, et al., 1984; Karson, LeWitt, et al., 1982; Tamer et al., 2005). Although a meta-analysis suggested this association to be not significant (Fitzpatrick et al., 2012), no data was reported and thus more research is required before drawing definitive conclusions. Consistent with the idea PD can be treated by DA-stimulating drugs, EBR typically increases following treatment (Agostino et al., 2008; Bologna et al., 2012; Karson, Burns, et al., 1984; Kimber & Thompson, 2000; Korsgaard, Noring, & Gerlach, 1984). Such treatment may also explain the existence of subgroups of patients with higher EBR than healthy controls (Karson, LeWitt, et al., 1982; Kimber & Thompson, 2000; Korošec et al., 2006). For instance, L-dopa is the most common drug for treating PD and its pulsatile, in contrast to continuous, stimulation of DA receptors can lead to dyskinesias (Thanvi, Lo, & Robinson, 2007), which may result in increased EBR. In patients with tardive dyskinesia the D2 antagonist sulpiride did not reduce blink rates despite slight increases in Parkinsonism in some patients (Casey, Gerlach, & Simmelsgaard, 1979), suggesting this side-

effect is not easily reversed. Despite these patients exhibiting dyskenisia, reduced EBR is considered characteristic of PD, leading to its common inclusion in both the diagnosis of the disorder, as well as assessment of patients' responses to drug treatment.

The second prominent disorder related to altered EBR is schizophrenia, which is linked to excessive DA activity in the striatum (Howes, McCutcheon, & Stone, 2015). Consistent with a hyperdopaminergic state, schizophrenia patients typically exhibit increased EBR (Adamson, 1995; Chen, Lam, Chen, & Nguyen, 1996; Helms & Godwin, 1985; Karson, Berman, Kleinman, & Karoum, 1984; Karson, Freed, Kleinman, Bigelow, & Wyatt, 1981; Karson et al., 1983; Kleinman et al., 1984; Mackert, Woyth, Flechtner, & Frick, 1988; Ostow & Ostow, 1945; Stevens, 1978b, 1978a; Swarztrauber & Fujikawa, 1998), although one study found increased EBR only after 3 years since the first episode (Chan et al., 2010), another found the increase was no longer significant once smoking behavior was controlled for (Klein, Andresen, & Thom, 1993), and one study found EBR was actually reduced in, perhaps due to antipsychotic treatment (Mackintosh, Kumar, & Kitamura, 1983). As in PD, EBR in schizophrenia is proposed to correlate with symptomology. Specifically, EBR has correlated positively with psychotic behavior (Owens, Harrison-Read, & Johnstone, 1994), negative symptoms (Chen et al., 1996), general psychopathology and disinhibition (Chan & Chen, 2004), as well as perseverative errors in the Wisconsin card sorting test (Chan et al., 2010), and risk of relapse (Chan et al., 2010; Hui et al., 2013). Again, EBR varied with drug treatment: DA antagonists reduce blink rate (Adamson, 1995; Karson, Freed, et al., 1981; Kleinman et al., 1984; Mackert et al., 1988), and this change can correlate with improvement in symptoms (Bartkó, Herczeg, & Zádor, 1990; Karson, Bigelow, Kleinman, Weinberger, & Wyatt, 1982) but baseline EBR itself did not predict response to treatment (Bartkó, Frecska, Horváth, Zádor, & Arató, 1990). In other drug studies, Lieberman et al. (1987) found methylphenidate (Ritalin) increased EBR in patients with schizophrenia. They also found larger increases predicted earlier relapse, suggesting a potential role for enhanced receptor sensitivity. Indeed, Strakowski et al. (1997) found

amphetamine increased EBR in patients with schizophrenia but, in contrast to studies in healthy humans, there were no sensitization effects for repeated doses, which was interpreted as the patients' receptors already being maximally sensitized. Lastly, one study found no change in the blink rate but reduced anxiety when the non-selective DA agonist apomorphine was administered (Ferrier, Johnstone, & Crow, 1984). To conclude, it is interesting schizophrenia is associated with an increase in D2 receptors (for a review, see Seeman, 2013) and, as previously discussed, D2 receptor availability correlated positively with EBR in monkeys at rest (Groman et al., 2014). Taken together, these findings suggest increased EBR in schizophrenic patients is D2-mediated.

EBR might also be altered in individuals not diagnosed with schizophrenia but who do exhibit psychotic behavior. Indeed, EBR was reported to be increased in an adult (Lovestone, 1992) and adolescents (Karson, Goldberg, & Leleszi, 1986) suffering from psychosis. In contrast, individuals suffering from psychotic depression did not differ from controls (Helms & Godwin, 1985), although one study that grouped a large variety of psychiatric disorders, amongst others psychotic depression, bipolar affective disorder, and atypical psychosis, did find increased EBR in this group relative to healthy controls (Swarztrauber & Fujikawa, 1998).

With respect to affective disorders, there is mixed evidence for elevated EBR. Although depression might be linked to reduced DA activity resulting in the characteristic inability to experience pleasure, compensatory mechanisms have been proposed such as upregulation of postsynaptic DA receptors and decreased DA transporter density that may account for increased DA transmission and/or sensitivity (Dunlop & Nemeroff, 2007). Consistent with these compensatory mechanisms, some have reported increased EBR in major depression (Giedke & Heimann, 1987; Mackintosh et al., 1983), but others found no difference as compared to controls (Ebert et al., 1996). EBR was also not associated with depressive symptomology in undergraduate students (Byrne, Norris, & Worthy, 2016), although this is perhaps because not all students demonstrated clinical levels of depression and symptoms were rated

only for the past seven days instead of a longer period of time. One study did find sleep deprivation increased EBR in depressed individuals, accompanied by an improvement in depressive state proportional to the increase in EBR (Ebert et al., 1996). With respect to a different affective disorder, there is evidence for elevated EBR in seasonal affective disorder (SAD), with one study reporting increased blink rate (Depue et al., 1990) and another reporting an increase that was reversed by light therapy (Depue, Iacono, Muir, & Arbisi, 1988). In contrast, Barbato et al. (1993) found no difference between SAD individuals and controls, although light therapy did reduce EBR in premenopausal women with SAD. Overall, these studies seem to point to increased blink rate in affective disorders, but inconsistent results prevent a conclusive answer. Perhaps more consistency might be obtained by associating EBR with specific depressive symptoms related to rather than a diagnosis that is likely to encompass a highly heterogeneous population.

There is also mixed evidence for increased EBR in individuals at risk for or having already developed Huntington's disease. Specifically, EBR was suggested to be increased in family members of patients (Valade, Davous, & Rondot, 1984) and in a child two years prior to developing Huntington's (Xing et al., 2008). However, Karson et al. (1984b) found only a nonsignificant increase. Notably, the latter study counted EBR during conversation, which is shown to be increased relative to rest (for a review, see Doughty, 2001), and this may have partially confounded the results. We come back to this point in the discussion. Lastly, for this disorder it is perhaps particularly important to distinguish between baseline blink rates in contrast to blinks made during ocular tasks such as smooth pursuit and saccade tasks, which have been shown to be abnormally high as a consequence of a form of ocular apraxia (Lasker & Zee, 1997) that might not necessarily reflect elevated DA levels. Future studies examining EBR of Huntington's patients in resting conditions might shed more light on the nature of blink rate abnormalities in this disorder and the relation to DA dysfunction.

Aside from the disorders discussed so far, EBR has also been examined to a lesser extent in other conditions. As the studies in each respective disorder

are not numerous, we summarize them here only briefly. First, although attention-deficit hyperactivity disorder (ADHD) is thought to be linked with reduced DA activity (del Campo, Chamberlain, Sahakian, & Robbins, 2011), evidence for lower EBR is inconsistent. Whereas Konrad et al. (2003) found decreased EBR in children with ADHD, others found no difference in ADHD, ADD, with/without conduct disorder (Daugherty, Quay, & Ramos, 1993; Groen, Börger, Koerts, Thome, & Tucha, 2015; Tantillo, Kesick, Hynd, & Dishman, 2002). One of these studies also found no difference in children with anxiety withdrawal disorder (Daugherty et al., 1993). Second, EBR was increased in women with restricting type anorexia nervosa and their blink rate correlated positively with the duration of illness (Barbato, Fichele, Senatore, Casiello, & Muscettola, 2006), although it should be noted this difference is potentially driven by the healthy control group in this study having a rather low EBR (11 p/min). Third, EBR is typically increased in Tourette's syndrome, (Schelkunov, Kenunen, Pushkov, & Charitonov, 1986; Tharp et al., 2015; Tulen et al., 1999) and although one study (Karson, Kaufmann, Shapiro, & Shapiro, 1985) found no difference, they and others (Tulen et al., 1999) did find EBR correlated with the frequency of tics. Further, whereas the non-selective DA antagonist pimozide did not affect blink rate in this patients (Karson et al., 1985), the alpha-adrenergic agonist clonidine did reduce it (Cohen, Detlor, Young, & Shaywitz, 1980). Fourth, there is mixed evidence for altered EBR in generalized dystonia, with one study finding an increase (Deuschl & Goddemeier, 1998) and another finding no difference with controls (Karson, Burns, et al., 1984). Fifth, blink rate is reduced in individuals exhibiting stereotypic behavior (Lee et al., 2010; MacLean Jr. et al., 1985; Roebel and MacLean Jr., 2007) and the severity of repetitive behavior has correlated negatively with blink rate (Bodfish, Powell, Golden, & Lewis, 1995). Sixth, mild cognitive impairment was associated with increased EBR and these rates correlated negatively with Montreal cognitive assessment test scores (Ladas, Frantzidis, Bamidis, & Vivas, 2014). Seventh, EBR was increased in boys with fragile X syndrome and smaller changes in EBR from resting conditions to active cognitive tasks was associated with more problem

behavior (Roberts, Symons, Johnson, Hatton, & Boccia, 2005). Finally, EBR was increased in children with autism (Goldberg, Maltz, Bow, Karson, & Leleszi, 1987), in individuals with panic disorder (Kojima et al., 2002), progressive supranuclear palsy (Bologna et al., 2009, 2016; Golbe et al., 1989; Karson, Burns, et al., 1984; Reddy et al., 2013), Prader-Willi syndrome as compared to those with intellectual disability (Holsen & Thompson, 2004), and in a patient with Wilson disease (Verma, Lalla, & Patil, 2012). On the other hand, EBR was reduced in iron-deficient anemic infants (Lozoff et al., 2010), in children with traumatic brain injury (Konrad et al., 2003) or epilepsy (Caplan, Guthrie, Komo, & Shields, 1998; Schelkunov et al., 1986), and in patients with amyotrophic lateral sclerosis (ALS; Byrne et al., 2013), whereas there was no altered EBR in patients with Graves' orbitopathy (Garcia, Pinto, Barbosa, & Cruz, 2011), or cerebrovascular lesions (Anagnostou, Kouzi, Vassilopoulou, Paraskevas, & Spengos, 2012).

Lastly, altered baseline EBR is associated not only with the aforementioned disorders, but may stem from recreational drug use as well. Whereas alcohol abuse in adolescents was not associated with EBR (Upadhyaya et al., 2003), recreational use of cocaine in otherwise healthy adults was associated with reduced EBR as compared to matched cocaine-free controls, with the highest reported dosage ever taken correlating negatively with EBR (Colzato, van den Wildenberg, & Hommel, 2008). Similarly, Kowal et al. (2011) found reduced EBR in cannabis users that was correlated negatively with years of exposure, monthly peak consumption, and lifetime consumption.

In sum, EBR can reflect altered DA activity in various disorders as well as response to certain treatments, although the findings vary in consistency among disorders. Additionally, studies in drug users suggest chronic use of recreational DA drugs can result in hypodopaminergic activity that is reflected in reduced EBR.

Table 4. Overview of studies on EBR in relation to healthy inter-individual characteristics.

Factor	EBR	Mean EBR	*N*	Recording method	Condition	Study
Age						
NA	=	NA	NA	EOG	Primary gaze	Kruis et al. 2016
0.53 ± 0.29 years average	↑	3.6	64	Video	NA	Lawrenson et al. 2005
1 vs. 0.33 years	↑	NA vs. NA	87 vs. 98	Video	Auditory and visual stimulation	Bacher 2014
1 to 4 vs. 0 to 0.16 years	↑	3.4 vs. 0.7	54 vs. 14	Observation	Conversation	Zametkin et al. 1979
4.5 ± 0.31 years average	=	7.1	54	EOG	Watching video	Lackner et al. 2010
5 to 10 vs. 1 to 4 years	↑	6.1 vs. 3.4	96 vs. 54	Observation	Conversation	Zametkin et al. 1979
5.1 years vs. ≤ 30 days	↑	8.0 vs. 62	200 vs. 50	Video	Primary gaze	Lavezzo et al. 2008
11 to 15 vs. 5 to 10 years	↑	10.3 vs. 6.1	78 vs. 96	Observation	Conversation	Zametkin et al. 1979
15 to 20 vs. 11 to 15 years	=	11.3 vs. 10.3	23 vs. 78	Observation	Conversation	Zametkin et al. 1979
20 to 25 vs. 15 to 20	=	17.8 vs. 11.3	33 vs. 23	Observation	Sitting in waiting room or church	Zametkin et al. 1979
21.6 ± 1.47 years average	=	18.5	61	EOG	Primary gaze	Zhang et al. 2015
25 to 30 vs. 20 to 25	=	14.1 vs. 17.8	27 vs. 33	Observation	Sitting in waiting room or church	Zametkin et al. 1979
30 to 35 vs. 25 to 30	=	15.4 vs. 14.1	23 vs. 27	Observation	Sitting in waiting room or church	Zametkin et al. 1979
33.4 ± 7.0 years average	=	13.8	100	Video	Primary gaze	Doughty et al. 2006
35 to 40 vs. 30 to 35	=	16.3 vs. 15.4	20 vs. 23	Observation	Sitting in waiting room or church	Zametkin et al. 1979
35.9 ± 17.9 years average	=	17	150	Video	Rest	Bentivoglio et al. 1997
40 to 45 vs. 35 to 40	=	15.0 vs. 16.3	30 vs. 20	Observation	Sitting in waiting room or church	Zametkin et al. 1979
45 to 50 vs. 40 to 45	=	17.5 vs. 15.0	19 vs. 30	Observation	Sitting in waiting room or church	Zametkin et al. 1979
50 to 60 vs. 45 to 50	=	16.2 vs. 17.5	24 vs. 19	Observation	Sitting in waiting room or church	Zametkin et al. 1979
50.2 ± 17.0 years average	↓	10.6	338	Observation	Rest	Chen et al. 2003
57.1 years average	=	11.3	156	Observation	Primary gaze	Deuschl and Goddemeier 1998
59.3 vs. 23.9 years	=	16.9 vs. 12.9	19 vs. 25	SMART	Primary gaze	Sforza et al. 2008
60+ vs. 50 to 60	=	16.3 vs. 16.2	7 vs. 24	Observation	Sitting in waiting room or church	Zametkin et al. 1979
80 to 89 vs. 40 to 49 years	=	31.3 vs. 23.5	8 vs. 8	Video	Conversation	Sun et al. 1997
Birth control pill (yes vs. no)						
Women (yes) vs. women (no)	↑	19.6 vs. 14.9	44 vs. 42	Observation	Primary gaze	Yolton et al. 1994
Women (yes) vs. men	↑	19.6 vs. 14.5	44 vs. 59	Observation	Primary gaze	Yolton et al. 1994
Women (no) vs. men	=	14.9 vs. 14.5	42 vs. 59	Observation	Primary gaze	Yolton et al. 1994
Depressive symptomatology	=	17.8	104	EOG	Primary gaze	Byrne et al. 2016
Gender (women vs. men)	=	NA vs. NA	NA vs. NA	EOG	Primary gaze	Kruis et al. 2016
	=	NA vs. NA	NA vs. NA	Observation	Primary gaze	Deuschl and Goddemeier 1998
	=	NA vs. NA	NA vs. NA	Video	Watching video	Berenbaum and Williams 1994

	=	NA vs. NA	11 vs. 32	Video	Interview	Declerk et al. 2006
	=	NA vs. NA	20 vs. 7	EOG	Primary gaze	Colzato et al. 2009b
	=	NA vs. NA	20 vs. 21	EOG	Primary gaze	Di Gruttola et al. 2014
	=	NA vs. NA	30 vs. 31	EOG	Primary gaze	Zhang et al. 2015
	=	NA vs. NA	35 vs. 19	EOG	Watching video	Lackner et al. 2010
	↑	NA vs. NA	40 vs. 24	EOG	Rest	Dreisbach et al. 2005
	↑	NA vs. NA	74 vs. 18	EOG	Rest	Müller et al. 2007a
	=	1.2 vs. 1.3	26 vs. 26	Video	Listening music	Bacher and Allen 2009
	↑	5.4 vs. 3.9	39 vs. 35	Video	Auditory and visual stimulation	Bacher 2014
	↑	6.2 vs. 3.0	80 vs. 70	Video	Reading	Bentivoglio et al. 1997
	↓	6.3 vs. 14.7	10 vs. 10	Observation	Facing mirror	Maclean, Jr. et al. 1985
	=	9.7 vs. 10.8	31 vs. 30	Video	Primary gaze	Doughty 2002
	↓	9.8 vs. 11.4	173 vs. 165	Observation	Rest	Chen et al. 2003
	=	18 vs. 15.6	80 vs. 70	Video	Rest	Bentivoglio et al. 1997
	↑	19 vs. 11	21 vs. 23	SMART	Primary gaze	Sforza et al. 2008
	↑	19.5 vs. 15.9	54 vs. 50	EOG	Primary gaze	Byrne et al. 2016
	=	19.8 vs. 15.7	40 vs. 23	EOG	Primary gaze	Barbato et al. 2012
	↑	22.0 vs. 8.6	14 vs. 16	Video	Primary gaze	Pult et al. 2013
	=	26.7 vs. 24	80 vs. 70	Video	Conversation	Bentivoglio et al. 1997
Hypnotizability	↓	NA	36	NA	Watching light box	Lindsay et al. 1993
	↑	NA	41	EOG	Primary gaze	Di Gruttola et al. 2014
High vs. medium hypnotizability	↓	15.3 vs. 19.2	13 vs. 22	Video	Rest, conversation, listening music	Lichtenberg et al. 2008
Internal locus of control	↑	19.1	43	Video	Interview	Declerk et al. 2006
Meditation						
Long-term meditators vs. meditation-naïve controls	↓	NA vs. NA	27 vs. 118	EOG	Primary gaze	Kruis et al. 2016
Mindfulness-based stress reduction meditation pre vs. post	=	NA vs. NA	36	EOG	Primary gaze	Kruis et al. 2016
Mood						
Negative mood induction (post vs. pre)	=	16.8 vs. 17.4	38	EOG	Primary gaze	Akbari Chermahini and Hommel 2012
Positive affect	∩	NA	54	Video	Auditory and visual stimulation	Bacher 2014
Positive mood induction (post vs. pre)	↑	18.8 vs. 14.1	43	EOG	Primary gaze	Akbari Chermahini and Hommel 2012
Personality						
Extraversion	=	NA	51	Video	Primary gaze	Tharp and Pickering 2011
	=	NA	40 (men)	Video	Watching video	Berenbaum and Williams 1994
	↑	NA	34 (women)	Video	Watching video	Berenbaum and Williams 1994

	=	15.1	28	EOG	Primary gaze	Colzato et al. 2009a
	=	NA	39	NA	Flash detection	Franks 1963
	=	18.3	63	EOG	Primary gaze	Barbato et al. 2012
Neuroticism	=	NA	51	Video	Primary gaze	Tharp and Pickering 2011
	=	15.1	28	EOG	Primary gaze	Colzato et al. 2009a
	↑	18.3	63	EOG	Primary gaze	Barbato et al. 2012
Psychoticism	=	NA	51	Video	Primary gaze	Tharp and Pickering 2011
	↑	15.1	28	EOG	Primary gaze	Colzato et al. 2009a
	=	18.3	63	EOG	Primary gaze	Barbato et al. 2012
Lie	=	15.1	28	EOG	Primary gaze	Colzato et al. 2009a
Schizotypal thinking						
Negative schizotypy	=	9.9	21 (placebo)	EOG	Rest	Mohr et al. 2005
	↑	11.0	18 (L-dopa)	EOG	Rest	Mohr et al. 2005
Positive schizotypy	=	10.4	39	EOG	Rest	Mohr et al. 2005

↓, decreased at $p < .05$; ↑, increased at $p < .05$; ∩, inverted-u-curve at $p < .05$; =, no difference; EBR, eye blink rate; EOG, electrooculography; NA, not available;

Healthy populations

In healthy humans, the EBR has been suggested to vary according to several factors. First of all, EBR and age seem to follow a non-linear relation where EBR initially increases from infancy to adulthood (Bacher, 2014; Lavezzo, Schellini, Padovani, & Hirai, 2008; Lawrenson, Birhah, & Murphy, 2005; Zametkin, Stevens, & Pittman, 1979; Zhang et al., 2015), which is proposed to reflect maturation of the dopaminergic pathways (Lawrenson et al., 2005; Zametkin et al., 1979). From adulthood onwards, findings are less clear. EBR has been reported stable (Bentivoglio et al., 1997; Deuschl & Goddemeier, 1998; Doughty, 2006; Kruis, Slagter, Bachhuber, Davidson, & Lutz, 2016; Sforza, Rango, Galante, Bresolin, & Ferrario, 2008; Sun et al., 1997; Zametkin et al., 1979), while others found a decline from 40 onwards and in particular in women (Chen et al., 2003). Although an age-related decline in EBR would be consistent with the idea dopaminergic systems degrade with aging (Bäckman, Nyberg, Lindenberger, Li, & Farde, 2006), the evidence for such a decline remains inconsistent.

Second, there are equivocal findings on gender differences in blink rate. While several studies report no or marginal effects of gender (Bacher & Allen, 2009; Barbato, della Monica, Costanzo, & de Padova, 2012; Berenbaum & Williams, 1994; Colzato, van den Wildenberg, van Wouwe, Pannebakker, & Hommel, 2009; Declerck, de Brabander, & Boone, 2006; Deuschl & Goddemeier, 1998; Di Gruttola, Orsini, Carboncini, Rossi, & Santarcangelo, 2014; Doughty, 2002; Kruis et al., 2016; Lackner, Bowman, & Sabbagh, 2010; Yolton et al., 1994; Zametkin et al., 1979; Zhang et al., 2015), others find women blink more often than men (Bacher, 2014; Byrne et al., 2016; Dreisbach et al., 2005; Lozoff et al., 2010; Müller, Dreisbach, Brocke, et al., 2007; Pult, Riede-Pult, & Murphy, 2013; Sforza et al., 2008), although one study found this was only significant while reading and not at rest (Bentivoglio et al., 1997). Yet other studies report EBR to be lower in females (Chen et al., 2003; MacLean Jr. et al., 1985). Several accounts have been put forward to explain potential gender differences. For example, in infancy a higher EBR in females was proposed to reflect their faster maturation of dopaminergic

systems (Bacher, 2014). In adulthood, differences might arise from fluctuations in DA associated with the menstrual cycle, possibly due to estrogen. In line with this idea, D2 receptor availability varies according to the menstrual cycle (Czoty et al., 2009), cognitive functions associated with DA may depend on estrogen level (Colzato & Hommel, 2014; Jacobs & Esposito, 2011), oral contraceptives were found to increase EBR (Yolton et al., 1994), and a marked drop in EBR in older Chinese women was suggested to coincide with an age-related decrease in estrogen (Chen et al., 2003). Given the possible influence of different phases in the menstrual cycle on DA, future studies investigating gender effects on EBR should distinguish between women who do and do not take hormonal contraceptives and, in case of the latter, distinguish between participants in different phases of the menstrual cycle.

Third, EBR might correlate with certain dimensions of personality, although again there are inconsistent results that may partly be attributed to the different questionnaires used to measure personality. Extraversion measured using the Eysenck personality inventory (EPI) correlated positively in women but not men (Berenbaum & Williams, 1994), but did not correlate with either gender using the same questionnaire (Barbato et al., 2012), a short (Colzato, Slagter, van den Wildenberg, & Hommel, 2009) or longer version (Tharp & Pickering, 2011) of the Eysenck personality questionnaire revised short scale (EPQ-RSS), or the Maudsley personality inventory (Franks, 1963). Neuroticism correlated positively using the EPI (Barbato et al., 2012), but not the EPQ-RSS (Colzato, Slagter, et al., 2009; Tharp & Pickering, 2011). Psychoticism was found to positively correlate using the short (Colzato, Slagter, et al., 2009) but not longer (Tharp & Pickering, 2011) version of the EPQ-RSS or EPI (Barbato et al., 2012). The social conformity dimension 'lie' was not unrelated as measured using the EPQ-RSS (Colzato, Slagter, et al., 2009). Lastly, an internal locus of control correlated positively as measured using the Rotter internal-external control scale (Declerck et al., 2006). Overall, findings on EBR and personality have been inconsistent and future research should aim to replicate these findings across multiple independent studies and use different questionnaires in the same study for systematic comparison.

Fourth, there is inconclusive evidence for a correlation between EBR and schizotypal thinking (Mohr et al., 2005). EBR correlated positively with negative schizotypal thinking after administration of L-dopa (which did not significantly increase EBR), but not after a placebo. On the other hand, there was no relation with positive schizotypal thinking after either L-dopa or placebo intake.

Fifth, EBR has been proposed predict hypnotizability, which is thought to relate to DA (Lichtenberg et al., 2008). Whereas two studies found a negative correlation between EBR and hypnotizability (Lichtenberg et al., 2008; Lindsay, Kurtz, & Stern, 1993), one found a positive relation that disappeared once controlling for mind wandering (Di Gruttola et al., 2014). The authors of the latter study suggested differences in mind wandering might accounted for the inconsistency with previous studies. As such, future studies should aim to consider individual differences in mind wandering to provide a clear picture of the relation between EBR and hypnotizability.

Lastly, EBR was found to relate to the lifestyle practice meditation, consistent with the finding meditation affects DA-related cognitive functions (Kruis et al., 2016). While long-term meditators had lower EBR than meditation-naïve participants, there was no effect of an eight week course of mindfulness-based stress reduction nor of a full day of meditation practices on EBR. As such, it has been suggested pre-existing differences in DA might predispose an individual to practicing meditation, or meditation must be practiced on the long term for it to affect EBR.

Eye blink rate and cognitive performance in healthy humans

Consistent with the idea spontaneous EBR reflects striatal DA activity, many studies find EBR predicts DA-related cognitive performance. In the following section we review these studies to illustrate the applicability and usefulness of EBR in cognitive research. Most of the available research can be grouped in two broad categories, which are (i) reinforcement learning and motivation, that is learning from positive or negative outcomes of actions and the effort punt in and vigor of actions, and (ii) cognitive flexibility, i.e. updating of

representations in frontal cortex in contrast to their stable maintenance. After these two categories we summarize a number of other studies that do not fit these categories. In Table 5 an overview of the following studies is provided.

Table 5. Overview of studies on EBR in cognitive research.

Paradigm/Task	EBR	Mean EBR	N	Recording method	Condition	Study
Attentional blink in rapid serial visual presentation task	= with size of attentional blink	15.2	39	EOG	Primary gaze	Slagter and Georgopoulou 2013
	↓ with size of attentional blink	16.8	20	EOG	Primary gaze	Colzato et al. 2008a
	Binaural beats eliminated attentional blink in low but not high blinkers	17.4 (median)	24	EOG	Primary gaze	Reedijk et al. 2015
Cognitive flexibility						
Distractibility and perseveration in task-switching task	↑ with bias towards novel information	NA	50	Video	Primary gaze	Tharp and Pickering 2011
	↑ with bias towards novel information	NA	64	EOG	Rest	Dreisbach et al. 2005
	↑ with bias towards novel information	10.0	87	EOG	Rest	Müller et al. 2007a
	= with bias towards novel information	14.6	70	EOG	Primary gaze	Müller et al. 2007b
Divergent thinking in alternative uses task	∩ with flexibility scores	NA	117	EOG	Primary gaze	Akbari Chermahini and Hommel (2010)
	∩ with flexibility scores	15.8	81	EOG	Primary gaze	Akbari Chermahini and Hommel (2012)
	Binaural beats enhanced flexibility scores in low but not high blinkers	NA	24	EOG	Primary gaze	Reedijk et al. 2013
Task-switching in dots-triangles task	↑ with accuracy scores	18.5	61	EOG	Primary gaze	Zhang et al. 2015
	↓ with switch costs	18.5	61	EOG	Primary gaze	Zhang et al. 2015
Task-switching in local-global task	= with accuracy scores and switch costs	18.5	61	EOG	Primary gaze	Zhang et al. 2015
Convergent thinking in remote associations task	↓ with accuracy scores	NA	117	EOG	Primary gaze	Akbari Chermahini and Hommel (2010)
Ego-depletion induced by Stroop task	∩ with antisaccade performance after incongruent-only but not congruent-only Stroop task	NA	84	EOG	Primary gaze	Dang et al. 2016
Inhibitory control						
Impulsivity in distribution of attention	↑ with more impulsive distribution of attention, i.e. more fixation and longer dwell times on erotic pictures	12.7	50	Eyetracking	Primary gaze	den Daas et al. 2013
Response inhibition in go/no-go task	↑ with accuracy scores	18.5	61	EOG	Primary gaze	Zhang et al. 2015
Response inhibition in stop-signal task	↓ with latency of inhibitory control	14.0	27	EOG	Primary gaze	Colzato et al. 2009b
Response inhibition in Stroop task	= with accuracy scores in incongruent trials	18.5	61	EOG	Primary gaze	Zhang et al. 2015
	↓ with latency in incongruent trials	18.5	61	EOG	Primary gaze	Zhang et al. 2015
Motivation and effort in finger-tapping task	↑ with exerted effort (button presses) in response to suboptimal reward cues	18.9	36	Eyetracking	Primary gaze	Pas et al. 2014
Pseudoneglect in greyscales task	↑ with rightward bias in spatial attention	13.6	23	EOG	Primary gaze	Slagter et al. 2010
Reinforcement learning						

Loss aversion in Iowa gambling task	↑ with gains through enhanced loss aversion (for individuals with higher depressive symptomatology)	17.8	104	EOG	Primary gaze	Byrne et al. 2016
Punishment avoidance (conflict-induced) in Simon task	↑ with drug-induced bias towards conflict-induced punishment avoidance	NA	27	EOG	Rest	Cavanagh et al. 2014
Reinforcement learning in probabilistic reinforcement learning task	↓ with learning from negative outcomes	14.3	38	EOG	Primary gaze	Slagter et al. 2015
	= with learning from positive outcomes	14.3	38	EOG	Primary gaze	Slagter et al. 2015
Sense of agency in intentional binding paradigm	↑ with strength of intentional binding, (but only) when presented with positive pictures	15.8	28	Eyetracking	Primary gaze	Aarts et al. 2012
Theory of mind in false-belief task	↑ with theory of mind performance	7.2	54	EOG	Watching video	Lackner et al. 2010
Visuomotor binding in feature-repetition task	↑ with strength of visuomotor binding of task-relevant but not task-irrelevant features	8.7	18	EOG	Rest	Colzato et al. 2007a
Working memory						
Updating in mental counters task	= with accuracy scores	18.5	61	EOG	Primary gaze	Zhang et al. 2015
Updating in 3-back task	↓ with accuracy scores	18.5	61	EOG	Primary gaze	Zhang et al. 2015
Working memory span	= with working memory span	NA	50	Video	Primary gaze	Tharp and Pickering 2011

↓, decreased at $p < .05$; ↑, increased at $p < .05$; ∩, inverted-u-curve at $p < .05$; =, no difference; EBR, eye blink rate; EOG, electrooculography; NA, not available

Eye blink rate and reward-driven behavior

As indicated by the studies reviewed below, EBR can predict the effect of reinforcement learning on reward-driven task performance. This is consistent with the idea outlined in the introduction that EBR reflects activation of the D2 receptor system, which regulates the balance between positive reinforcement of behavior through Go learning and negative reinforcement through NoGo learning. Whereas the prediction errors driving such reinforcement learning depend on phasic (burst) DA release, background (tonic) DA level in the striatum is thought to be associated not with reward-driven learning per se but instead motivational aspects that determine the effort expended in and vigor of responding, as demonstrated in mice (Beeler, Daw, Frazier, & Zhuang, 2010; Niv, Daw, Joel, & Dayan, 2006) and humans (Treadway et al., 2012).

Evidence for an association between EBR and reinforcement learning comes from two studies that reveal EBR predicts learning from negative outcomes in particular. First, Slagter et al. (2015), using a probabilistic reinforcement learning task, found individuals with lower EBR tended to avoid choosing stimuli that were often unrewarded, but individuals with a higher EBR did not tend to choose regularly-rewarded stimuli more often. Consistent with these findings, Cavanagh et al. (2014) showed that pharmacologically reducing DA tone, as indicated by lower EBR, led to an increased aversion of punishment following response conflict. The authors administered low-dose cabergoline, which preferentially binds to D2 autoreceptors and thus reduces striatal DA release. Participants then performed a Simon task in which stimuli could lead to reward or punishment. Notably, for half of the stimuli the probability of reward and punishment was contingent on the congruency and thus associated conflict of the stimulus, although these stimuli were equally often rewarded. After completing the task, it was found lowering EBR increased the tendency to evaluate a stimulus whose incongruent trials always led to reward as being more rewarding than a stimulus whose incongruent trials never led to reward. Given that these stimuli was equally often rewarded, this finding suggests reduced striatal DA tone led to increasing the impact of punishment over reward.

The results of Slagter et al. (2015) and Cavanagh et al. (2014), showing a distinct relation of EBR with learning from negative versus positive outcomes, concur with the model for basal ganglia-mediated reinforcement learning as described in the introduction (Frank & O'Reilly, 2006; Maia & Frank, 2011). In this model a D1-rich direct pathway mediates Go learning driven by positive prediction errors (i.e. outcomes better than expected; reward) and a D2-rich indirect pathway mediates NoGo learning driven by negative prediction errors (i.e. outcomes worse than expected; punishment). Under the assumption EBR reflects D2 receptor function more than that of D1 (Groman et al., 2014) and given the D2-driven pathway mediates learning from negative outcomes (Maia & Frank, 2011), it is unsurprising lower EBR should predict learning from negative rather than positive outcomes. Although one might expect higher EBR to nevertheless promote learning from positive outcomes via stimulation of the D1/Go pathway and strengthened inhibition of the D2/NoGo pathway, this might not be the case because D2 receptors are more sensitive to DA than D1 (Frank & O'Reilly, 2006) and they have stronger inhibitory effects on the D1-driven pathway than vice versa (Bahuguna et al., 2015). As such, it might be DA levels in the healthy upper range are not high enough for sufficient stimulation of the D1 pathway to overcome its D2-driven inhibition, which would be consistent with the fact drug induced D1-activity affects EBR but D1 receptor availability is not related to resting EBR (Groman et al., 2014).

In apparent contrast to the findings of Slagter et al. and Cavanagh et al. is a study by Byrne et al. (2016), which investigated EBR and self-reported depressive symptomatology in undergraduate students in relation to performance on the Iowa gambling task (IGT). They found high but not low EBR, albeit combined with elevated depressive symptoms, was associated with increased loss-aversive behavior. Although a higher EBR was only marginally related to better IGT performance, i.e. making choices that lead to net gains, EBR and depressive symptomatology interacted such that individuals with more symptoms and high EBR performed better on the task. Modelling the data revealed having more depressive symptoms was associated with loss-

aversive behavior and individuals with high EBR persevered in choices that lead to net gains, speculated by the authors to be due to enhanced learning which options led to net losses and then avoiding those options.

These results contrast with those of Slagter et al. and Cavanagh et al. showing low instead of high EBR predicts aversion-avoidant behavior, as is expected from the basal ganglia Go/NoGo model. In light of these contradictory results it is important to consider two points on which these studies differed. First, Byrne et al. suggested differences in the format of the given reward may render these studies not comparable. Whereas Slagter et al. used the word 'Correct!' as a positive outcome and 'Incorrect' as a negative outcome, Cavanagh et al. used earning points as a reward and the absence of this reward as punishment, and Byrne et al. rewarded and punished participants by adding or subtracting points, respectively. Although these outcomes may be considered different based on the distinction in operant conditioning between 'positive' punishment by applying a stimulus vs. 'negative' punishment by removing a stimulus (Lieberman, 2000), both should lead to negative prediction errors (i.e. worse outcomes than expected) whose DA dips and pauses stimulate NoGo learning. Future studies might want to directly compare the relation between EBR and different forms of punishment in an attempt to resolve these inconsistent results. A second important difference is the results of Byrne et al. applied only to individuals who reported high depressive symptomatology, whereas no such distinction was made amongst the participants of Slagter et al and Cavanagh et al. Individuals with high depressive symptomatology might not be comparable to participants who did not report depressive symptoms, as processing of reward and punishment seems to be altered in depression (Ubl et al., 2015). Although highly speculative, perhaps Byrne et al.'s finding of increased loss-aversive behavior can be attributed not to DA but to a serotonin-mediated increase in learning of aversive outcomes that is associated with depression (Cools, Roberts, et al., 2008). On the other hand, the combination of high EBR and depressive symptoms, the latter possibly related to a compensatory increase in DA transmission (Dunlop & Nemeroff, 2007), may have led to sufficient D1

stimulation to account for the perseverative choosing of options leading to net gains. This explanation remains highly speculative and would require further investigation.

EBR has also predicted the amount of effort people are willing to spend on rewarded behavior. This was demonstrated by Pas et al. (2014), who showed EBR correlates positively with the amount of effort individuals exert in response to suboptimal reward cues. They used a finger-tapping task in which participants needed to carry out a high number of button presses in a short amount of time to earn money. The amount (low vs. high) was indicated at the beginning of a trial by a cue that could be considered optimal when presented supraliminal or suboptimal when presented subliminal through the use of masking. Consistent with the idea DA motivates reward-driven behavior, individuals with a higher EBR experienced a stronger reward effect for suboptimal cues. That is, the difference in exerted effort (button presses) between the two reward conditions (low and high) was larger for individuals with high EBR and this effect was only present for suboptimal cues. As such, this study indicates individuals with presumably higher striatal DA level exert more effort in reward-driven behavior under suboptimal conditions.

Further support for a role of DA in motivated behavior comes from a study by Aarts et al. (2012). They argued increasing DA activity could promote more motivated behavior and consequently increase the sense of agency over effects produced by this behavior. They measured sense of agency in the intentional binding paradigm, wherein increased sense of agency is indicated by stronger intentional binding. At the beginning of each trial participants saw either a neutral or positively valenced picture, the latter being considered rewarding and hence expected to induce phasic DA bursts. Results showed EBR was associated with stronger intentional binding, but only when positive but not neutral pictures were presented. Furthermore, positive pictures enhanced intentional binding in high but not low EBR individuals. These results suggest EBR is associated with motivated behavior but they also indicate it might be more accurate to say EBR modulates the effect of phasic DA bursts on motivated behavior.

Eye blink rate and cognitive flexibility

As discussed in the introduction, cognitive control benefits from a delicate balance between maintaining task-relevant representations in the face of interference and flexibly updating these representations when situational demands change (Cools & D'Esposito, 2011). DA gates the signal that elicits updating in frontal cortex by modulating the decision threshold in the basal ganglia such that a higher DA level facilitates updating by reducing the threshold (Frank & O'Reilly, 2006; Maia & Frank, 2011). In line with this model, the studies discussed below demonstrate EBR as indicator of DA level predicts task performance dependent on gating of representations.

A study by Zhang et al. (2015) demonstrated EBR can predict the efficiency of updating task-goal representations by showing high EBR was associated with increased accuracy and reduced switching costs. Although this result was obtained in a task in which participants needed to switch attending to dots and triangles, this was not replicated in a global-local task in which participants switch attending from larger, global stimuli to its comprising smaller, local stimuli. Curiously, scores on the two tasks did not correlate despite both presumably measuring task switching performance, suggesting certain differences between the tasks, e.g. level of difficulty, may have led to different associations with EBR. As such, this study suggests higher EBR, signaling a reduced threshold for updating task goals, predicts better cognitive flexibility performance but not in every kind of task.

Further support for a relation between EBR and cognitive flexibility comes from a series of studies showing high EBR is associated with improved task switching at the cost of increased distractibility, both being consistent with a reduced threshold for updating cortical representations in high EBR individuals. Dreisbach et al. (2005) had participants perform a classification task in which targets and distractors were signaled by different colors. When the target color switched to a novel one, higher EBR was associated with better performance, but performance worsened when the distractor rather than the target color became novel. This concurs with the idea higher EBR is associated with a reduced threshold for updating cortical representations, thereby

inducing a bias towards novel information that may or may not facilitate performance depending on the situational demands. These findings were replicated by Tharp and Pickering (2011) and Müller et al. (2007a), with the latter also finding this effect to be stronger in men than women. However, a follow-up study that adapted the paradigm to include reward found the effect of EBR was in the same direction but not statistically significant (Müller, Dreisbach, Goschke, et al., 2007). Although the authors proposed insufficient power as an explanation of this nullfinding, perhaps the presence of reward overshadowed the effect of color novelty in this task, leading to its weakened association with EBR. This nullfinding notwithstanding, the three studies that did report a significant effect confirm EBR predicts performance dependent on the updating of task goals.

In a different approach to cognitive flexibility, Akbari Chermahini and Hommel (2012, 2010) showed in several experiments EBR predicts divergent thinking, a crucial component of creativity thought to rely on the ability to flexibly switch between mindsets to generate many diverse ideas (Guilford, 1967). Given this description, divergent thinking would be expected to benefit from a reduced threshold for updating representations. Participants performed an alternative uses task (AUT) in which they need to list as many, preferably unconventional and original, uses for common household objects. The answers are rated, amongst others, according to how many different categories of uses are listed. This score, referred to as 'flexibility', was found to follow an inverted-u-shaped relation with EBR in each of four experiments. That is, scores were higher for intermediate blink rates and lower for low and high blink rates. Additionally, it was reported positive mood induction increased EBR and this was associated with enhanced flexibility scores but only for low-EBR individuals (Akbari Chermahini & Hommel, 2012). Furthermore, a follow-up study that also used the AUT found cognitive and neural entrainment through presentation of binaural beats could improve divergent thinking scores but only for individuals with a low EBR (Reedijk, Bolders, & Hommel, 2013), perhaps because they have most room for improvement. The AUT and EBR being non-linearly related suggests perhaps low blink rates are associated with

an inability to flexibly update the current category of use for the household item, whereas very high blink rates are associated with excessive triggering of categories inappropriate for the current item. Regardless, the fact EBR related to cognitively flexibility in a non-linear fashion is highly relevant for future studies on EBR, who need to consider not only linear correlations or median-split groups but quadratic relations as well.

Eye blink rate and other cognitive measures

Aside from the more-investigated topics of reward and cognitive flexibility, EBR has also been related to performance in a variety of other paradigms. In addition to task-switching as discussed in the previous section, two other key cognitive control processes are inhibitory control and (updating of) working memory (Miyake et al., 2000), both of which would require appropriate thresholding and gating for proper performance. Indeed, several studies indicate a relation between EBR, impulsivity, and inhibitory control, i.e. the ability to withhold prepotent responses, fitting the idea changes in the basal ganglia's response threshold affects the ability to inhibit responses. Colzato et al. (2009b) first showed EBR was related to inhibitory control as assessed in a stop-signal task, reporting higher EBR to be associated with increased latency of inhibitory processes, i.e. reduced inhibitory efficiency. Curiously, Zhang et al. (2015) found opposite results when using different tasks to measure inhibition. Using a go/no-go task, in which a go or no-go cue precedes an imperative go or no-go stimulus, they found EBR correlated positively with accuracy scores. They also found lower inhibition costs in a Stroop task, i.e. smaller differences in reaction time on incongruent as compared to congruent trials. In light of these contradictory findings it is interesting to note a study by den Daas et al. (2013) who investigated the relation between EBR and impulsivity as assessed through eye-tracking. The authors presented participants with side-by-side pictures of naked and clothed individuals and found high EBR individuals showed longer dwelling times and higher fixation counts on naked targets, which they interpreted as reflecting an impulsive state of attention distribution towards the most salient information. While this study

uses a very different methodology to assess inhibition, its findings are in line with Colzato et al. (2009b) to the extent that both show a high EBR predicts a reduced threshold for responding, either to the saliency of erotic pictures or an imperative stimulus.

Colzato et al.'s finding that higher EBR is associated with worse inhibitory control fits the idea higher striatal DA activity is associated with a reduced threshold for responding. Hence Zhang et al.'s finding that EBR was positively related with inhibitory performance in a go/no-go and Stroop task is striking. The Stroop result is especially surprising as EBR was previously associated with increased distractibility (Dreisbach et al., 2005; Müller, Dreisbach, Brocke, et al., 2007; Tharp & Pickering, 2011), suggesting higher EBR should impair performance in an incongruent Stroop condition in which semantic meaning is a salient distractor. Although Colzato et al. reported a lower mean EBR and less variation therein (M = 14.0, SD = 7.9) than Zhang et al. (M = 18.5, SD = 11.0), based on an inverted-u-shaped relation between EBR and inhibitory control this difference should have led Zhang et al. to find even larger impairments rather than improvements. This raises the possibility the inhibitory control processes tapped by the stop-signal task and go/no-go task are actually related to different optimal levels of DA and thus have different associations with EBR. This idea is tentatively supported by Fillmore et al. (2006), who found an inverted-u-shaped dose-response relationship between cocaine and stop-signal performance but a linear relationship between the same doses of cocaine in the same individuals and go/no-go performance. To validate this explanation future research should examine stop-signal and go/no-go performance in the same individuals in relation to EBR.

Zhang et al. (2015) also reported a relation between EBR and working memory, but only in one of two tasks. First, they found no effect in a mental counter task in which participants had to simultaneously keep track of the values of three independent counters that could each go up or down several times during a trial. However, they did find an effect in a visual, letter-based N-back task. The results showed a negative correlation between EBR and 3-back accuracy scores, indicating a lower threshold for updating information in

working memory led to levels of distractibility that impaired performance, consistent with the increased distractibility found in other studies (Dreisbach et al., 2005; Müller, Dreisbach, Brocke, et al., 2007; Tharp & Pickering, 2011). Given the characteristic inverted-u-shaped relation between DA and working memory (Cools & D'Esposito, 2011), it would be interesting to see whether future research can demonstrate a quadratic relation between EBR and *N*-back performance, perhaps by including a wider range of EBR values.

In one of the aforementioned studies on EBR and divergent thinking, another aspect of creativity was also examined (Akbari Chermahini & Hommel, 2010). This aspect is convergent thinking, which relies on finding the single correct answer for a constrained problem and as such requires narrow focus and online stabilization of task goals rather than flexible updating of representations. It was assessed using the remote associations task in which three unrelated words are shown and one must identify a single word that fits them all. Convergent thinking followed a negative linear correlation with EBR, but this effect was not very strong as it was only significant when data from three experiments was pooled. Although the mapping of convergent thinking on stability of representations versus divergent thinking on flexibility of representations is probably too restrictive to be completely accurate, these results fit the idea performance requiring a narrow focus and more stable representation of task goals suffers from an increased tendency to gate representations as indicated by higher EBR.

Recently, Dang et al. (2016) showed EBR might predict the ego-depletion effect on task performance. This effect refers to the idea exerting self-control depletes certain resources, which accounts for impaired self-regulation on a subsequent task (Baumeister, Bratslavsky, Muraven, & Tice, 1998). Participants completed either an easy, non-depleting version of the Stroop task consisting only of congruent trials, or a difficult, depleting version consisting only of incongruent trials. Subsequently, all participants completed an anti-saccade task in which strong attentional control is needed to prevent attention being drawn towards a distractor and away from a difficult-to-detect target. Hence this performance would be susceptible to depletion of self-

control. Contrary to their expectations, there was only an inverted-u-shaped relation between EBR and anti-saccade after the difficult, depleting version of the Stroop task but no association at all after completing the easy version of the Stroop task. Because a medium level of DA might be most beneficial to cognitive flexibility (Akbari Chermahini & Hommel, 2010, 2012), the authors argued participants with a medium EBR showed no cost from switching between the difficult Stroop and anti-saccade task, whereas those with a low or high EBR had less efficient task switching and thus performed worse on attentional control after a depleting task. In an attempt to validate this interpretation of the findings, future research could investigate an association between individual switching performance and the susceptibility to ego-depletion as measured by Dang et al. Until then, this study provides first, albeit tentative, evidence EBR can predict susceptibility to ego-depletion.

EBR has also been related to the attentional blink, which occurs when stimuli are presented in rapid succession and two to-be-detected stimuli are in close temporal proximity. Typical findings are the first target T1 is adequately detected but detection of the second target T2 is severely impaired when presented 200-500 ms after T1 (Raymond, Shapiro, & Arnell, 1992). One potential explanation for this phenomenon is T1 processing and consolidation in working memory occupies attentional mechanisms that are consequently unavailable when T2 follows shortly after T1 (Shapiro, 2001). Reasoning working memory is modulated by DA, Colzato et al. (2008a) found EBR predicts the size of the attentional blink. Specifically, individuals with a higher EBR had a smaller attention blink, i.e. better detection of T2. This suggests the reduced gating threshold in high EBR individuals may have facilitated the processing of T2 in frontal cortex, thereby increasing the odds participants are able to detect it. This effect was not replicated in a more recent study by Slagter and Georgopoulou (2013). Although this may have been due to technical differences such as stimulus duration and refresh rate of the computer screen, a probably more important difference is Colzato et al. used distractors and targets that were all colored black whereas Slagter and Georgopoulou used white distractors, a red T1, and a green T2. The latter methodology implies a

set switch between T1 and T2 is required, with participants needing to switch attending from the color red to green, which might introduce an additional bottleneck in the processing stream that masks individual differences in the attentional blink (Dale, Dux, & Arnell, 2013; Potter, Chun, Banks, & Muckenhoupt, 1998) and leads to a nonsignificant relationship with EBR. On a different topic, similar to a study on divergent thinking discussed above (Reedijk et al., 2013), binaural beats have been shown to completely eliminate the attentional blink but only in individuals with low EBR (Reedijk, Bolders, Colzato, & Hommel, 2015), again indicating a potential ceiling effect wherein high EBR individuals may not have enough room for improvement due to the binaural beats. Overall, these studies indicate a relation between EBR and the attentional blink but also highlight a need to consider experimental design choices (stimulus duration, presence of a set-switch between T1 and T2) that might affect the detectability of this relation.

Colzato et al. (2007a) found EBR can predict the strength of visuomotor binding, which is proposed to be driven by DA (Colzato, van Wouwe, & Hommel, 2007a). This was demonstrated using a task in which participants respond with a left or right keypress to stimuli with varying features (color, shape, location). Only one feature was responded to whereas the rest were irrelevant. Carrying out a response to a stimulus leads to concurrent activation of motor and sensory representations thought to result in bi-directional associations between motor and sensory representations, even those of task-irrelevant features, such that activation of either the motor or sensory code primes activation of the other (Hommel, 1998, 2004). Hence, in this task the repetition of a stimulus feature across trials can facilitate or impair the response to the current stimulus depending on whether its feature was previously associated with the correct or incorrect response on the current trial. The authors found individuals with a high EBR experienced greater impairment when feature-repetition primed an incorrect response, suggesting a stronger binding of response and sensory features in these individuals. Notably, this effect was restricted to repetition of the task-relevant feature, possibly due to a burst of DA triggered by the task-relevant feature leading this

feature to be processed more readily in prefrontal cortex, leading to its stronger binding with motor representations.

Interestingly, Slagter et al. (2010) showed EBR can predict individual differences in subtle biases in spatial attention. Such pseudoneglect is thought to be related to asymmetries of the DA system (Tomer, 2008). In particular, it is thought a high EBR might reflect higher activity in the left basal ganglia that leads to a contralateral, rightward shift in spatial attention (Slagter et al., 2010). This was confirmed using a greyscales task in which two black-to-white gradients are shown side by side, starting of as white in the middle and turning progressively darker towards the outer sides. Participants judged which of the two gradients was darker overall, although unbeknownst to them the gradients were identical in one condition of the task. As hypothesized, higher EBR was associated an increased tendency to judge the gradient on the right as darker. This finding confirms EBR can predict the direction of a subtle attentional bias and, perhaps more interestingly, this tentatively suggests a particular role of the left basal ganglia in EBR.

Lastly, a study by Lackner et al. (2010) showed EBR predicts representational theory of mind (RTM) performance in infants, which is consistent with a role for maturation of the DA system in theory of mind. Infants ranging from 4 to 6 years old performed a variety of RTM tasks, such as false-belief tasks that require them to consider others do not necessarily have access to the same information as they themselves do. As hypothesized, infants with higher EBR demonstrated more accurate performance, supporting the idea EBR in infants can reflect maturation of DA systems and development of RTM. This finding also concurs with the idea that if there is any relationship between EBR and age, it might be most pronounced and reliable in children (see section 3.2 on age and EBR).

Discussion

This review provided an overview of research on spontaneous EBR as indicator of DA function. Here we summarize the most important conclusions,

consider the different methodologies used to assess EBR, and give suggestions for future research.

The reviewed literature indicates, first of all, pharmacological activation of either D1 or D2 receptors can affect EBR, although baseline EBR seems positively related to availability of striatal D2 but not D1 receptors. As such, resting EBR might primarily reflect D2 receptor activity in the striatum, perhaps because D2 receptors are more sensitive to low DA levels than D1 receptors (Frank & O'Reilly, 2006). The reviewed cognitive literature supports this idea by showing EBR predicts learning mediated by the D2 receptor system in the basal ganglia, but not learning thought to be driven by D1 (e.g. Slagter et al., 2015). The drug literature also indicates the effects of drugs on DA activity and EBR are not always straightforward, as low and high-dose agonists might have opposite, counterintuitive effects on DA activity and EBR (Cavanagh et al., 2014; Frank & O'Reilly, 2006). Second, a large body of literature shows EBR can serve as a marker of DA function in neurological and psychiatric disorders or recreational drug users, reflecting dopaminergic hypo- or hyperactivity as well as response to drug treatment. Additionally, there is research suggesting EBR can co-vary with factors such as age, gender, and personality, although findings so far have been equivocal. In an attempt to provide more consistent results, future research should aim to use comparable measurement tools to assess personality across studies and distinguish between women in different phases of the menstrual cycle or taking hormonal contraceptives. Lastly, studies employing a variety of cognitive paradigms show EBR is a useful predictor of cognitive-behavioral performance. It appears most reliably related to reward-driven behavior and cognitive flexibility, consistent with the idea increased DA as reflected by higher EBR is accompanied by facilitated gating of cortical representations.

Methodologies of eye blink rate assessment

As revealed by the tables listed in this review, there is considerable variability in the methods used to record EBR and the conditions under which these recordings took place. It is important to consider these differences, as they may

contribute to variability in EBR data not related to DA. The most often-used recording methods are direct observation and counting by a researcher, and visual inspection of a video recording or electrooculography (EOG) measurement. Less-often used methods are magnetic search coils applied to the eyelid that produce a digitally-recorded current upon blinking, a SMART analyzer motion system that tracks a reflective marker taped on the eyelid, and eyetrackers for which signal loss lasting between 200 and 500 ms is considered a likely blink.

Each method may raise concerns, although none seem grave enough to warrant dismissal as for most concerns alleviating factors are proposed. That is not to say some methods might be best suited for different conditions or populations. For example, there is the possibility of human error or interrater differences when assessing EBR via direct observation or visual inspection of recordings and this might be especially problematic when for high EBR the blinks are difficult to visually separate (Zaman & Doughty, 1997). In these cases search coils, a SMART system, or EOG might be preferable for their ability to depict sensitive measurements of eyelid movement. A concern regarding search coils and reflecting markers from SMART may be that their placement on eyelids could affect blinking, but one study reported the coil did not impair eyelid movement and participants become unaware of its presence quickly (Garcia et al., 2011), whereas studies with SMART reported there are no adverse effects related to the experimental procedures (Bologna et al., 2014, 2012). The third alternative, which is by far the most often-used method in cognitive research, is EOG in which blinking results in a brief, high-amplitude shift of opposite polarity in signals recorded by electrodes positioned above and below the eyes (see Lackner et al. (2010) for a visual representation of blinks in EOG channels). This can provide a sensitive measure of muscle movement near the eyes, which also means movement of nearby muscles, e.g. from speaking, may create noise from which blinks are difficult to distinguish. The susceptibility of the EOG signal to participants' movements means this method may be best suited to conditions involving as little movement as possible and individuals/patients who are able to sit still. This ties into an

obvious caveat in studies estimating blinks as brief signal loss during eyetracking, which is many other events may account for such signal loss. Although these studies count only brief intervals of signal loss that befit the swift nature of blinking, this does not discount the possibility of technical issues nor the fact that individuals might exhibit movements that lead to signal loss without necessarily reflecting a blink. This might be especially relevant for movement disorders such as PD and tic behavior in Gilles de la Tourette. Nevertheless, eyetracking has provided theory-driven findings with EBR in healthy adults (Aarts et al., 2012; den Daas et al., 2013; Pas et al., 2014), tentatively suggesting this method produces reliable measurements under the right circumstances.

Another highly variable factor in methodology is the duration of recordings, which range from a single minute to an hour or longer. If reliable estimates of EBR are to be obtained in only a few minutes, as most studies attempt, it is important blinking behavior is stable throughout this period. However, as noted by Doughty (2016) reports on this stability have been mixed, with some indicating increasing variability throughout the measurement period or starting after three minutes (Depue et al., 1990; Doughty, 2013, 2014; Zaman & Doughty, 1997). Such claims raise concern about the reliability of brief measurements. However, Doughty (2016) found EBR variability to be stable when measuring the first 35 blinks during a maximum of 5 minutes. Importantly, this only applied when participants maintained primary gaze, i.e. looking straight ahead at a fixation point, whereas variability fluctuated significantly if a chin support was used. Although these results support the idea short measurements can result in reliable estimates, they also indicate variability in EBR can be determined in part by the recording methodology. This calls for more systematic studies to reveal how conditions other than primary gaze can be adjusted to allow reliable brief measurements. Related to this point, Doughty (2016) notes some studies advocate for an initial adjustment period of several minutes for participants to acclimate to the recording room, but more research is necessary to

systematically investigate the potential effect of different adjustment periods on (variability in) EBR.

With respect to the conditions under which EBR has been measured, it is important to acknowledge a distinction between two types of EBR that may have different relations with DA. On the one hand there is 'tonic' EBR, referring to baseline rates of blinking at rest, and 'phasic' EBR, referring to blink rates in response to stimulus conditions (Bacher & Allen, 2009). Tonic EBR is typically assessed in primary gaze, that is by having subjects not perform any kind of task and instead look straight ahead at a neutral, white wall or fixation point, whereas phasic EBR is assessed while subjects for example watch a video, read, or converse. The distinction between tonic and phasic EBR is important because numerous activities alter EBR relative to rest, thereby limiting the comparability of results acquired under different conditions. For example, reading and conversing reduce and increase EBR, respectively (for a review, see Doughty, 2001), and increased mental workload and task difficulty reduce EBR (for a review, see Lean & Shan, 2012). Several studies in this review have measured EBR under conditions such as watching a video or during an interview, and these studies present a potentially confounded association between DA, EBR, and the population or cognitive measure of interest. In particular studies on atypical populations have examined EBR in various conditions other than primary gaze, e.g. during an interview or watching a video. This methodology might have contributed to variation in results across studies because changes in EBR due to the measurement condition, e.g. during an interview, might have masked differences in EBR as compared to controls. As such we recommend future studies to include assessment of EBR during primary gaze, i.e. in silent rest and looking straight ahead, to provide a reliable baseline measurement for comparison across studies.

Lastly, it is not only important *how* EBR is measured but also *when*, as the circadian rhythm seems to affect DA and therewith EBR. Blink rates are found to be stable between 10 and 17 h (Barbato et al., 2000; Doughty, 2006) but to increase in the evening, paralleling an increase in subjective sleepiness

(Barbato et al., 2000). This finding is consistent with sleep deprivation leading to an increase in both DA and EBR (Barbato et al., 1995, 2007; Crevits, Simons, & Wildenbeest, 2003; Doughty, 2006; Ebert et al., 1996). Because EBR might relate to an individual's subjective sleepiness, perhaps the most reliable estimate of basal DA function and EBR for comparison across studies is obtained during the day rather than the evening or night. Correspondingly, the majority of reviewed studies report measuring EBR only between 9 and 17 h and we highlight the need for future studies to keep accurately reporting the time-of-day for EBR measurements to facilitate between-study comparison.

Future research

We would like to end with several recommendations for future research that hopefully stimulate new lines of research as well as help address unresolved issues and facilitate between-study comparison, some of which have already been mentioned briefly. First of all, drug studies should aim to consider baseline EBR as a determinant of drug-induced change in blink rate. Certain studies present inconsistent results that might be reconciled by distinguishing drug response from low and high baseline blinkers. For example, van der Post et al. (2004) found no change in EBR following administration of drugs known to affect EBR in animals, which might be explained by the finding of Cavanagh et al. (2014) that drug-induced change in EBR can be opposite for low and high baseline blinkers.

Second, we recommend researchers to explicitly report EBR values and associated levels of significance both for baseline conditions and every drug and dose combination they employ, to facilitate between-study comparison. As is evident from this review's tables, it is often not clear which drug and dose combinations yielded significant effects, for example because researchers present their findings only in small figures (plotting drugs and doses against EBR) without clearly flagging all significant changes. Presenting detailed information, e.g. tables that list all drugs, doses, EBR values and significance levels, in addition to figures would allow readers to benefit more from the huge amount of information these studies can provide.

Third, the majority of EBR studies examine only linear correlations or use a median split to distinguish groups of low and high blinkers. Although this is often sufficient to find a distinction in performance, DA and cognitive performance, in particular working memory, often follow a characteristic inverted-u-shaped function (Cools & D'Esposito, 2011). However, the only study so far examining EBR and working memory reported solely linear relations. Such an approach potentially ignores non-linear patterns in the data, leading to loss of valuable information. Indeed, a select few studies have established nonsignificant linear but significant quadratic relations. Specifically, an intermediate EBR might be associated with optimal performance, whereas low and high blink rates are associated with lower performance (Akbari Chermahini & Hommel, 2010, 2012; Dang et al., 2016). Therefore we strongly advise future studies to also consider regression analyses of data instead of only median-split grouping and to report on quadratic relations albeit to confirm their non-significance.

Fourth, to allow unconfounded investigation of EBR and individual differences in cognitive performance, researchers should carefully screen participants not only for neurological and psychiatric conditions known to affect DA but smoking behavior as well. Nicotine, presumably through actions on DA, can affect excitability in the trigeminal complex (Evinger et al., 1993, 1988), which is a proposed neural circuitry for DA modulation of EBR (Kaminer et al., 2011, 2015). Indeed, smoking has been associated with increased blink rates (Klein et al., 1993). Hence, to promote reliable results with a little noise as possible due to smoking behavior, this characteristic ought to either be carefully monitored in participants or be included in the exclusion criteria.

Fifth, an important topic of investigation for future research would be the test-retest reliability of EBR within an individual. After all, if EBR is to be a predictor of individual differences in cognitive function then EBR itself needs to be a stable, reliable measure. Studies investigating the effects of large age ranges (e.g. Zametkin et al., 1979) often present cross-sectional data and thus do not speak to this issue. Although some studies report no significant

differences in baseline EBR between several sessions, few have provided detailed measures of reliability. One recent study explicitly addressing this issue found a high level of consistency in long-term meditators and a healthy control group (Chronbach's alpha of .79 and .85, respectively) in three measurements spaced eight to ten weeks apart (Kruis et al., 2016).

Sixth, although the present review has focused solely on the single, average spontaneous EBR value, there is evidence to suggest *patterns* of blinking might represent a novel informative characteristic. EBR patterns can be based on the time between blinks, i.e. the inter-blink-interval, and have shown to vary between healthy individuals, even in a single experimental condition, while being comparable in terms of average EBR. Three patterns have been proposed: an irregular, a J-type, and a symmetrical pattern (Doughty, 2002). They are characterized, respectively, by longer intervals interspersed with short ones, by progressively longer intervals, and by more constant, regular intervals. So far, no studies have associated these patterns with measures of cognitive performance, even though they might constitute more informative and sensitive markers of individual differences by including both mean of and variance in EBR.

Lastly, whereas studies so far typically calculated an average EBR value across several minutes under constant conditions, a promising novel line of research looks at trial-to-trial changes in EBR to track fluctuations in DA related to ongoing task demands. Two examples of such event-based EBR research are by van Bochove et al. (2013) who showed the Gratton effect (i.e. a conflict-sequence effect) was larger after a blink trial than after a non-blink trial, and by Rac et al. (submitted) who found an association between trial-to-trial EBR and changes in working memory updating and gating demands. These studies suggest EBR can be used to track transient changes in striatal DA activity in response to real-time fluctuations in cognitive demands. As such, event-based EBR might present a unique method of investigating the role of DA in cognitive performance on a trial by trial basis, which is not easily permitted by traditional methods with low temporal resolution such as PET.

Conclusion

To conclude, the present review provides a comprehensive overview or research showing EBR is a useful, easily-accessible marker of DA function with promising utility for a wide variety of research. Although equivocal findings are still present and more systematic research is necessary to resolve these inconsistencies, we strongly encourage future studies to examine the role of EBR in cognitive research.

Chapter Two

Color vision predicts processing modes of goal activation during action cascading

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Abstract

One of the most important functions of cognitive control is action cascading: the ability to cope with multiple response options when confronted with various task goals. A recent study implicates a key role for dopamine (DA) in this process, suggesting higher D1 efficiency shifts the action cascading strategy toward a more serial processing mode, whereas higher D2 efficiency promotes a shift in the opposite direction by inducing a more parallel processing mode (Stock, Arning, Epplen, & Beste, 2014). Given that DA is found in high concentration in the retina and modulation of retinal DA release displays characteristics of D2-receptors (Peters, Schweibold, Przuntek, & Müller, 2000), color vision discrimination might serve as an index of D2 efficiency. We used color discrimination, assessed with the Lanthony Desaturated Panel D-15 test, to predict individual differences (N = 85) in a stop-change paradigm that provides a well-established measure of action cascading. In this task it is possible to calculate an individual slope value for each participant that estimates the degree of overlap in task goal activation. When the stopping process of a previous task goal has not finished at the time the change process towards a new task goal is initiated (parallel processing), the slope value becomes steeper. In case of less overlap (more serial processing), the slope value becomes flatter. As expected, participants showing better color vision were more prone to activate goals in a parallel manner as indicated by a steeper slope. Our findings suggest that color vision might represent a predictor of D2 efficiency and the predisposed processing mode of goal activation during action cascading.

Introduction

One of the most important functions of cognitive control is action cascading, that is the ability to cope with multiple response options when confronted with various task goals. In such a situation, successful action control would require efficient activation of and switching between different task goals in order to properly organize behavior. This can be achieved via distinct strategies that are thought to lie on a continuum; on the one end is a more serial processing mode in which the next task goal is activated only when the previous task goal has finished, and on the other end is a more parallel processing mode in which the next task goal is activated when the previous task goal is still active (Mückschel, Stock, & Beste, 2014; Verbruggen, Schneider, & Logan, 2008).

Neurobiological models of action selection indicate dopamine (DA) plays a key role in action cascading and individual differences in DA function might predict the preferred (i.e., serial vs parallel) action cascading strategy. One particularly prominent model is the dual-state theory, which proposes that different DA receptors in the prefrontal cortex (PFC) exert opposite effects on activity states and cognition (Durstewitz & Seamans, 2008). This model, which is supported by a wealth of behavioral and electrophysiological data, suggests the dynamics of PFC activity lie on a continuum ranging from (i) a D1-dominated state that inhibits spontaneous but enhances task-related neural firing, thereby favoring robust online maintenance of representations, to (ii) a D2-dominated state that facilitates spontaneous neural firing and shifting between activity patterns, thereby allowing fast switching between representations. The dual-state theory has been successfully applied to action cascading performance, supporting the idea that different DA receptors shift the action cascading strategy into different directions: individuals with a genetic predisposition towards higher D1 efficiency demonstrated a more serial, step-by-step processing mode, whereas higher D2 efficiency predicted a more parallel, overlapping processing mode (Stock et al., 2014). Taken together, these findings suggest an individual with higher D1 efficiency is predisposed to a more serial action cascading strategy due to a stable but potentially rigid PFC processing state, whereas an individual with higher D2

efficiency is predisposed to a more parallel strategy due to a flexible but interference-prone PFC state.

In the present study we investigated whether individual differences in DA function could indeed predict the processing mode of goal activation in action cascading, using color vision (CV) discrimination as an indirect but low-cost and non-invasive marker of DA function. The retina is rich in DA and dysregulated retinal DA function is associated with impaired CV (Brandies & Yehuda, 2008). This is illustrated by populations thought to suffer from dysregulated DA function and who demonstrate impaired CV, such as patients with Parkinson's disease (Büttner et al., 1995; Büttner, Patzold, Kuhn, Müller, & Przuntek, 1994; Kertegle et al., 2010; Müller, Kuhn, Büttner, & Przuntek, 1997; Oh et al., 2011; Pieri, Diederich, Raman, & Goetz, 2000; Price, Feldman, Adelberg, & Kayne, 1992), attention-deficit/hyperactivity disorder (Banaschewski et al., 2006; Soyeon Kim et al., 2014; Roessner et al., 2008), Gilles de la Tourette syndrome (Melun, Morin, Muise, & DesRosiers, 2001), cocaine users (Desai, Roy, Roy, Brown, & Smelson, 1997; Hulka, Wagner, Preller, Jenni, & Quednow, 2013), and in normal aging (Jackson & Owsley, 2003; Melun et al., 2001). One study so far has demonstrated CV can predict DA-related cognitive performance. Specifically, CV in healthy young adults predicted individual differences in the cognitive control of response conflict, with better CV predicting reduced response conflict in an auditory Simon task (Colzato, Sellaro, Hulka, Quednow, & Hommel, 2014).

Although the exact nature of the link between CV and DA-related performance is unclear, the modulation of DA release in the retina displays characteristics of D2 receptors (Peters et al., 2000), raising the possibility that CV can predict individual differences in performance related to D2 efficiency. Consistent with this idea, administration of D2-like receptor antagonists leads to impaired retinal function (Fornaro, Calabria, Corallo, & Picotti, 2002) as observed in schizophrenia (Shuwairi, Cronin-Golomb, McCarley, & O'Donnell, 2002). Additionally, cocaine treatment decreases D2 receptor function (Madhavan, Argilli, Bonci, & Whistler, 2013), leading to increased cocaine craving (Volkow et al., 2006) which in turn is associated with CV

impairment (Roy, Roy, Smelson, Brown, & Weinberger, 1997; Roy, Smelson, & Roy, 1996). Although these findings may point towards a particular role of D2 receptors in CV, it should be acknowledged that D1 receptors likely also play a role (Brandies & Yehuda, 2008) and to date it has been difficult to disentangle the exact contributions of D1 and D2 receptors. However, the aforementioned study relating action cascading performance to gene polymorphisms indicated that D1 and D2 exert opposite effects on the processing mode of goal activation (Stock et al., 2014). Hence, the present study can provide further insight into the role of D1 and D2 receptors in CV by comparing the relationship between CV and action cascading to the previous findings on action cascading and gene polymorphisms. Specifically, assuming CV indeed indicates D2 function and given that increased D2 efficiency is associated with a more parallel processing mode in action cascading (Stock et al., 2014), we would expect individuals with better CV to demonstrate a more parallel action cascading strategy. In contrast, if CV were to primarily reflect D1 receptor function then we would expect better CV to predict a more serial strategy. Because previous studies indicate DA function might be particularly related to CV in the blue-yellow domain (Banaschewski et al., 2006; Colzato, Sellaro, et al., 2014; Desai et al., 1997; Hulka et al., 2013; Melun et al., 2001; Roessner et al., 2008), we also investigated whether blue-yellow CV in particular predicts the action cascading strategy.

To assess action cascading performance we used the stop-change paradigm (Verbruggen et al., 2008), an established diagnostic measure of action cascading. In this task participants need to respond as fast as possible to a GO stimulus, but in some trials a STOP signal, presented after a variable delay, indicates the need to withhold this response. A subsequent CHANGE signal indicates the new response rule that must be used to respond to the GO stimulus. In this task it is possible to calculate an individual slope value for each participant that estimates the degree of overlap in activation of the STOP and CHANGE goal. When the STOP process has finished at the time the CHANGE process is initiated, the slope value becomes flat and indicates more serial processing. In contrast, when the STOP process has not yet finished at

the time the CHANGE process is initiated, the slope value becomes steeper and indicates more parallel processing. In line with our expectations, we predicted participants with better CV to be prone to activating goals in a parallel manner and thus demonstrate a steeper slope.

Methods

Participants

Eighty-five undergraduate students from Leiden University (71 females, 14 males, mean age 20.00 years, standard deviation 2.09) were recruited to participate in this study for partial course credit. Participants were screened individually using the Mini International Neuropsychiatric Interview (MINI), a short, structured interview of approximately 15 min that screens for several psychiatric disorders and drug use (Sheehan et al., 1998) and is typically used in clinical and cognitive research (Colzato, de Bruijn, & Hommel, 2012; Colzato, van den Wildenberg, & Hommel, 2013). Participants were included if they met the following criteria: (i) between 18 and 30 years; (ii) no personal or family history of neurological or psychiatric disorders; (iii) no history of substance abuse or dependence; (iv) no chronic or acute medication; (v) no known color blindness; and (vi) females were actively taking hormonal contraceptives.

Informed consent was obtained from all participants upon arrival in the lab. The study conformed to the ethical standards of the Declaration of Helsinki and the protocol was approved by the local ethical committee (Leiden University, Institute for Psychological Research).

Lanthony Desatured Panel D-15 Test

In order to test CV discrimination we used the Lanthony Desaturated Panel D-15 test. This test comprises a fixed reference cap and 15 changeable color caps that need to be ordered in sequence. The color caps are of low saturation (decreased chroma) and increased lightness. The test was carried out under a daylight fluorescent lamp with an illumination of 1400 lx. Participants did not have a time limit but typically took at most 5 min to complete the test.

Quantitative scoring was based on the color scoring method proposed by Geller (2001) and resulted in a total color distance score (TCDS). A perfect TCDS score of 56.41 indicates all caps are arranged in the correct order and higher scores indicate CV impairment.

Qualitative scoring was based on the method outlined in (Hulka et al., 2013), which comprises plotting the individual participant's cup order on a template that describes a hue circle containing four reference axes. These axes are named protan, deutan, tritan and tetartan, and reflect the red, green, blue and yellow color domains respectively. Cap reversals that are parallel to one of the axes indicate an error in the respective color domain. Single cap inversions (e.g. 1-3-2-4-…) are classified as minor errors or normal confusion, whereas cap reversals spanning two or more caps are considered major errors. Two or more major errors indicate a CV disorder, which was classified based on Verriest's classification (Verriest, 1963): type I reflects CV impairment along the red-green axes; type II is a combined impairment of the red-green and blue-yellow axes; type III reflects impairment along the blue-yellow axes; type IV is diagnosed when no clear pattern can be determined.

Stop-Change Paradigm

The paradigm was adapted from Steenbergen, Sellaro, Stock, Beste, & Colzato, 2015 and Verbruggen et al., 2008, and was previously used to investigate the relation between D1 and D2 efficiency and action cascading (Stock et al., 2014). For an illustration of the task, see Figure 1.

The task consisted of a total of 864 trials divided in six equally sized blocks. Throughout each trial a white rectangle of 20 x 96 mm was presented on a black background. Within the rectangle were three horizontal reference lines (line thickness 1 mm, width 13 mm) that divided four vertically aligned circles (diameter 7 mm). At 250 ms after the onset of each trial one of the circles was filled white, thus becoming the GO target stimulus. In 67% of trials (the GO condition) participants needed to indicate whether the GO stimulus was located above or below the middle reference line, using a button press with the right middle finger to indicate "above" and the right index finger to indicate

"below". The GO stimulus was either response terminated or disappeared after 2500 ms had elapsed. If a response had not been given at 1000 ms after GO stimulus onset, the word "Quicker" was presented above the rectangle until the participants responded.

The remaining 33% of trials comprised the stop-change (SC) condition, which started with presentation of the GO stimulus but a STOP signal was also presented after a variable stop-signal delay (SSD). The STOP signal comprised the border of the rectangle turning red, which indicated to participants to try and withhold their right-handed response. Every STOP signal was followed by a CHANGE signal, which was a sine tone presented via headphones for 100 ms at 75 dB SPL and could be either high (1300 Hz), medium (900 Hz), or low (500 Hz) in pitch. This CHANGE signal indicated which of the three reference lines had to be used on the current trial to evaluate the GO stimulus' location (above vs below). The high, medium and low tones indicated the upper, middle and bottom reference lines respectively. All three lines were used with equal frequency. The CHANGE response had to be carried out with the left hand, using a button press with the left middle finger to indicate "above" and the left index finger to indicate "below". Crucially, in half of the SC trials the stimulus onset asynchrony (SOA) for the CHANGE signal was set to 300 ms (SCD300 condition), in the other half the STOP and CHANGE stimuli were presented simultaneously (i.e., SOA of 0 ms; SCD0 condition). If reaction time (RT) to the CHANGE signal was longer than 2000 ms, the word "Quicker" was presented above the rectangle.

After each SC trial, the SSD was adapted using a staircase algorithm to yield an approximate 50% of successfully inhibited GO responses (Logan & Cowan, 1984). The SSD was initially set to 250 ms and after an entirely correct SC trial (successful inhibition of the initial GO response before the CHANGE signal and subsequent correct left-handed response), the SSD was lowered by 50 ms. If these criteria were not met, the SSD was increased by 50 ms. This was constrained such that the SSD never became lower than 50 or higher than 1000 ms. This procedure allows an accurate estimate of the stop-signal reaction time (SSRT), an index of the duration of the covert response inhibition process

(Logan & Cowan, 1984). After each trial, a fixation cross was presented in the middle of the screen for the duration of the inter-trial interval (ITI; fixed duration 900 ms). All conditions were presented in a random order and participants were instructed to respond as fast and accurate as possible.

To estimate the degree of overlap between activation of the STOP goal and the CHANGE goal, we calculated an individual slope value for each participant (Stock et al., 2014; Verbruggen et al., 2008). This measure reflects the difference in RT between the SCD0 and SCD300 condition divided by the difference in SOA (i.e., -300). Following the line of argumentation in (Stock et al., 2014), RT is expected to be higher in the SCD0 condition, resulting in a typically negative slope that becomes steeper as the difference in RT increases. In brief, assuming that participants are able to resolve the stopping process at least partly during the SCD in the SCD300 condition, this promotes a more serial processing mode of the STOP and CHANGE goals. On the other hand, in the SCD0 condition participants have the option of either processing these goals serially or in parallel. If processed in parallel, there is a risk of interference between the two task goals that results in increased RT in the SCD0 relative to the SCD300 condition and thus leads to a steeper slope (for further elaboration, see Stock et al., 2014; Verbruggen et al., 2008).

Procedure

Participants arrived in the lab to be tested individually. After informed consent was obtained they completed the D-15 test to measure CV, which took on average 5 min. Participants then practiced the stop-change before completing the real task in approximately 45 min. Subsequently they were debriefed, rewarded with partial course credit and thanked for their participation.

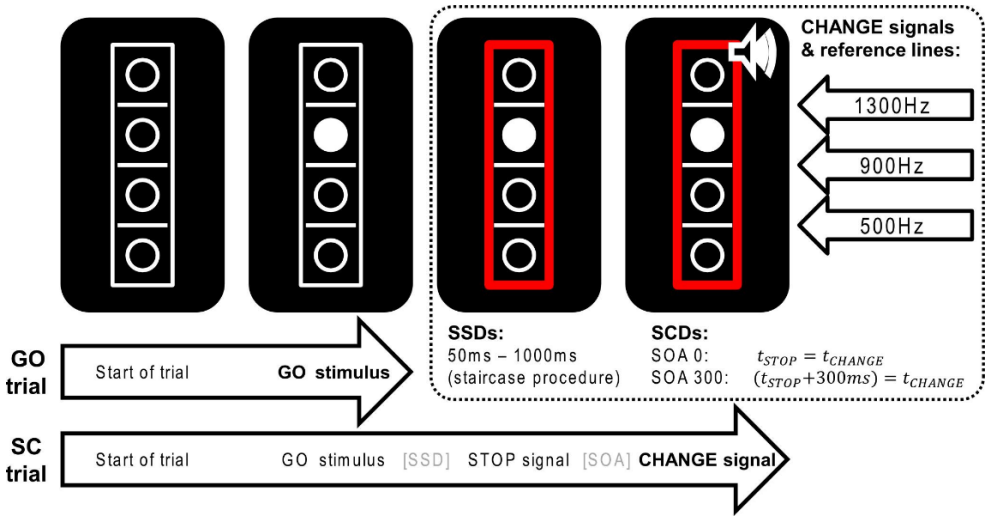

Figure 1. Schematic illustration of the stop-change (SC) paradigm. GO trials end after the first response to the GO stimulus, whereas SC trials end after the first response to the CHANGE signal. The stop-signal delay (SSD) was adjusted on a trial-by-trial basis using a staircase procedure. The stop-change delay (SCD) was set to either 0 or 300 ms, divided randomly and equally over all SC trials. As illustrated on the right, three tones of distinct pitch indicated the new reference line on SC trials. Figure adapted from Steenbergen, Sellaro, Stock, Beste, & Colzato, 2015.

Statistical analysis

For the behavioral data of the stop-change task, we first calculated individual mean RT separately for the three conditions (GO, SCD0, SCD300) and regardless of accuracy. Then for each condition we excluded trials with errors or RT above or below 2 standard deviations of the individual's mean RT in that condition. The SSRT was then calculated by subtracting the participant's mean SSD from the mean RT in the GO condition. Lastly, the individual slope was calculated by subtracting mean RT in the SCD300 from mean RT in the SCD0 condition and dividing the difference by -300. This results in a slope value that typically but not necessarily lies between 0 and -1, with lower values indicating more parallel processing.

To assess whether CV predicts a more serial or parallel processing mode of goal activation while accounting for a potentially skewed distribution of TCDS, we computed Spearman correlation coefficients between the TCDS and slope values. To determine whether impairment in the blue-yellow domain in particular predicts action cascading strategy, we investigated whether participants showing a specific blue-yellow disorder demonstrate a steeper slope than participants who did not show any CV disorder. For this comparison, a nonparametric one-tailed Mann-Whitney's U test was used to compensate for unbalanced sample sizes. To provide further insight in the relationship between CV and performance, we also computed Spearman correlation coefficients between TCDS, SSRT and mean RT in the GO, SCD0 and SCD300 conditions. A significance threshold of .05 was adopted for all statistical tests.

Results

For the quantitative analysis, TCDS (M = 76.50, SD = 12.28) was significantly positively correlated with the individual slope (M = -.62, SD = .23), $r_s(85)$ = .244, p = .025, see Figure 2. This indicates better CV (lower TCDS) is associated with a more parallel processing mode of goal activation (more negative, steeper slope). On the other hand, TCDS did not demonstrate significant Spearman correlations with mean RT in the GO (M = 629, SD = 202), SCD0 (M = 1138, SD = 295) or SCD300 (M = 952, SD = 310) condition or with SSRT (M = 280, SD = 65), all ps > .05. These null-findings suggest CV is not associated with a general change in response speed across conditions or a change in inhibitory control.

For the qualitative analysis, no participants demonstrated a type I disorder along the red-green domain, 1 demonstrated a type II disorder along the red-green and blue-yellow domain, 17 demonstrated a type III disorder along the blue-yellow domain, 4 demonstrated a type IV unclassified disorder, and 63 demonstrated only minor errors or 1 major error. No participants achieved a perfect score, i.e., all participants made at least a single minor or major error. Due to low sample sizes in the type II and IV disorder groups, we

only compared participants with a type III disorder along the blue-yellow axes with those who did not show any CV disorder. A nonparametric one-tailed Mann-Whitney's U test reveals no significant difference between the two groups in terms of slope, $U = 504$, $p = 0.356$, suggesting specific impairments in the blue-yellow domain do not explain the relationship between CV and the action cascading strategy.

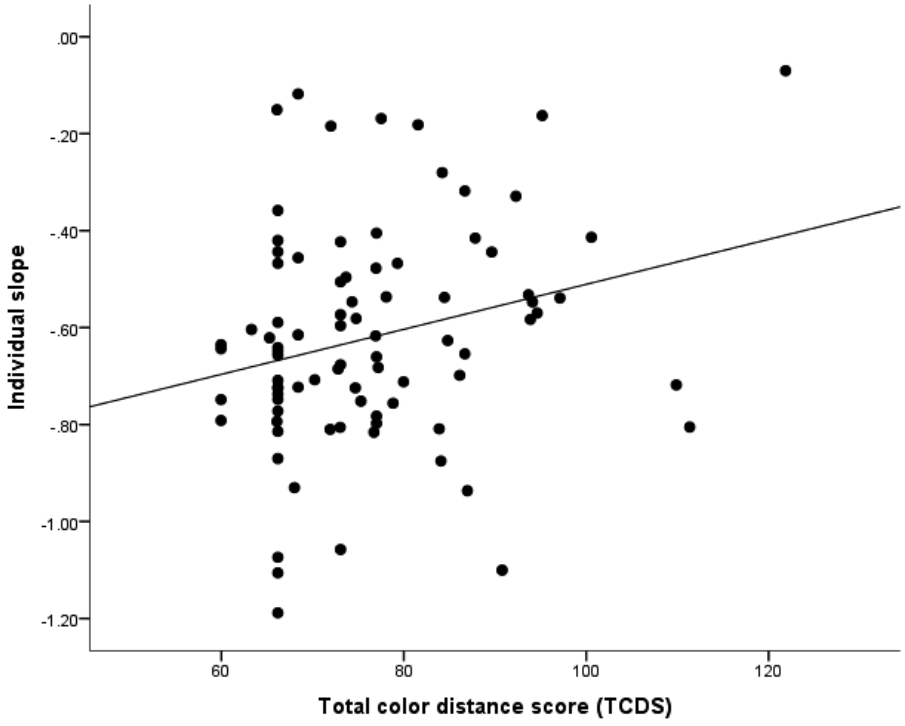

Figure 2. Scatter diagram of individual total color distance score (TCDS), with lower scores reflecting better performance, against the individual slope value from the stop-change paradigm, with lower scores indicating a more parallel processing mode.

Discussion

The present study investigated whether color discrimination can serve as an indirect marker of DA receptor function and therefore predict individual differences in the processing modes of goal activation in action cascading. So

far, little is known about the exact link between CV and DA, and it is likely that both D1 and D2 receptors contribute to visual function (Brandies & Yehuda, 2008). However, modulation of DA release in the retina resembles characteristics of D2 receptors (Peters et al., 2000), D2-like antagonists impair CV (Fornaro et al., 2002), and cocaine craving has been associated with both reduced D2 function (Volkow et al., 2006) and impaired CV (Roy et al., 1997, 1996). Although not conclusively, these findings support the hypothesis that CV might reflect D2 efficiency in particular. Given that increased D2 receptor efficiency has been shown to predispose individuals to a more parallel processing mode (Stock et al., 2014) and assuming CV reflects D2 function, we thus expected better CV to predict a more parallel action cascading strategy. Consistent with this hypothesis, individuals with lower TCDS (indicative of better color discrimination) were prone to activate goals in a parallel manner as indicated by a steeper slope value in the stop-change paradigm. This can be understood in light of the dual-state theory of PFC activation (Durstewitz & Seamans, 2008), which proposes increased D2 functioning (here indexed by CV) is associated with a more D2-dominated PFC activity state in which spontaneous firing is enhanced and representations are easily switched between. A downside of such a flexible activity state is that it may be particularly prone to interference when processing goals in parallel, resulting in delayed responses as compared to when goals are processed in a serial manner. Based on this reasoning, the present study provides converging evidence that CV is associated with D2 receptor efficiency, as indicated by a more parallel processing mode of goal activation.

Previous research has suggested CV in the blue-yellow domain is particularly indicative of DA function (Banaschewski et al., 2006; Colzato, Sellaro, et al., 2014; Desai et al., 1997; Hulka et al., 2013; Melun et al., 2001; Roessner et al., 2008), although this is not always the case (Barbato, Rinalduzzi, Laurenti, Ruggieri, & Accornero, 1994; Büttner et al., 1995; Shuwairi et al., 2002). However, our results do not indicate a relationship between specific blue-yellow impairment and stop-change performance. Although one might interpret non-specific CV impairment to reflect reduced

attentional performance rather than specific DA impairments (Bertrand et al., 2012), we argue an explanation of our data in these terms is unlikely. If CV performance in our sample indeed reflected only attentional performance then this impairment should have extended to general task performance, especially since the task is often experienced as very demanding. However, CV did not reliably predict RT in the three conditions (GO, SCD0, SCD300) separately, nor does this account explain why impaired CV would have predisposed individuals to activate goals in a more parallel rather than serial manner. Instead, it is important to note that only 17 out of 85 participants were classified as having a disorder in the blue-yellow domain. This suggests the present study has less than 50% power to detect an effect with a small to medium effect size, as might be expected based on the strength of the correlation between TCDS and the slope values. Hence it would be informative for future studies to revisit this issue with a sample that includes more individuals with a blue-yellow CV disorder. In this context, it is interesting to note that an age above 25 years was previously associated with a drop in CV performance, especially for the blue-yellow domain (National Research Council, 1981). Hence, it may be considered a strength of the present study that our sample barely meets this cut-off (mean age = 20.00 years, standard deviation = 2.09), suggesting age did not confound our results. Indeed, calculating a partial Pearson correlation between TCDS and slope value while controlling for age results in near identical results to those reported above ($r(85) = .249$, $p = .022$). Nevertheless, it would be interesting for future research to include a wider age range to see if the relationship between blue-yellow CV and action cascading is modulated by age.

As the present study is the first to indicate CV may predict performance related to D2-functioning in particular, it is important future studies seek to validate this link. For example, previous studies have found that spontaneous eye blink rate (EBR), another presumed marker of DA and possibly D2 function (Jongkees & Colzato, 2016), reliably predicts performance on the alternative uses task, a test of creativity that strongly depends on divergent thinking and the fast and flexible switching between representations (Akbari

Chermahini & Hommel, 2010, 2012). Similarly, EBR predicts performance on an attentional set-shifting paradigm in which enhanced flexibility promotes performance in one condition but the coinciding increased distractibility impairs performance in another condition (Dreisbach et al., 2005; Müller, Dreisbach, Brocke, et al., 2007; Tharp & Pickering, 2011). Given these results, it would be interesting to establish whether CV as predictor of a more D2-dominated PFC activity state is also associated with enhanced divergent thinking, cognitive flexibility and distractibility.

Moreover, our results indicate a large spread in action cascading performance in individuals with near-perfect CV, whereas this variation seems to decrease as CV worsens, see Figure 2. This may suggest the relationship between CV, action cascading, and presumably the predisposed PFC activity state, increases as CV is further impaired. This pattern would resemble the dedifferentiation hypothesis, put forward to explain the age-related increase in the correlation between performance on different cognitive tasks (Baltes, Cornelius, Spiro, Nesselroade, & Willis, 1980), or between sensory and cognitive functions (Baltes & Lindenberger, 1997). The hypothesis states this increased correlation may be the result of degradation of specialized neural structures due to a shared cause (e.g. loss of catecholamine function or white matter), leading these functions to share more variance in performance. The D2 system may be one factor in such dedifferentiation (cf. Papenberg, Lindenberger, & Bäckman, 2015), as this system is known to degrade with age and mediate related cognitive decline (Bäckman et al., 2000). In light of our results, we speculate CV as predictor of D2 function may predict dedifferentiation of cognitive functions even in healthy young adults. Hence, it would be interesting for future research to examine whether the correlation between performance on different cognitive tasks indeed increases as a function of CV.

Lastly, the present study has focused exclusively on color discrimination performance as a measure of visual function, but there are other conceivable measures such as visual acuity and motion discrimination that remain open to investigation. Given the widespread influence of DA on visual

function (Brandies & Yehuda, 2008), it would be interesting for future research to validate whether action cascading and other DA-driven processes are predicted only by color discrimination or instead relate to visual function in general.

To conclude, our results indicate CV discrimination in healthy humans is related to individual differences in DA function and D2 efficiency in particular. Better CV predicted a more parallel processing mode of goal activation, consistent with a more D2-dominated PFC activity state. As such, this study is a first step towards elucidating the link between CV, DA and cognitive-behavioral performance.

Chapter Three

Effect of tyrosine supplementation on clinical and healthy populations under stress or cognitive demands

Jongkees, B. J., Hommel, B., Kühn, S., & Colzato, L. S. (2015). Effect of tyrosine supplementation in clinical and healthy populations under stress or cognitive demands—a review. *Journal of Psychiatric Research, 70*, 50-57.

Abstract

Consuming the amino-acid tyrosine (TYR), the precursor of dopamine (DA) and norepinephrine (NE), may counteract decrements in neurotransmitter function and cognitive performance. However, reports on the effectiveness of TYR supplementation vary considerably, with some studies finding beneficial effects, whereas others do not. Here we review the available cognitive/behavioral studies on TYR, to elucidate whether and when TYR supplementation can be beneficial for performance. The potential of using TYR supplementation to treat clinical disorders seems limited and its benefits are likely determined by the presence and extent of impaired neurotransmitter function and synthesis. Likewise, the potential of TYR supplementation for enhancing physical exercise seems minimal as well, perhaps because the link between physical exercise and catecholamine function is mediated by many other factors. In contrast, TYR does seem to effectively enhance cognitive performance, particularly in short-term stressful and/or cognitively demanding situations. We conclude that TYR is an effective enhancer of cognition, but only when neurotransmitter function is intact and DA and/or NE is temporarily depleted.

Introduction

The amino-acid L-Tyrosine (TYR) is the biochemical precursor of the catecholamines dopamine (DA) and norepinephrine (NE). Given the right circumstances TYR supplementation can enhance DA and NE levels in the brain (Cuche et al., 1985; Gibson & Wurtman, 1978; Tam, Elsworth, Bradberry, & Roth, 1990) and this possibility has led numerous studies to investigate whether administration of TYR can positively influence cognitive or behavioral performance that relies on catecholamine function. Unfortunately, reports on the effectiveness of TYR supplementation have varied greatly, with some studies showing a marked positive effect, whereas others report no significant changes. In the present paper we provide a summary review of the available cognitive and behavioral TYR studies and their main results, to gain a better understanding of the conditions under which TYR has a positive effect and whether TYR may be useful in a clinical context. Further, we hope to help inform future studies on how to best design and analyze experiments regarding TYR.

Before we review the behavioral and cognitive studies on TYR, we will first elaborate on the mechanism through which TYR presumably enhances brain physiology. As we will argue, the nature of this mechanism might play a crucial role in determining whether and to what extent supplementation can benefit performance. Plasma TYR levels peak between 1 and 2 hours after consumption and can remain significantly elevated up to 8 hours (Glaeser, Melamed, Growdon, & Wurtman, 1979). Correspondingly, in rats it was shown prefrontal DA increased 1 hour after TYR administration, but not earlier (Tam et al., 1990). Once it has passed the blood-brain barrier (BBB)and is taken up by the appropriate brain cells, TYR is converted into L-DOPA through an enzyme called tyrosine-hydroxylase (TH; Daubner, Le, & Wang, 2011). TH activity initially increases upon consumption of TYR, but it is regulated by end-product inhibition (Daubner et al., 2011; Tam et al., 1990), preventing large increases in catecholamine release. L-DOPA is converted into DA, resulting in an increase in DA level. In turn, DA can be converted into NE

through the enzyme dopamine beta-hydroxylase (Kaufman & Friedman, 1965).

Importantly, TYR has been found to enhance neurotransmitter synthesis only in actively firing neurons (Fernstrom & Fernstrom, 2007; Lehnert, Reinstein, Strowbridge, & Wurtman, 1984; Tam et al., 1990). This suggests TYR can reverse a process called neurotransmitter depletion, in which increased brain activity leads to decreased DA and NE levels, with behavioral performance levels declining accordingly. This role as a depletion reverser is best illustrated by the following example. When exposed to stress or a cognitively challenging task, catecholamine neurons become more active and their synthesis rate increases (Kvetnansky, Sabban, & Palkovits, 2009; Lehnert et al., 1984; Mahoney, Castellani, Kramer, Young, & Lieberman, 2007). As more neurotransmitters are synthesized to meet the situational demands, the resource from which they are synthesized, namely TYR, is expended. Synthesis becomes limited once TYR runs low, leading to less neurotransmitter availability and corresponding decrements in performance (Goldman-Rakic et al., 2000; Muly, Szigeti, & Goldman-Rakic, 1998). In this situation TYR might benefit brain function by providing the resources necessary to allow neurotransmitter synthesis to continue and maintain catecholamine levels needed to ensure optimal performance (Wurtman, Hefti, & Melamed, 1980). On the contrary, one may assume when the rate of synthesis is not elevated then TYR supplementation amounts to providing unnecessary extra resources from which to synthesize DA and NE, which should not impact these neurotransmitters levels or their associated performance. Indeed, in rats it was shown that TYR administration only enhanced DA synthesis in the striatum when this region was pharmacologically activated (Tam et al., 1990). In other words, TYR supplementation seems to have a beneficial effect only in situations that stimulate neurotransmitter synthesis, i.e., situations that are sufficiently stressful or challenging. Indeed, in the present review we will demonstrate that TYR's role as a depletion reverser fits well with the pattern of results found in the literature.

To date there is not yet a single, agreed upon effective dose for TYR supplementation and thus administered doses have varied from 500 mg to 12 g per day (see Table 1). To put these numbers in perspective, the World Health Organization's daily upper requirement of TYR is 14mg/kg (Deijen, 2005), meaning an individual weighing 70 kg needs to consume approximately 1 g of TYR per day for normal functioning. Doses far exceeding 1 g are unlikely to confer any additional benefits, as the rate-limiting TH enzyme is assumed to be close to saturation under normal circumstances (Brodnik, Bongiovanni, Double, & Jaskiw, 2012). Consistent with this idea, TYR transport across cell membranes decreases in healthy individuals after TYR supplementation (Wiesel et al., 1999). Any excess TYR would thus be metabolized rather than converted into L-DOPA. Future studies may wish to examine reductions in TYR transport with varying doses of TYR, to shed light on what an optimal dose might be. While too much TYR might not be extra beneficial, consuming too little might also be possible. In rats a dose of 50 but not 25 mg/kg was successful at increasing prefrontal DA level (Tam et al., 1990), although how these numbers translate to humans remains unclear. Complicating matters further, TYR shares a transporter across the BBB with several other large neutral amino-acids such as phenylalanine and tryptophan (Fernstrom, 1990). Hence the amount that crosses the BBB instead of being metabolized peripherally depends on the consumer's levels of these other amino-acids. For this reason studies investigating the effect of acute TYR supplementation should and often do have their subjects fast overnight to reduce competition from other amino-acids. This has important implications for the efficacy of TYR supplementation in a long-term setting, since completely avoiding these other amino-acids for longer periods might not be practical nor recommendable.

Such issues could be avoided by opting for L-DOPA rather than TYR administration, which acts more downstream and avoids the rate-limiting TH factor as well as the competition from other amino-acids. However there are a number of reasons why TYR supplementation might be preferable. First of all, the TH enzyme already being near saturation under normal circumstances

(Brodnik et al., 2012) can be considered a positive characteristic, as it prevents large amounts of TYR being converted. In contrast, the conversion of L-DOPA into DA does not depend on such a rate-limiting factor, allowing far larger increases in catecholamines but also significantly increasing the risk of inducing levels that are detrimental for performance (Goldman-Rakic et al., 2000; Muly et al., 1998). Given the characteristic inverted-U profile of DA (Cools & D'Esposito, 2011), it would be easy for L-DOPA administration to push individuals to the lower right end of the curve, whereas the subtle increase from TYR is far less likely to do so. Given TYR's hypothesized role as a depletion reverser, it may even be that TYR maintains rather than changes an individual's position on this curve in the face of stress or cognitive demands. This also means the beneficial effects of TYR are likely smaller than L-DOPA's, especially for disorders associated with severe hypodopaminergic states. In TYR's favor, there are currently no established side-effects of long-term TYR supplementation, although one study showed increased saccadic intrusions during smooth-pursuit eye movement performance in patients with schizophrenia (Deutsch et al., 1994). On the other hand, chronic L-DOPA administration is associated with adverse symptoms such as dyskinesia, insomnia, nausea, and sometimes even psychosis (Foster & Hoffer, 2004; Liggins, Pihl, Benkelfat, & Leyton, 2012). However it should be noted studies on the chronic effect of TYR supplementation are still scarce and its potential long-term side-effects should be more extensively investigated before drawing definitive conclusions. Lastly, TYR might be preferred to L-DOPA simply because it is readily accessible to general public, being sold in regular drug stores.

As mentioned earlier, most of the presently reviewed studies, especially those in healthy individuals, have focused on short-term effects of TYR and therefore our conclusions should mainly be considered in this context. The benefits of TYR during long-term stress are less investigated, although still promising. Prolonged stress exposure (e.g. low temperatures) can increase TH activity up to one month, suggesting TYR might be especially useful during this period. After one month, however, TH activity is no longer significantly

elevated (Kvetnansky et al., 2009). Nevertheless, catecholamine neurons can remain highly active, leading to a depletion of DA and NE levels (Kvetnansky et al., 2009). This suggests TYR can also be beneficial in long-term settings. Indeed, a study in Antarctica residents (Palinkas et al., 2007) showed TYR has measurable benefits even after 7 weeks of exposure to extreme cold. Still, studies on short-term conditions far outweigh those on long-term effects and future studies should aim to lessen this discrepancy.

The available studies on TYR come from three major domains of research. First, many psychiatric disorders are associated with decreased DA and/or NE availability in the brain. Therefore, TYR has been investigated as a potential treatment for clinical symptoms associated with suboptimal catecholamine levels. Second, stress is thought to reduce catecholamine levels in the brain through increased turnover rates, leading to impairments in performance (Lehnert et al., 1984; Mahoney et al., 2007). As such, TYR supplementation has also been proposed as a potential reverser of stress-induced decrements in performance. Lastly, TYR also has promising implications for healthy individuals without overt exposure to stress, provided that high demands on cognitive performance create a stress-like state that might induce neurotransmitter depletion—the detrimental consequences of which might be counteracted by TYR supplementation (Colzato, Jongkees, et al., 2014; Steenbergen, Sellaro, Hommel, et al., 2015). We will use these three research areas to structure our overview, starting with studies on TYR and clinical populations, followed by studies on stressed but otherwise healthy individuals, after which we review studies on healthy humans in cognitively demanding situations. Characteristics and main outcomes of the reviewed studies are presented in Table 1.

Table 1. Characteristics and main outcomes of the reviewed studies.

Authors	Sample	Dose of TYR	Findings
Banderet and Lieberman, 1989	Healthy, cold exposure (*N*=23)	100 mg/kg	Reduced symptoms, improved mood, reaction times and vigilance
Chinevere et al., 2002	Healthy, physically exerted (*N*=9)	150 mg/kg	No effect of TYR
Colzato et al., 2013	Healthy, cognition challenged (*N*=22)	2.0 g	Improved working memory updating
Colzato et al., 2014a	Healthy, cognition challenged (*N*=22)	2.0 g	Improved inhibitory control
Colzato et al., 2014b	Healthy, cognition challenged (*N*=32)	2.0 g	Improved convergent thinking
Deutsch et al., 1994	Schizophrenia patients (*N*=11)	10.0 g	Increased saccadic intrusions, no effect on behavior or cognition
Deijen and Orbleke, 1994	Healthy, auditory stress (*N*=16)	100 mg/kg	Improved working memory and Stroop performance
Deijen et al., 1999	Healthy, intensive combat training (*N*=21)	2.0 g	Improved memory and tracking performance
Eisenberg et al., 1988	ADHD patients (*N*=7)	100 mg/kg	No effect of TYR
Gelenberg et al., 1980	Depressive patients (*N*=1)	100 mg/kg	Self-rated improvement of depression
Gelenberg et al., 1990	Depressive patients (*N*=65)	100 mg/kg	No effect of TYR
Goldberg et al., 1980	Depressive patients (*N*=2)	100 mg/kg	Improvement of symptoms
Growdon et al., 1982	Parkinson's patients (*N*=23)	100 mg/kg	Increased levels of TYR and homovanillic acid.
Kishore et al., 2013	Healthy, heat exposure (*N*=10)	6.5 g	Reduced delay in event related potentials
Leathwood and Pollet, 1983	Healthy, no manipulation (*N*=60)	500 mg	No effect of TYR on mood
Lemoine et al., 1989	Parkinson's patients (*N*=10)	1.6-4.0 g	Improvement of symptoms
Lieberman et al., 1983	Healthy, no manipulation (*N*=16)	100 mg/kg	No effect of TYR on mood
Magill et al., 2003	Healthy, sleep deprivation (*N*=76)	150 mg/kg	Improved working memory, reasoning and vigilance
Mahoney et al., 2007	Healthy, cold exposure (*N*=19)	150 mg/kg	Improved working memory
Nemzer et al., 1986	ADHD patients (*N*= 14)	140 mg/kg	No effect of TYR
O'Brien et al., 2007	Healthy, cold exposure (*N*=15)	300 mg/kg	Improved working memory
Palinkas et al., 2007	Healthy, in Antarctica (*N*=43, 42)	12 g	Improved mood during winter
Pietz et al., 1995	Phenylketonuria patients (*N*=24)	100 mg/kg	No effect of TYR
Pollin et al., 1961	Schizophrenia patients (*N*=12)	285 mg/kg	No effect of TYR
Posner et al., 2009	ADHD patients (*N*=1)	100 mg/kg	Improvement of symptoms
Reimherr et al., 1987	ADHD patients (*N*=12)	50-150 mg/kg	Short term, unsustained clinical response
Shurtleff et al., 1994	Healthy, cold exposure (*N*=8)	150 mg/kg	Improved working memory

Smith et al., 1998	Phenylketonuria patients (*N*=21)	100 mg/kg	No effect of TYR
Sutton et al., 2005	Healthy, physically exerted (*N*=20)	150 mg/kg	No effect of TYR
Thomas et al., 1999	Healthy, cognition challenged (*N*=20)	150 mg/kg	Improved working memory
Tumilty et al., 2011	Healthy, heat exposure (*N*=8)	150 mg/kg	Increased endurance capacity
Tumilty et al., 2014	Healthy, heat exposure (*N*=7)	150 mg/kg	No effect of TYR
Watson et al., 2012	Healthy, heat exposure (*N*=8)	150 mg/kg	No effect of TYR
Wood et al., 1985	ADHD patients (*N*=12)	150 mg/kg	Short term, unsustained clinical response

Studies reviewed in the present article, listing author names, publication years, type and size of sample, as well as potential stressor, dose of L-Tyrosine and the study's main outcomes.
TYR, L-Tyrosine

Literature Overview

Clinical Populations

Many clinical conditions are characterized by suboptimal catecholamine levels, and this has led to the idea that TYR supplementation could alleviate catecholamine-related symptoms. Many clinical studies on TYR have concentrated on impaired DA function, whereas NE is less often investigated. Therefore the focus of this section will be on DA, while NE will be discussed only briefly.

One of the most prominent psychiatric disorders is depression and it has been linked to impaired DA and perhaps NE, function (Dunlop & Nemeroff, 2007). The possibility of using TYR as a treatment was proposed some decades ago already (Gelenberg & Gibson, 1984). This treatment was reported to help in very small samples (Gelenberg, Wojcik, Growdon, Sved, & Wurtman, 1980; Goldberg, 1980), but a larger, randomized study was unable to replicate this result (Gelenberg et al., 1990). This led to the conclusion that treating depression with TYR is not very promising (Fernstrom, 2000; Parker & Brotchie, 2011). However, depression is a complex and varied disorder that likely has many subtypes defined by different etiologies (e.g., DA versus serotonin deficiencies), as well as varying symptoms (e.g., lack of motivation versus anxiety) and these may differ per individual patient (Harald & Gordon, 2012). It is therefore unlikely a non-specific and simple treatment like food supplementation has a marked effect on samples that may be highly heterogeneous. Studies investigating the effect of TYR on depression may prove more fruitful if they take into account these different etiologies and symptoms while clearly distinguishing the response of different patient groups. For example, we speculate perhaps those depressed individuals experiencing a lack of motivation, which may result from DA deficiency (Dunlop & Nemeroff, 2007), are the ones who could benefit most from TYR supplementation. On the other hand, individuals with psychotic depression may have excess DA, perhaps in part because their DBH enzyme, which converts DA into NE, is less active (Sapru, Rao, & Channabasavanna, 1989). Such individuals would be unlikely to benefit from a further boost in DA

activity and therefore TYR supplementation may not be recommendable for them, even though their NE levels need to be increased. Indeed, inducing higher DA levels in psychotic patients may even exacerbate their symptoms rather than improving them.

TYR supplementation might be of interest for schizophrenia, although studies on this topic are scarce. Enhancing DA function in this disorder might seem counter-productive, given it is strongly associated with increased DA signaling, sensitivity, and synthesis capacity (Howes et al., 2015; Seeman, 2013). However, in this regard it is important to distinguish between striatal areas, often demonstrating a hyperdopaminergic state in schizophrenia, and extrastriatal, prefrontal regions showing, in contrast, a marked reduction in DA activity (Davis, Kahn, Ko, & Davidson, 1991; Finlay, 2001; Slifstein et al., 2015). Although not meant as a strict dichotomy, striatal hyperactivity is strongly linked to positive, i.e. psychotic symptoms, whereas prefrontal hypoactivity is associated with negative symptoms and cognitive deficits (Guillin, Abi-Dargham, & Laruelle, 2007). For this reason it is possible TYR supplementation could exacerbate positive symptoms by further fueling the striatum, while alleviating negative symptoms and cognitive deficits by facilitating prefrontal cortex (PFC) function. Consistent with this hypothesis, DA agonists increase psychotic symptoms in certain patients (Lieberman, Kane, & Alvir, 1987), yet they can also improve cognitive task performance (Barch & Carter, 2005; Daniel et al., 1991). Another interesting possibility is TYR's enhancement of PFC function might even downregulate the striatal hyperactivity and thereby improve positive symptoms as well. This hypothesis is based on the idea that there is strong dopaminergic reciprocity between the PFC and striatum, with increases and decreases in prefrontal DA being associated with decreases and increases in striatal DA, respectively (Akil et al., 2003; Cools & D'Esposito, 2011; Meyer-Lindenberg et al., 2005).

To date, however, the few conducted studies do not support these speculations. One study assessing the effects of 3 weeks of TYR supplementation revealed no changes in behavior (Pollin, Cardon Jr, & Kety, 1961), although the study reported solely qualitative observations made during

interviews. The only study providing quantitative data reported no effect of 3 weeks of TYR supplementation on positive or negative symptoms, nor cognitive capacity as measured by the Wisconsin card sorting test and a memory test, despite increased plasma levels of TYR (Deutsch et al., 1994). This study did reveal an increase in saccadic intrusions during smooth-pursuit eye movement performance. It should be noted both aforementioned studies examined only 11-12 individuals and, given the possible heterogeneity of schizophrenia's etiology (Tandon, Keshavan, & Nasrallah, 2008), it is unlikely the patients within and across samples were comparable to each other. Therefore, we argue it is not yet warranted to dismiss TYR's potential for alleviating symptoms of schizophrenia. Instead, more studies are needed in which samples are larger and heterogeneity is kept as small as possible or response to TYR is distinguished between patients. Other issues deserving further investigation are (1) whether TYR should be administered during periods of remission, psychosis, or both, since striatal hyperactivity seems most prominent and reliably found during psychosis (Howes et al., 2015), (2) if and how TYR supplementation interacts with DA antagonists, i.e. antipsychotics, and (3) whether a dysregulation of tyrosine transport across cell membranes (Bongiovanni, Leonard, & Jaskiw, 2013) influences effects of TYR supplementation in patients with schizophrenia, as this dysregulation is associated with impaired cognitive function (Wiesel et al., 2005).

The third disorder we review in relation to TYR is attention deficit hyperactivity disorder (ADHD) as its etiology and symptoms strongly relate to reduced DA levels and impaired cognitive function (del Campo et al., 2011). However, results from studies on amino-acid supplementation in ADHD are again not straightforward. Some individuals benefit from TYR (J. Posner, Gorman, & Nagel, 2009; Reimherr, Wender, Wood, & Ward, 1987; Wood, Reimherr, & Wender, 1985), while some studies report no change in behavioral or cognitive function at all (Eisenberg, Asnis, van Praag, & Vela, 1988; Nemzer, Arnold, Votolato, & McConnel, 1986). As is the case with depression, schizophrenia, and presumably most other psychiatric disorders, many different risk factors are associated with ADHD (Pellow, Solomon, &

Barnhard, 2011) and they may all have different implications for potential effects of TYR supplementation. One such ADHD risk factor is impaired neurotransmitter metabolism (McConnel, 1985) and it has been proposed individuals suffering from this factor are the ones who can benefit from TYR supplementation, although they may account for only 5 to 10% of ADHD cases (Pellow et al., 2011). Therefore, future work on TYR and ADHD should consider these different risk factors, to elucidate whether and for whom TYR supplementation could be beneficial.

Parkinson's disease is characterized by a severe loss of dopaminergic neurons and decreased DA in many brain areas (Dauer & Przedborski, 2003), leading to impairments of behavior, emotion and cognition. Interestingly, an early study showed administering TYR to Parkinson's patients raised levels of DA's metabolite homovanillic acid, suggesting TYR effectively promoted DA function (Growdon, Melamed, Logue, Hefti, & Wurtman, 1982). Another early study reported clinical improvement in some, but not all patients after three years of TYR treatment (Lemoine, Robelin, Sebert, & Mouret, 1989). However, the reliability of the latter finding is debatable given a small sample of 10 patients was investigated and to the best of our knowledge these results have not been replicated or followed up on thus far. One reason TYR may not actually benefit every, if any, case of Parkinson's may be that the disorder is associated with reduced expression of the TH enzyme (Zhu, Zhang, & Zeng, 2012), which converts TYR into L-DOPA. Low TH activity would contribute to decreased DA synthesis. If little TYR is converted into L-DOPA, then any DA deficits may not be due to a *shortage* of TYR, but rather its reduced conversion. Therefore, supplementing Parkinson's patients with TYR may amount to providing them with an unnecessary surplus of DA precursor. Furthermore, TYR stimulates neurotransmitter production only in already active neurons (Fernstrom & Fernstrom, 2007; Lehnert et al., 1984), yet Parkinson's is associated with a loss of dopaminergic neurons, thereby reducing TYR's site of action. Thus, the benefits of TYR observed in the aforementioned studies may have been either chance findings or fortunate cases in which TH expression was still relatively high. These possibilities

underscore the need for considering factors such as reduced TH expression when investigating whether TYR supplementation can benefit Parkinson's patients or any other clinical population.

Lastly, TYR has also been investigated in relation to phenylketonuria, a disorder characterized by a shortage of TYR (Hanley et al., 2000). It sounds quite plausible TYR supplementation would benefit individuals suffering from this disorder, since supplementation is presumed to specifically remedy a shortage of TYR that limits neurotransmitter synthesis. However, several studies failed to find behavioral or cognitive improvements after administrating TYR to patients (e.g. Pietz et al., 1995; Smith et al., 1998) and indeed, simple TYR supplementation might not be recommendable for these patients (van Spronsen, van Rijn, Bekhof, Koch, & Smit, 2001). Many rationales exist for the relation between amino-acid supplementation and phenylketonuria (van Spronsen, de Groot, Hoeksma, Reijngoud, & van Rijn, 2010). One proposed reason why supplementation is ineffective for these patients is that their elevated phenylalanine levels compete too strongly with TYR for access through the blood-brain barrier (Kalsner, Rohr, Strauss, Korson, & Levy, 2001), thus preventing TYR from being converted into DA and NE. This possibility highlights the fact that compromised integrity of any step in neurotransmitter synthesis may lead to a counterintuitive effect of TYR, even when individuals have suboptimal catecholamine levels. Wherever possible, studies on TYR and clinical populations should keep in mind such limiting factors of TYR conversion.

In sum, using TYR to treat psychiatric and neurological disorders associated with suboptimal catecholamine levels is less promising and more complex than initially thought. The effect of TYR on clinical symptoms may critically depend on whether a shortage of TYR contributes to the pathology, as well as whether and how neurotransmitter synthesis is affected by the disorder.

Stressed Individuals

Stress induces increased catecholamine activity and turnover rates in the brain, leading to depletion of neurotransmitter levels as well as behavioral depression (Kvetnansky et al., 2009; Lehnert et al., 1984). However, studies that administered TYR to rats prior to stress exposure have shown neurotransmitter depletion and decrements in performance can be reversed (Lehnert et al., 1984; Harris R Lieberman, Georgelis, Maher, & Yeghiayan, 2005; Rauch & Lieberman, 1990; Shurtleff, Thomas, Ahlers, & Schrot, 1993; Yeghiayan, Luo, Shukitt-Hale, & Lieberman, 2001). These findings fueled numerous studies investigating whether and under which conditions TYR can also improve human performance during stress. These studies often focused on either physical exercise or cognitive functions.

Studies on TYR and physical exercise have primarily focused on endurance exercise, hypothesizing that maintaining adequate DA levels in the brain can support the motivation to keep on performing well. Two studies have examined TYR and endurance exercise without an additional overt stressor, but neither found any improvement following TYR administration (Chinevere, Sawyer, Creer, Conlee, & Parcell, 2002; Sutton, Coill, & Deuster, 2005). Other studies have looked at exercise performance during heat exposure, but the results were inconsistent. One study found TYR led to longer exercise times in the absence of an increase in ratings of perceived exertion and thermal sensation (Tumilty, Davison, Beckmann, & Thatcher, 2011), suggesting TYR enhanced endurance. However, these results were not replicated in two later studies (Tumilty, Davison, Beckmann, & Thatcher, 2014; Watson, Enever, Page, Stockwell, & Maughan, 2012). Therefore, there is no consistent evidence TYR improves physical exercise performance, e.g. endurance, during stress. In contrast, we propose physical performance *can* benefit from TYR supplementation, but only under specific conditions that activate catecholamine neurons and recruit higher cognitive functions such as attention and cognitive control. In other words, we speculate physical performance only benefits from TYR supplementation when it places high enough cognitive demands on the individual that induce catecholamine depletion. Studies

showing no effect of TYR might then have examined exercise that failed to sufficiently engage cognitive processes. This hypothesis is motivated by the numerous findings that TYR is effective at enhancing cognition during stress, as discussed below.

In remarkable contrast to the aforementioned studies, TYR consistently has an effect on cognitive performance when healthy humans consume TYR prior to stress exposure. In such studies hypothermia is often used as a stressor and TYR has been repeatedly shown to reverse the impairments in cognition it may cause (Mahoney et al., 2007; O'Brien, Mahoney, Tharion, Sils, & Castellani, 2007; Shurtleff, Thomas, Schrot, Kowalski, & Harford, 1994). These studies investigated the crucial cognitive aspect working memory, which involves actively maintaining and updating information in memory (Baddeley & Hitch, 1974; Miyake et al., 2000). It is unsurprising working memory is often investigated in relation to TYR, given this function is closely associated with DA level (Cools, Gibbs, Miyakawa, Jagust, & D'Esposito, 2008; Goldman-Rakic et al., 2000; Moustafa, Sherman, & Frank, 2008; Siessmeier et al., 2006) and changes in catecholamine levels due to TYR are therefore likely to alter working memory performance. All studies reported cold exposure reduced working memory performance, yet this decline was reversed when subjects were supplemented with TYR. Another study looking into TYR and exposure to cold was performed by Banderet and Lieberman (1989), who considered a wider range of cognitive functions. They reported improvements in, for example, vigilance and reaction times on several tasks. Interestingly, these authors limited their analysis to "those individuals most affected by exposure to the cold" (p. 760), suggesting TYR only benefits individuals who would otherwise have been stressed by the coldness. This possibility underscores the usefulness of taking into account individual differences when investigating the effects of TYR (Jongkees, Hommel, & Colzato, 2014). Furthermore, TYR has been beneficial for performance on the Stroop task and working memory while participants were exposed to an auditory stressor (Deijen & Orlebeke, 1994), as well as for working memory, reasoning, and vigilance during sleep deprivation (Magill et al., 2003). Lastly,

TYR has also been shown to improve both cognitive and behavioral performance in army cadets following an intensive combat training course. However, it is difficult to draw conclusions from this study, given that many other amino-acids were supplemented along with TYR and the placebo-group was not given a completely neutral placebo (Deijen, Wientjes, Vullinghs, Cloin, & Langefeld, 1999).

Lastly, some studies have investigated whether TYR can reverse stress-induced mood decrements. Palinkas et al. (2007) supplemented TYR to residents of Antarctica, with the aim to improve mood during prolonged cold climate exposure. The adverse environment of Antarctica can be considered a multi-stressor intervention involving confinement and isolation as well as the inherent cold. Interestingly, Palinkas et al. found TYR improved mood, but only during the winter. This study is one of few that examines TYR supplementation in healthy individuals in a long-term setting. The result is consistent with the finding by Banderet and Lieberman (1989) that TYR reversed cold-induced reductions in mood. In contrast, TYR has been found not to influence mood without stress exposure (Leathwood & Pollet, 1983; Lieberman, Corkin, Spring, Growdon, & Wurtman, 1983). These findings suggest TYR might be beneficial for mood, but only in stressful situations such as extreme cold.

Overall, the positive effects of TYR on cognitive performance are likely due to TYR preventing a decline of catecholamine availability during stress (Banderet & Lieberman, 1989; Kvetnansky et al., 2009; Lehnert et al., 1984), which prevents decrements in higher cognitive functions such as attention and working memory. Unfortunately, it is as of yet unclear whether these effects are attributable specifically to a modulation of DA, NE, or both. It is also still unknown what changes in cognition mediate the repletion of catecholamines and the reported improvements. For example, TYR's beneficial effect on working memory, found in many studies, might be due to TYR allowing maintenance of the optimal level of DA necessary for a balance between stable cognitive representations versus flexible updating of said

representations (Cools & D'Esposito, 2011). However, this remains speculative until further investigation.

Alternatively, one study by Kishore et al. (2013) suggests at least part of TYR's mechanism of action may be enhancing NE function. They showed TYR supplementation during heat exposure had a beneficial effect on the event related potential P300 and reaction times on a auditory stimuli discrimination task. The P300 may reflect phasic activity of NE in the locus coeruleus (LC-NE) system (Nieuwenhuis, Aston-Jones, & Cohen, 2005), indicating TYR's effect on the P300 may have occurred by facilitating the function of this LC-NE system. This is a plausible biological mechanism of the aforementioned positive effects of TYR during stress, particularly on attention and vigilance. However, it is important to keep in mind that any effect of TYR on NE is mediated by TYR's conversion into DA and therefore a role for DA in these improvements should not be excluded. Indeed, in the next section we will report evidence showing TYR also enhances functions thought to be strongly related to DA.

Cognitive Demands and Healthy Individuals

Lastly we review studies on TYR supplementation in healthy individuals who were not explicitly and overtly exposed to stressors that may impact their performance. Instead these studies all used rather challenging cognitive tasks, which were presumed to be demanding enough to induce a stress-like state leading to neurotransmitter depletion and reduced levels of performance, an effect TYR may counteract.

Consistent with the aforementioned studies on stress, two studies to date have shown TYR can enhance working memory performance, but in the absence of an overt exposure to stress. Instead, improvements were only found under particularly challenging conditions. Specifically, Thomas, Lockwood, Singh, & Deuster (1999) found TYR only promoted working memory when other tasks were performed simultaneously, a feat that would normally degrade performance. Colzato, Jongkees, Sellaro, & Hommel (2013) found TYR improved working memory even in single-task conditions, but only in the

task's more demanding condition that placed a stronger load on memory. These results confirm TYR can benefit cognitive performance and highlight the importance of a performance-degrading factor for TYR to counteract.

Aside from working memory, TYR has also been shown to selectively improve inhibitory control without influencing response execution (Colzato, Jongkees, et al., 2014). Furthermore, research has found TYR can promote cognitive flexibility as measured in a task-switching paradigm. In this study TYR presumably improved performance by facilitating conflict-resolving processes that may have otherwise induced a stressful state (Steenbergen, Sellaro, Hommel, et al., 2015). Recently, TYR has also been shown to promote performance on a convergent-thinking task, which is thought to rely on DA-driven cognitive control (Colzato, de Haan, & Hommel, 2014). This finding in particular points to the possibility some benefits of TYR may be mediated by enhanced DA function. None of the aforementioned studies reports an influence of TYR on mood, but this is unsurprising given that mood was not reduced even in the placebo conditions, so there was no decrement to counteract.

All in all these results suggest TYR can improve cognitive performance without overt exposure to stress, but only if performance would normally be degraded by high cognitive demands. This is likely because high cognitive demands induce a stress-like state leading to catecholamine depletion, the negative consequences of which can be counteracted by TYR.

Conclusion

As the biochemical precursor of DA and NE, TYR has the potential to enhance catecholamine function in the brain when situational demands are particularly high. TYR's general mechanism of action is presumably helping the brain keep up with the need for elevated rates of neurotransmitter synthesis. Unfortunately, the potential of TYR supplementation as a treatment for psychiatric disorders seems limited at best. This may be due to the fact that the suboptimal catecholamine levels in many disorders are due to an impairment of DA and/or NE synthesis and metabolism. For example, in Parkinson's

disease the enzyme that converts TYR into L-DOPA is less active. Such complications limit TYR's ability to promote optimal brain function (Zhu et al., 2012). In other words, disorders characterized by suboptimal DA and NE do not necessarily benefit from TYR supplementation. The potential of TYR for improving physical exercise during stress seems equally limited; perhaps because physical performance is not as directly linked to catecholamine levels in the brain as cognitive performance is. As such we hypothesize physical exercise may benefit from TYR only to the degree that it creates a stressful state in which cognitive control functions are challenged and neurotransmitter levels are depleted. By far the clearest picture arises from studies on TYR and cognitive performance. When healthy subjects are exposed to either external stressors or cognitively demanding situations, catecholamine levels in the brain decline and performance on cognitive tasks decreases accordingly. However, TYR supplementation seems to replete neurotransmitter levels in the brain and reverses the stress-induced degradation of a variety of cognitive functions. It is not yet known whether this improvement is because of enhancement of phasic NE firing in the locus coeruleus, which would benefit functions such as attention, or due to boosting DA function in striatal or prefrontal areas, which is closely associated with working memory function. In the end it may be both, or it may differ between different cognitive functions and specific tasks of interest.

In sum, the cognitive changes mediating performance improvements after TYR supplementation remain unknown and, unfortunately, most of the literature focuses on short rather than long-term settings. Nevertheless, based on this overview of the literature we conclude TYR is very promising as an enhancer of cognition and perhaps mood, but only when (healthy) individuals find themselves in stressful or cognitively demanding situations.

Chapter Four

People are different: tyrosine's modulating effect on cognitive control in healthy humans may depend on individual differences related to dopamine function

Jongkees, B. J., Hommel, B., & Colzato, L. S. (2014). People are different: tyrosine's modulating effect on cognitive control in healthy humans may depend on individual differences related to dopamine function. *Frontiers in Psychology, 5:1101.*

Introduction

The amino-acid tyrosine (TYR) is thought to modulate cognitive functions that are driven by dopamine (DA), as consumption of TYR enhances DA levels in the brain (Cuche et al., 1985; Gibson & Wurtman, 1977). It could therefore reverse decreases in DA level that are detrimental for cognitive performance (Goldman-Rakic et al., 2000; Muly et al., 1998; Nieoullon, 2002). So far, TYR has been considered not so much as an enhancer of healthy cognitive functioning but rather as a means to reduce the negative side-effects of dopamine-related pathologies, such as Parkinson's disease (Growdon et al., 1982; Lemoine et al., 1989), phenylketonuria (van Spronsen et al., 1996), depression (Gelenberg & Gibson, 1984), and attention deficit hyperactivity disorder (Wood et al., 1985). However, the outcomes were mixed: some patients reported significant improvements, while other did not. In clinical samples some variation in response may be explained by impaired processes such as DA synthesis, which would lessen or even completely prevent an effect of TYR. But even healthy samples differ in response to TYR supplementation, which suggests that clinically impaired DA function is not the only source of variation. In healthy individuals, TYR has often been used to reduce the negative effects of conditions that deplete the brain's dopaminergic resources, such as extreme stress. The supply of TYR was found to reduce stress-induced impairments of working memory and attentional tasks, but more so in individuals who were particularly sensitive to the stressors (Deijen & Orlebeke, 1994; Mahoney et al., 2007; Shurtleff et al., 1994). Even without exposure to stress, administration of TYR has been shown to have an acute beneficial effect on task-performance thought to be related to DA, e.g. simultaneously performing multiple tasks (Thomas et al., 1999), the updating and monitoring of working memory (Colzato, Jongkees, et al., 2013), and inhibitory control (Colzato, Jongkees, et al., 2014). Taken together, in healthy humans tyrosine seems to work against what has been coined "ego-depletion"—the exhaustion of limited cognitive control (CC) resources (Baumeister et al., 1998). Demanding tasks may deplete the available control resources more, especially in individuals having fewer resources and/or those

that suffer more from the situational demands, and tyrosine may be able to replete the missing resources to some degree. This possibility should not be surprising given that CC relies on DA (Cools, 2006). The hypotheses that DA is one of the depleted resources and TYR reverses its depletion are consistent with the idea that there is an optimal level of DA at which cognitive performance peaks while it suffers at lower levels (Goldman-Rakic et al., 2000; Muly et al., 1998; Nieoullon, 2002). Given that TYR raises the DA level, we argue that TYR can enhance cognitive performance in healthy individuals whenever one has a lower than optimal DA level. Although somewhat less straightforward, we argue that this should also be the case for clinical patients as long as impairments of DA function are held constant across samples.

Besides individual differences in the response to task-induced depletion, DA level also seems to vary between healthy individuals in a more stable and enduring fashion (Cools, Gibbs, et al., 2008; Cools, 2006; Cools et al., 2009). This suggests that individuals differ in how far away they are from their optimum, i.e. some individuals have a lot of room for improvement, while others may already have an optimal, or even a higher-than-optimal DA level. We expect that individuals with an optimal baseline have little left of the enzyme called tyrosine-hydroxylase, which converts TYR into DA (Daubner et al., 2011). This means that they have little risk of overdosing from TYR supplementation, instead they should experience hardly any change in performance.

Given that individuals can vary in their response to TYR supplementation, it is necessary that future studies on TYR take into account individual differences, so to ensure that samples are comparable and results are generalizable. To this end we discuss a number of DA-related measures and factors that could predict or modulate the effect of TYR supplementation. This is by no means an exhaustive list; the aim of this opinion article is rather to point out and highlight some accessible predictors of DA function that may help to improve designing future TYR studies and making the analyses of their outcomes more informative. To this date, the individual differences discussed below have not yet been investigated in combination with TYR. However,

based on literature that details their relation to DA function we argue that these individual differences will prove fruitful for future research.

Indicators and modulators of DA function

At present, DA can only be measured (relatively) directly using positron emission tomography (PET), which is rather expensive and invasive as it involves injecting a radioactive substance into the bloodstream (Volkow, Fowler, Wang, Baler, & Telang, 2009). Noninvasive and cheap alternatives to estimate DA function exist, and some can be found in our eyes. The amacrine and interplexiform cells of the retina contain a high concentration of DA (Bodis-Wollner & Tzelepi, 1998; Witkovsky, 2004), and disorders associated with DA dysfunction have been related to abnormal color discrimination (Hulka et al., 2013; Pieri et al., 2000; Tannock, Banaschewski, & Gold, 2006). It has been proposed that deficits in color vision, particularly blue-yellow impairment, indicate a central hypodopaminergic state (A. Roy, Roy, Berman, & Gonzalez, 2003). This proposition is consistent with the recent finding that color discrimination predicts cognitive control, with better discrimination being associated with more efficient conflict-resolution in an auditory Simon task (Colzato, Sellaro, et al., 2014). Given the relation between color vision and DA level, we argue that color vision can predict the effect of TYR supplementation. Particularly individuals with impaired color vision could benefit from TYR, as they are likely to have less DA than non-impaired peers.

Another interesting aspect of our eyes is the spontaneous eye blink rate (EBR), which has been found to reliably indicate the striatal DA level (Karson, 1983). Specifically, a higher EBR is associated with more striatal DA. As expected, disorders related to abnormal DA function show atypical EBRs: Parkinson's disease is associated with decreased DA levels, and correspondingly with decreased EBRs (Deuschel & Goddemeier, 1998), while schizophrenia is associated with increased DA levels and increased EBRs (Freed, 1980). Also, EBR has been successfully used to predict individual differences in cognitive performance (Colzato, Slagter, et al., 2008; Colzato, van den Wildenberg, et al., 2008, 2009; Colzato, van Wouwe, et al., 2007b;

Dreisbach et al., 2005). Given the relation between DA and EBR, it follows that EBR can predict the benefit of TYR supplementation. We suggest that individuals with a low EBR, indicative of a low DA level, stand to benefit most from TYR, as it will bring their DA level closer to the optimum that is associated with peaking performance (Goldman-Rakic et al., 2000; Muly et al., 1998). Of further interest, striatal DA is thought to be particularly involved in cognitive flexibility (Cools, 2006)—the ability to update and switch between mental representations (Miyake et al., 2000). Therefore, EBR might predict improvement in performance especially on tasks that require a flexible mind.

A third useful indicator of DA function is its metabolite homovanillic acid (HVA), which has been shown to relate to cognitive performance (Nagy et al., 2007), and is elevated in schizophrenia patients (Sumiyoshi et al., 2000) and reduced in individuals with ADHD (Gerra et al., 2007). Given that plasma levels of HVA indicate the DA level, HVA could be used to predict individual differences in response to TYR supplementation. Specifically, we expect individuals with a low HVA level, which indicates a low DA level, to benefit most from TYR.

Moving on to gene polymorphisms, the Val158Met-polymorphism in the catechol-O-methyltransferase (COMT) gene could also predict the effect of TYR supplementation, as this gene is involved in DA degradation in the prefrontal cortex. Specifically, the Met allele is associated with slower degradation of DA and high levels of prefrontal DA as the result, whereas the Val allele is associated with faster DA degradation and less prefrontal DA (Chen et al., 2004). As expected, several studies have shown Val-carriers to be less adept at cognitive tasks than Met-carriers (Egan et al., 2001; Goldberg & Weinberger, 2004; Mattay et al., 2003), which suggests that Val-carriers have lesser DA. This again implies that Val-carriers stand to benefit from TYR supplementation. Of further interest is the fact that prefrontal DA is tightly associated with cognitive stability (Cools, 2006), i.e. the ability to maintain task-relevant representations in the face of distractors or interference (Miyake et al., 2000). Given that the COMT gene modulates prefrontal DA level, its

polymorphism could predict improvement especially on tasks that require cognitive stability.

Another candidate for modulating the effect of TYR supplementation is the C957T polymorphism in the DRD2 gene, which is linked to messenger RNA stability (Duan et al., 2003), leading to variation in extrastriatal D2 receptor availability (Hirvonen, Lumme, et al., 2009) and striatal DA level (Hirvonen, Laakso, et al., 2009). Specifically, T-carriers have reduced messenger RNA stability, which results in less striatal DA than homozygotic C-carriers. Correspondingly, T-carriers show less inhibitory control (Colzato, van den Wildenberg, et al., 2013; Colzato, van den Wildenberg, Van der Does, & Hommel, 2010) and worse memory performance (Li et al., 2013). Also related to striatal DA is a second gene called DAT1, which is involved in DA reuptake (Lewis et al., 2001). Although there have been contradictory findings on how the varying number of base pair repeats in this gene relates to DA transporter (DAT) availability, recent studies suggest that 10-repeat homozygotes have lesser DAT availability, and consequently more striatal DA, than 9-repeat carriers (Shumay, Chen, Fowler, & Volkow, 2011; van de Giessen et al., 2009). This is consistent with the finding that 10-repeat carriers are better than 9-repeat carriers at updating stimulus-response bindings (Colzato, Zmigrod, & Hommel, 2013), which is thought to be driven by DA (Colzato, van Wouwe, et al., 2007a, 2007b). Given that both T-carriers of the DRD2 gene and 9-repeat carriers of the DAT1 gene are likely to have less striatal DA, we argue that especially they can benefit from TYR supplementation, again perhaps more so on tasks that require flexible cognition.

Interestingly, the aforementioned effect of the DRD2 polymorphism on inhibitory control and memory performance is stronger in older individuals than in younger ones (Colzato, van den Wildenberg, et al., 2013; S.-C. Li et al., 2013). This is consistent with the resource-modulation hypothesis (Lindenberger et al., 2008), which states that aging-related changes in neurophysiology enlarge the effect of polymorphisms on cognition. Given that DA systems deteriorate with old age (Bäckman et al., 2000, 2006; Erixon-

Lindroth et al., 2005; Volkow et al., 1998), it is important to take characteristics such as age into consideration when investigating the modulating effect of TYR on DA and cognitive functions.

Last but not least is the tyrosine-hydroxylase (TH) gene, which codes for the enzyme that converts TYR into L-DOPA (Daubner et al., 2011). The C-824T-polymorphism in the TH gene has been shown to influence urinary excretion of norepinephrine, with carriers of the T allele excreting more norepinephrine (Rao et al., 2007). Horiguchi et al. (2014) found that in patients with schizophrenia the T allele is associated with increased TH transcription activity and higher IQ. These authors proposed that the resulting increase in norepinephrine levels in the brain may have protected the patients from cognitive decline. Higher levels of norepinephrine suggest higher levels of DA as well, since DA is the precursor of norepinephrine (Buu & Kuchel, 1979). Therefore, these findings have two interesting implications for studies on TYR. First, the higher TH transcription activity associated with the T allele in the C-824T polymorphism could mean that low baseline DA individuals who carry this allele can benefit especially from TYR supplementation, as more TYR can be converted into DA. Second, the idea that the T allele protects against cognitive decline in schizophrenia could imply that it does so in old age as well. In that case, the effect of this polymorphism on TYR supplementation might be strongest in older individuals, as might be the case with the DRD2 gene. This possibility again underscores the need to keep age and age-related changes in neurophysiology in mind when investigating TYR.

Conclusion

The amino-acid TYR is a promising cognitive enhancer, yet studies on how TYR supplementation can benefit cognitive performance are still scarce. We suggest that future studies on TYR should take into account individual differences related to DA function, and to that end we have listed several indicators and modulators of DA that could predict the effect of TYR supplementation. It should be noted that these factors are unlikely to operate independently from each other. For example, individuals carrying the Val

allele of the COMT polymorphism, which indicates a low prefrontal DA level, could benefit especially from TYR when also carrying the T allele of the TH gene, since that would allow more conversion of TYR into DA. As such, interactions between the presently suggested modulators should be taken into account, to achieve a better understanding of their implications for the effect of TYR supplementation.

Chapter Five

Eating to stop: tyrosine supplementation enhances inhibitory control but not response execution

Colzato, L. S., Jongkees, B. J., van den Wildenberg, W. P. M., & Hommel, B. (2014). Eating to stop: tyrosine supplementation enhances inhibitory control but not response execution. *Neuropsychologia, 62*, 398-402.

Abstract

Animal studies and research in humans have shown that the supplementation of tyrosine, or tyrosine-containing diets, increase the plasma tyrosine and enhance brain dopamine (DA). However, the strategy of administering tyrosine (and the role of DA therein) to enhance cognition is unclear and heavily debated. We studied, in a healthy population, whether tyrosine supplementation improves stopping overt responses, a core cognitive-control function. In a double-blind, placebo-controlled, within-subject design, one hour following the administration of tyrosine (corresponding to the beginning of the 1h-peak of the plasma concentration) or placebo, participants performed a stop-signal task—which taps into response inhibition and response execution speed. Participants in the tyrosine condition were more efficient in inhibiting unwanted action tendencies but not in reacting to go signals. This is the first demonstration that the supplementation of tyrosine selectively targets, and reliably improves the ability to stop overt responses.

Introduction

Tyrosine is one of the most investigated amino acids, the building blocks of proteins. It is contained in food such as fish, soy, eggs, milk and bananas, and it is the precursor (the chemical that precedes another compound in the biochemical pathway) of the neurochemical dopamine (DA). Animal studies and research in humans have shown that the supplementation of tyrosine, or tyrosine-containing diets, increase the plasma tyrosine and enhance brain DA release, in particular from activated neurons (Acworth, During, & Wurtman, 1988; Deijen, 2005; During, Acworth, & Wurtman, 1988). Even though the neurobiology of tyrosine supplementation is not yet completely understood, this phenomenon does not seem to be subject to dose-dependent effects (Deijen & Orlebeke, 1994; Shurtleff et al., 1994). This indicates that the relation between tyrosine and cognitive performance does not follow the inverted U-shaped dose-effect curve that is typical for dopaminergic agonists (Cools, 2006). Once the optimal level is reached, higher levels of tyrosine will thus no longer increase DA levels, as the enzyme tyrosine hydroxylase, which converts tyrosine into DA, will be inhibited (Gibson & Wurtman, 1977). Therefore, even excessive levels of tyrosine administration are not expected to impair cognitive processes.

Previous literature has mainly focused on the supplementation of tyrosine to reverse conditions associated with dopaminergic-based pathologies, such as Parkinson's disease (Growdon et al., 1982; Lemoine et al., 1989), phenylketonuria (van Spronsen et al., 1996), depression (Gelenberg & Gibson, 1984; Gelenberg et al., 1990; Gelenberg, Wojcik, Gibson, & Wurtman, 1983) and attention deficit disorder (Reimherr et al., 1987; Wood et al., 1985). Furthermore, the role of tyrosine as "counteractor" has been largely investigated under conditions that cause brain DA depletion, such as stress. In humans, tyrosine has been shown to reverse stress-induced deficits in working memory and attentional tasks (Deijen & Orlebeke, 1994; Mahoney et al., 2007; Shurtleff et al., 1994). Only in one study tyrosine has been administered without exposure to stress, revealing beneficial effects, but only when performing more tasks at the same time (Thomas et al., 1999). This indicates

that tyrosine may reverse "ego-depletion" (Baumeister et al., 1998) (i.e. reduced self-control after a depleting task), but only when cognitive control is required. This should not be surprising given that executive control is considered to emerge from the interplay between the prefrontal cortex (PFC) and the striatum, which both are driven by DA (Cools, 2006)—the precursor of which is tyrosine.

The current study focused, for the first time, on the acute effect of tyrosine supplementation on the inhibition of behavioral responses—a key cognitive control function (Logan & Cowan, 1984; Logan, 1994) that is known to be modulated by DA. Indeed, inhibitory control is enhanced after the acute intake of d-amphetamine and cocaine, drugs that stimulate DA release (Fillmore, Rush, & Abroms, 2005; Fillmore et al., 2006). Along the same line, Colzato, van den Wildenberg, & Hommel (2007) reported response inhibition (assessed by means of the stop-signal task developed by Logan & Cowan, 1984) to be impaired in chronic recreational users of cocaine, who are likely to suffer from reduced dopamine D2 receptors in the striatum (Volkow, Fowler, & Wang, 1999). Participants pressed a left or right button as soon as a green left- or right-pointing arrow appeared (go trials). However, in some trials the color of the arrow suddenly changed to red, in which case the participants were supposed to refrain from responding (stop trials). This stop-signal task measures both the efficiency of response execution (by means of reaction time to go-signals) and the efficiency in inhibitory control (by means of the stop signal reaction time or SSRT, where longer SSRT reflect general slowing of inhibitory processes and indicate a lower level of inhibitory efficiency). Cocaine users needed significantly more time to inhibit responses to stop-signals than non-users.

Given the role of DA in modulating response inhibition, we expected the supplementation of tyrosine to enhance stopping control. Moreover, based on the ego-depletion hypothesis (Baumeister et al., 1998), we expected this effect to be limited to stopping overt responses without affecting response execution speed. Demanding tasks, such as stopping on time, may deplete the available control resources more than easy tasks, such as reacting to go signals.

Accordingly, we assumed tyrosine to be able to replete the missing resources when more control is needed to carry out the task, as in the case of inhibiting unwanted action tendencies.

Methods

Participants

Twenty-two healthy female adults (mean age = 20,4 years; mean Body Mass Index = 21,5) with no cardiac, hepatic, renal, neurological or psychiatric disorders, personal or family history of depression, migraine and medication or drug use participated in the experiment and served in two experimental sessions separated by 3–7 days. A double blind, placebo-controlled, randomized cross-over design with counterbalancing of the order of conditions was used to avoid expectancy effects. Placebo and L-Tyrosine dose corresponded to oral dose (powder) of 2.0 gr of microcrystalline cellulose (Sigma-Aldrich Co. LLC) and of 2.0 gr of tyrosine (supplied by Bulkpowders Ltd.) dissolved in 400 ml of orange juice. Following Markus, Firk, Gerhardt, Kloek, & Smolders (2008), women using contraception were tested when they actually used the contraception pill. On each experimental morning, participants arrived at the laboratory at 9:30 a.m. Participants had been instructed to fast overnight; only water or tea without sugar was permitted. In addition, subjects were not allowed to use any kind of drugs before and during the experiment or to drink alcohol the day before their participation and arrival at the laboratory. Written informed consent was obtained from all subjects; the protocol and the remuneration arrangements of 20 euro were approved by the local ethical committee (Leiden University, Institute for Psychological Research).

Apparatus and stimuli

The experiment was controlled by a ACPI uniprocessor PC running on an Intel Celeron 2.8 gHz processor, attached to a Philips 109B6 17 inch monitor (LightFrame 3, 96 dpi with a refresh rate of 120 Hz). Responses were made by pressing the “Z” or “?” of the QWERTY computer keyboard with the left and

right index finger, respectively. Participants were required to react quickly and accurately by pressing the left and right key in response to the direction of a left- or right-pointing green arrow (go trials) of about 3.5 X 2.0 cm with the corresponding index finger.

Stop-signal task

Each experimental session consisted of a 30-min session in which participants completed a version of the stop-signal task adopted from (Colzato, van den Wildenberg, et al., 2007, 2013, 2010). Arrows were presented pseudo-randomly for maximal 1500 ms, with the constraint that they signaled left- and right-hand responses equally often. Arrow presentation was response-terminated. Intervals between subsequent go signals varied randomly but equiprobably, from 1250 to 1750 ms in steps of 125 ms. During these interstimulus intervals, a white fixation point (3 mm in diameter) was presented. The green arrow changed to red on 25% of the trials, upon which the choice response had to be aborted (stop trials). A staircase-tracking procedure dynamically adjusted the delay between the onset of the go signal and the onset of the stop signal to control inhibition probability (Levitt, 1971). After a successfully inhibited stop trial, stop-signal delay in the next stop trial increased by 50 ms, whereas the stop-signal delay decreased by 50 ms in the next stop trial when the participant was unable to stop. This algorithm ensured that motor actions were successfully inhibited in about half of the stop trials, which yields accurate estimates of SSRT and compensates for differences in choice RT between participants (Band, van der Molen, & Logan, 2003). Individual SSRTs were calculated according to the *integration method* (see Logan & Cowan, 1984, see also Figure 1). The stop task consisted of five blocks of 104 trials each, the first of which served as a practice block to obtain stable performance.

Physiological and mood measurements

Heart rate (HR) and systolic and diastolic blood pressure (SBP and DPB) were measured from the non-dominant arm with a OSZ 3 Automatic Digital

Electronic Wrist Blood Pressure Monitor (Speidel and Keller). Mood was rated on a 9 × 9 Pleasure × Arousal grid (Russel, Weis, & Mendelsohn, 1989) with values ranging from –4 to 4.

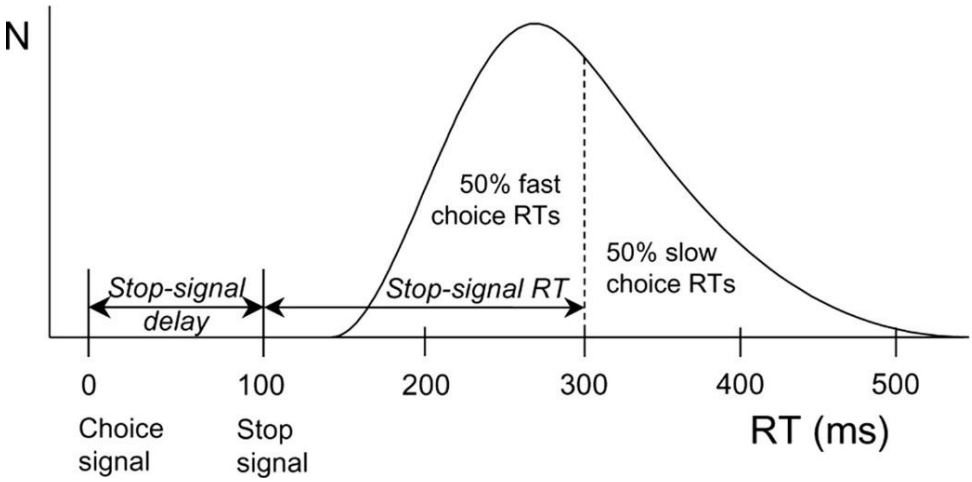

Figure 1. Calculation of stop-signal RT (SSRT) according to a race model. Following the race model assumption of independence (Logan & Cowan, 1984), the RT distribution of the go process is the same whether or not a stop signal is presented. The left side of the go RT distribution represents fast responses that escape inhibition. The right side represents slow responses that will be inhibited. If participants failed to stop on n% of the stop trials (here 50%), the finishing time of the stop process was on average equal to the nth percentile of the go RT distribution (here 300 ms). The mean stop signal delay (SSD, 100 ms) was then subtracted from the nth percentile of the go RT distribution, resulting in the estimate of the mean SSRT (200 ms).

Procedure and design

All participants were tested individually. Upon arrival, they were asked to rate their mood and HR, SBP and DPB were collected. One hour following the administration of tyrosine (corresponding to the beginning of the 1h-peak of the plasma concentration; Glaeser et al., 1979) or placebo, participants rated again their mood before having HR, SBP and DBP measured for the second

time. Next, participants were presented with the stop-signal task (Logan & Cowan, 1984). After the behavioral task, participants again rated their mood before having HR, SBP and DBP measured for the third time.

Statistical analysis

A significance level of $p<.05$ was adopted for all statistical tests.

Stop-signal task. Individual SSRTs for stop-signal trials and mean RT to go-signals were calculated to index response inhibition and response execution speed for all participants. Mean SSRTs and mean RT to go-signals were analyzed separately by means of repeated measure ANOVAs with condition (Placebo vs. Tyrosine) as within-subject factor. Additionally, to evaluate the robustness of our results, for both mean SSRTs and mean RTs to go-signals we calculated Bayesian information criteria (BIC) values to estimate a Bayes factor and generate the posterior probability associated with the occurrence of the null (H_0) and alternative (H_1) hypotheses, given the observed data (Masson, 2011; Wagenmakers, 2007). This method allows making inferences about both significant and nonsignificant effects by providing the exact probability of their occurrence. Furthermore, to investigate the effect of tyrosine supplementation on post-error slowing, we computed RTs for Go trials that immediately followed a stop-signal trial. More specifically, after having taken into account trial sequence, we split stop-signal trials according to inhibition success by comparing post-stop trial adjustments immediately after a successful stop trial vs. after a failed stop trial. That is, post-stop trials were sorted into mapping repetitions (a stop trial with an arrow pointing to the left is followed by a go trial with an arrow pointing also to the left) vs. alternations (a stop trial with an arrow pointing to the left is followed by a go trial with an arrow pointing to the right). This way we were able to test the effect of stopping success (successful stop vs. failed stop) and arrow repetition (repetition vs. alternation) on RT on the subsequent Go trial.

Physiological and mood measurements. Mood, HR, BPS and BPD were analyzed separately by means of repeated-measures ANOVAs with condition (Placebo vs. Tyrosine) and effect of time (first vs. second vs. third measurement) as within-subjects factor.

Results

Stop-signal task. All participants were able to stop their responses on stop-signal trials successfully in about half of the time a stop signal instructed them to do so (51.8% in the placebo and 51.6% in the tyrosine condition), indicating that the dynamic tracking algorithm worked well in both conditions. According to the race model that predicts inhibitory success, the stop process and the go process should run independently (Logan & Cowan, 1984). The race model predicts that the RT derived from stop trials that escaped inhibition (failed-stop RT) is shorter than the mean Go RT. This prediction was confirmed for both the Tyrosine Condition and the Placebo condition. On average, failed-stop RT was about 43 ms shorter than mean Go RT, $F(1, 21) = 128.11$, $p < .001$, MSE = 306.77, $\eta 2p = 0.859$. The percentage of choice errors to go-signals was low and did not discriminate between placebo (1.1%) and tyrosine condition (1.2%). Most importantly, SSRTs were significantly longer in the placebo (228 ms) than in the tyrosine condition (214 ms), $F(1,21)=5.83$, $p < 0.05$, MSE = 456.57, $\eta^2 p = 0.217$, see Figure 2. The Bayesian probability associated with H_1 was .76 which, on the basis of the guidelines proposed by Raftery (1995), represents positive evidence in favor of H_1 (the same probability of H_0 was complementary, i.e., .24). Analyses of mean RT to go-signals showed that participants did not react faster in the placebo (397 ms) than in the tyrosine condition (401 ms), $F < 1$. Bayesian analysis revealed that, based on our data, the posterior probability of H_0 was .81, which represents positive evidence for H_0 (cf. Raftery, 1995). This is consistent with our expectation that tyrosine supplementation would enhance response inhibition while leaving performance relating to response execution unaffected.

This same pattern of results was obtained after controlling for the order in which sessions were administered, $F(1,20)=4.58$, $p < 0.045$, MSE = 304.607,

$\eta 2p = 0.186$ (SSRTs), and was confirmed by additional analyses run separately for the Go RTs and the SSRTs when controlling for the analogous effect on the complementary measure, calculated as the difference in performance between Placebo and Tyrosine sessions: $F<1$ (Go RTs), and $F(1,20)=5.39$, $p < 0.05$, MSE = 367.124, $\eta 2p = 0.212$ (SSRTs).

To investigate the effect of tyrosine on response inhibition more thoroughly, for both Placebo and Tyrosine sessions we estimated the entire distribution of SSRTs– a procedure that has been found to provide a more detailed description of the differences between two experimental conditions or groups (Heathcote, Popiel, & Mewhort, 1991; Matzke & Wagenmakers, 2009). To this end we used the Bayesian parametric approach (BPA) developed by Matzke, Dolan, Logan, Brown, & Wagenmakers (2013), which assumes that SSRTs are ex-Gaussian distributed and uses Markov chain Monte Carlo sampling (MCMC; e.g., Gamerman & Lopes, 2006) to obtain posterior distributions for SSRT parameters. In fitting the ex-Gaussian distribution (the convolution of normal and exponential functions), three parameters representing different parts of the curve are obtained: mu (μ) and sigma (σ) corresponding to the mean and standard deviation of the normal component, respectively, and tau (τ), corresponding to both the mean and standard deviation of the exponential component—thus representing the positive skew of the distribution. The BPA was implemented using the BEESTs (Bayesian Ex-Gaussian Estimation of Stop-Signal RT distributions) software developed by Matzke, Dolan, et al. (2013), see also Matzke, Love, et al., (2013) for details on the procedure. The BPA was applied to hierarchical stop-signal data after having removed outliers (RT slower and faster than two standard deviations from a participant's mean). For the MCMC sampling, the following values were specified in the input arguments of the software: number of chains = 3; samples = 36,000; burn-in = 12,000; thinning = 12; predictions = 1000. Results revealed that the difference between Placebo and Tyrosine sessions was mainly captured by the μ component of the ex-Gaussian distribution (see Table 1), thus suggesting that tyrosine influences the latency but not the variability of the SSRTs.

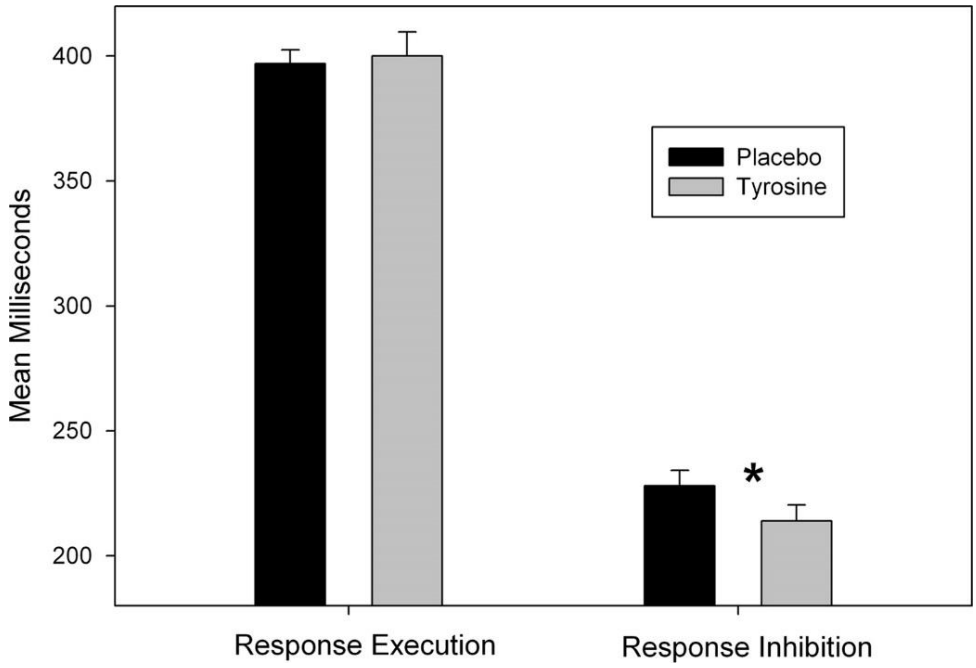

Figure 2. Mean SSRT (response inhibition) and Mean Go RT (response execution speed) as a function of condition (Placebo vs. Tyrosine). Asterisk indicates significant (* p<.05) effect of tyrosine on mean SSRT. Vertical capped lines atop bars indicate standard error of the mean.

Table 1. Summary statistics of the posterior SSRTs distribution of the group-level mean and standard deviation (SD) parameters for the Placebo and Tyrosine sessions.

SSRT distribution	**Placebo**	**Tyrosine**
Parameters estimation		
μ	190	179
SD(μ)	20.6	19.5
σ	22	21
SD(σ)	45.5	45.5
τ	26	31
SD(τ)	54.6	45.1

Post-error slowing. The only significant effect obtained was a main effect of Mapping; Go RT immediately following a stop trial with a repeating arrow was longer compared to trials with alternating arrows, 445 vs. 415 ms, $F(1, 21) = 48.50$, $p < .001$, MSE = 805.34, $\eta 2p = 0.698$. Go RT adjustments did not

depend on stopping success, F < 1, and no significant effects of Tyrosine were obtained on post-stop adjustments (Fs < 1).

Physiological and mood measurements. ANOVAs revealed that HR (74 vs. 71 vs. 67 and 75 vs. 70 vs. 65 after placebo and tyrosine, respectively), BPD (70 vs. 69 vs. 67 and 69 vs. 68 vs. 68 after placebo and tyrosine), BPS (111 vs. 111 vs. 110 and 115 vs. 113 vs. 109 after placebo and tyrosine), and mood (1.1 vs. 1.5 vs. 1.0 and 1.4 vs. 1.3 vs. 0.9 after placebo and tyrosine) did not significantly change after the intake of tyrosine, *F's*<1.

Conclusions

This study tested, for the first time, whether the supplementation of tyrosine, the precursor of DA, is associated with a detectable selective enhancement in response inhibition. As expected, in the tyrosine condition participants were more efficient in inhibiting unwanted action tendencies than in the placebo condition while response execution was unaffected.

Our results fit with the idea that, in healthy humans, tyrosine works against the phenomenon of "ego-depletion"—the exhaustion of limited cognitive control resources (Baumeister et al., 1998). Demanding tasks, such as stopping on time, may deplete the available control resources more than easy tasks, such as reacting to go signals. Accordingly, tyrosine may be able to replete the missing resources when more control is needed to carry out the task.

Our results are also consistent with the idea that DA plays a key role in stopping overt responses. Indeed, a number of patient studies have provided converging evidence for the involvement of DA in response inhibition. Compared to healthy controls, Parkinson's patients, who suffer from loss of dopaminergic cells in the basal ganglia, show difficulties in inhibiting unwanted action tendencies (Gauggel, Rieger, & Feghoff, 2004; Wylie, Ridderinkhof, Bashore, & van den Wildenberg, 2010). In line with this picture, Colzato, van den Wildenberg, et al. (2013, 2010) reported response inhibition to be predicted by the C957T polymorphism at the DRD2 gene in a young and in aging populations. In contrast, COMT Val58/108Met polymorphism seems

to have little if any association with cognitive function (Barnett, Scoriels, & Munafo, 2008, for a recent review). Moreover, very recently, Ghahremani et al. (2012) have found that striatal dopamine D2/D3 receptor availability was negatively correlated with SSRTs and positively correlated with inhibition-related fMRI activation in frontostriatal neural circuitry. Most importantly, correlations involving D2/D3 receptor availability were more robust in the dorsal regions (caudate and putamen) of the striatum, in line with previous findings of striatal activation accompanying stopping (Aron & Poldrack, 2006; Vink et al., 2005; Zandbelt & Vink, 2010). Finally, ADHD patients (see Alderson, Rapport, & Kofler, 2007, for a recent review) and recreational users of cocaine (Colzato, van den Wildenberg, et al., 2007), who are likely to suffer from reduced dopamine D2 receptors in the striatum (Volkow et al., 1999), need significantly more time to inhibit responses to stop-signals than non-users.

The present findings raise the question whether tyrosine supplementation might also enhance other cognitive control functions, such as the "shifting" between tasks and mental sets (also called "flexibility"), and the "updating" (and monitoring of) working memory (WM) representations (Miyake et al., 2000). Moreover, it would be very useful to explore the direct effect of prolonged use of tyrosine supplementation on the brain. It remains to be demonstrated, for instance, that tyrosine use produces long-term changes at the neuromodulatory (enhanced functioning of DAD2 receptors) and at functional level (in PFC and striatum) proportionally to the degree of behavioural performance enhancements.

Future research needs also to take individual differences into account. There is ample evidence suggesting a considerable role for individual differences with respect to the efficiency of cognitive control processes and the neurotransmitter systems driving them (Cools, 2006). Furthermore, in healthy humans tyrosine has been shown to reverse stress-induced deficits in working memory and attentional tasks, but in particular in individuals who were most affected by the stressors (Deijen & Orlebeke, 1994; Mahoney et al., 2007; Shurtleff et al., 1994)—suggesting individual differences in the reactivity to

tyrosine. It makes sense to assume that preexisting neuro-developmental factors (such as genetic variability related to levels of the neurotransmitter systems) affect the degree to which individuals can benefit from tyrosine supplementation, especially because many of them are arguably tapping into cognitive control processes.

Taken altogether, our results support the materialist approach that "you are what you eat" (Feuerbach, 1862/1960)—the idea that the food one eats has a bearing on one's state of mind. The food we intake may thus act as a cognitive enhancer that modulates the way we think, perceive and react to the physical world. In particular, the supplementation of tyrosine, or tyrosine-containing diets, may promote cognitive enhancement in inexpensive, efficient, and healthy ways.

Chapter Six

L-tyrosine administration modulates the effect of transcranial direct current stimulation on working memory in healthy humans

Jongkees, B. J., Sellaro, R., Beste, C., Nitsche, M. A., Kühn, S., & Colzato, L. S. (2017). L-Tyrosine administration modulates the effect of transcranial direct current stimulation on working memory in healthy humans. *Cortex, 90*, 103-114.

Abstract

Transcranial direct current stimulation (tDCS) is an increasingly popular method of modulating cognitive functions in humans. However, some doubt its efficacy as findings are inconsistent or remain unreplicated. It is speculated dopamine (DA) might play an important role in this inconsistency, by determining the direction and strength of the cognitive-behavioural effects of tDCS. However, so far evidence for this hypothesis has been correlational in nature, precluding definitive conclusions. The present proof-of-principle study aimed at investigating a potentially causal role for DA in the effect of tDCS on cognition in healthy humans. In experiment 1 we aimed to replicate previous findings showing administration of DA's precursor L-Tyrosine (Tyr), presumably by inducing a modest increase in DA level, can enhance working memory performance as assessed with a verbal *N*-back task. In experiment 2 we investigated the effect of Tyr administration on bilateral tDCS over dorsolateral prefrontal cortex (DLPFC) and working memory. Experiment 1 showed Tyr administration enhances performance in a verbal *N*-back task. Experiment 2 showed Tyr modulates the effect of bilateral tDCS over DLPFC on working memory. Specifically, tDCS had opposite effects on performance depending on current direction through the brain and Tyr administration. The present study provides two major findings. First, we replicate Tyr's beneficial effect on verbal working memory. Second, our results indicate a causal role for DA in the effect of tDCS on cognition. For this reason, we encourage future studies to consider the modulating effect of DA, as a step towards more consistent and replicable results regarding the efficacy of tDCS.

Introduction

Transcranial direct current stimulation (tDCS) is an increasingly popular, non-invasive method for modulating cognitive functions in healthy individuals and psychiatric patients (Plewnia, Schroeder, & Wolkenstein, 2015). tDCS induces a polarity-dependent shift in the resting membrane potential of cortical neurons, altering their likelihood of firing (Nitsche & Paulus, 2000) and longer stimulation results in neuroplastic after-effects (Nitsche & Paulus, 2001; Nitsche, Nitsche, et al., 2003). However, some still doubt the efficacy of tDCS in enhancing cognitive-behavioral performance, as many findings have so far not been subject of replication, and the effects of tDCS have some variability (Horvath, Forte, & Carter, 2015a, 2015b), but see (Antal, Keeser, Priori, Padberg, & Nitsche, 2015). Although much variation in results could be due to methodological differences between studies (e.g. stimulation duration, electrode placement) (Plewnia et al., 2015), another source of variance might stem from dopamine (DA) function (Li, Uehara, & Hanakawa, 2015). This idea is corroborated by the finding that tDCS differentially affects individuals carrying certain DA-related genetic polymorphisms (Nieratschker, Kiefer, Giel, Krüger, & Plewnia, 2015; Plewnia et al., 2013). However, given the inherently correlational nature of genetic studies, it remains unconfirmed whether DA plays a causal role in modulating the cognitive-behavioral effects of tDCS. Previous studies investigating the effect of DA manipulations on tDCS focused only on the electrophysiological effects of tDCS in the motor cortex (Fresnoza, Paulus, Nitsche, & Kuo, 2014; Fresnoza, Stiksrud, et al., 2014; Kuo, Paulus, & Nitsche, 2008; Monte-Silva et al., 2009; Monte-Silva, Liebetanz, Grundey, Paulus, & Nitsche, 2010; Nitsche et al., 2006; Nitsche, Kuo, Grosch, et al., 2009). In contrast, no studies have investigated the effect of DA manipulations on the cognitive-behavioral effects of tDCS. Hence, it remains unclear whether DA can indeed contribute to variability of results obtained with prefrontal tDCS.

Although available evidence suggests DA modulates the physiological and behavioral effects of tDCS, it remains speculative why this is the case. One reason DA might interact with the acute effects of online tDCS (i.e. stimulation

coinciding with task performance) might be because both tDCS and DA target resting membrane potentials. Anodal (excitatory) and cathodal (inhibitory) stimulation increase and decrease resting membrane potentials respectively (Nitsche & Paulus, 2000), whereas DA enhances and reduces firing of neurons with high and low membrane potentials, respectively (Frank, 2005; Hernández-López et al., 1997). As such, DA is known to modulate task-induced cortical activity (Egan et al., 2001; Mattay et al., 2003) and, in turn, task-induced activity has been identified as a possible determinant of tDCS effects (Antal, Terney, Poreisz, & Paulus, 2007; Bortoletto, Pellicciari, Rodella, & Miniussi, 2015). This suggests changes in background and task-dependent neural firing might mediate an effect of DA on online tDCS. On the other hand, DA might modulate the after-effects of tDCS on cortical excitability by also acting on N-methyl-d-aspartate (NMDA) receptors. These receptors mediate neuroplasticity via long-term potentiation (LTP) and depression (LTD) (Lüscher & Malenka, 2012) and are thought to underlie the neuroplastic after-effects of tDCS (Liebetanz, Nitsche, Tergau, & Paulus, 2002; Nitsche et al., 2004; Nitsche, Fricke, et al., 2003). Previous animal studies show DA can facilitate the induction and consolidation of LTP and LTD, but results have varied for different receptor subtypes (Gurden, Takita, & Jay, 2000; Huang, Simpson, Kellendonk, & Kandel, 2004; Spencer & Murphy, 2000). In line with these findings, Nitsche et al. (2006) reported that pharmacologically blocking D2 receptors nearly abolished the after-effects of tDCS on cortical excitability, whereas activation of D2, and to a lesser-extent D1, prolonged these effects. Additionally, Kuo et al. (2008) demonstrated L-dopa administration turned an excitability enhancement due to anodal tDCS into a diminishment, whereas the inhibitory after-effects of cathodal tDCS were prolonged. Taken together these findings establish a role for DA in the acute and long-term physiological effects of tDCS, but so far studies investigating if and how this translates to cognition and behavior remain scarce.

Hence, in the present study we set out to clarify the (potentially causal) relation between DA and the cognitive-behavioral effects of prefrontal tDCS.

To this end we investigated whether a modest increase in DA in healthy individuals modulates the effect of tDCS on (verbal) working memory (WM), a core cognitive function (Miyake et al., 2000) often investigated in relation to tDCS. Slight increases in DA level can be achieved by administration of DA's biochemical precursor l-tyrosine (Tyr), which can enhance a variety of DA-related cognitive functions in humans (Jongkees, Hommel, Kühn, & Colzato, 2015). Thus we reasoned if a simple DA manipulation such as Tyr administration would modulate the effect of tDCS on WM, then this would provide first tentative support for a causal role of DA in the cognitive-behavioral effects of tDCS. Following earlier studies on a DA manipulation on tDCS (Kuo et al., 2008; Nitsche et al., 2006), we have restricted our investigation to the after-effects of tDCS.

We applied bilateral tDCS over the dorsolateral prefrontal cortex (DLPFC), a region strongly implicated in WM (Curtis & D'Esposito, 2003) and often targeted with tDCS. Many studies show WM improvements with tDCS by applying anodal stimulation over the left DLPFC, with the cathodal return electrode being placed over the contralateral orbital region (Fregni et al., 2005; Hoy et al., 2013; Jeon & Han, 2012; Mulquiney, Hoy, Daskalakis, & Fitzgerald, 2011; Ohn et al., 2008; Oliveira et al., 2013; Teo, Hoy, Daskalakis, & Fitzgerald, 2011) or symmetrically over the right DLPFC (Oliveira et al., 2013). Stimulation with the opposite montage, i.e. with the cathode over left DLPFC, is less-investigated but may impair performance (Marshall, Mölle, Siebner, & Born, 2005), although this result was obtained with intermittent (15s on/off) rather than the more common continuous stimulation. Given this pattern of results we hypothesized that, after placebo intake, individuals receiving anodal over left, cathodal over right (AL-CR) stimulation would show higher WM performance than those receiving cathodal over left, anodal over right (CL-AR) stimulation. Given the hypothesized causal role of DA in the effects of tDCS, we expected a modulation of this pattern of results after administration of Tyr.

In short, we aimed to provide first tentative evidence supporting a causal role for DA in the cognitive-behavioral effects of tDCS by investigating

whether administration of Tyr modulates the effects of two tDCS montages on WM as assessed in a verbal *N*-back task.

Methods

Overview

We performed two separate experiments. To support the notion Tyr modestly enhances WM performance, in experiment 1 we aimed to replicate previous findings showing beneficial effects of Tyr administration on WM performance (Colzato, Jongkees, et al., 2013) in a double-blind between-subjects design. Participants consumed either 2.0 g of Tyr or placebo and 1 h later, when plasma Tyr levels start to peak (Glaeser et al., 1979), their WM performance was tested on a verbal *N*-back task. In experiment 2 we assessed the interaction between two tDCS montages (AL-CR vs. CL-AR) and administration of Tyr or placebo in a single-blind, between-subjects design. As in experiment 1, participants consumed either 2.0 g of Tyr or placebo. 1 h later they received 15 min of either AL-CR or CL-AR stimulation over bilateral DLPFC and subsequently their WM performance was tested using the same *N*-back task as in experiment 1. The studies conformed to the ethical standards of the declaration of Helsinki, the protocols were approved by the local ethical committee (Leiden University, Institute for Psychological Research), and volunteers signed an informed consent form before participation.

Experiment 1

Participants

36 students were recruited from Leiden University and randomly assigned to one of two groups: placebo or Tyr. Each group consisted of 18 participants. The two groups were comparable with respect to age, $F(1, 34) = 3.42$, $p = .073$, weight, $F(1, 34) = .44$, $p = .513$, body-mass index (BMI), $F(1, 34) = .287$, $p = .595$, and identical with respect to gender distribution. Group demographics are shown in Table 1. Participants were screened individually using the Mini International Neuropsychiatric Interview (M.I.N.I.) (Sheehan et al., 1998). The M.I.N.I. is a well-established brief diagnostic tool in clinical, drug, and stress

research that screens for several psychiatric disorders and drug use (Colzato & Hommel, 2008; Colzato, Kool, & Hommel, 2008; Sheehan et al., 1998). As such, all participants were screened for physical and mental health problems. Individuals with recent or regular drug use were excluded from participation. One exception to this was hormonal contraceptive medication, which all female participants had to be using regularly to limit fluctuations in hormone levels associated with the menstruation cycle as these can influence DA function and thereby confound results related to DA (Colzato & Hommel, 2014; Czoty et al., 2009; E. Jacobs & Esposito, 2011). The specific type or brand of contraception was not recorded. None of the participants reported any health problems.

Table 1. Group demographics for Experiment 1 and 2.

	Experiment 1				Experiment 2							
					AL-CR				CL-AR			
	Placebo		Tyrosine		Placebo		Tyrosine		Placebo		Tyrosine	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (years)	20.8	1.9	22.2	2.4	20.8	2.0	19.7	1.7	20.7	2.3	20.9	1.4
Weight (kg)	62.7	7.6	64.1	4.7	63.2	6.1	62.5	8.1	65.8	9.3	68.5	9.7
BMI (kg/length2)	21.6	2.3	22.0	2.1	22.3	2.5	21.5	2.1	22.6	2.3	22.7	2.4
Female/male ratio	17/1		17/1		15/3		16/2		15/3		15/3	

Task design

The *N*-back task is conceptualized to assess WM performance (Kane, Conway, Miura, & Colflesh, 2007) and, indeed, is often used in tDCS studies investigating WM (Au et al., 2016; Fregni et al., 2005; Hoy et al., 2013; Mylius et al., 2012; Ohn et al., 2008; Oliveira et al., 2013; Teo et al., 2011; Zaehle, Sandmann, Thorne, Jäncke, & Herrmann, 2011). The present study used a letter-based, i.e. verbal version of the task (Colzato, Jongkees, et al., 2013). A 1-back condition might be too easy to find positive effects of Tyr (Colzato, Jongkees, et al., 2013) and previous studies on tDCS and the *N*-back task have primarily used 2-back and 3-back conditions. Hence to keep the experiments comparable we included both 2-back and 3-back conditions in experiment 1 and 2. The 2-back condition was always presented first.

Stimuli were presented in the middle of a computer screen with a refresh rate of 60 Hz and a 800 x 600 resolution using E-Prime 2.0 software. Participants were comfortably seated approximately 50 cm from the screen while wearing headphones. Responses were given using the 'z' and 'm' buttons of a QWERTY keyboard. Mapping of response buttons to target (i.e. repetition) and non-target (i.e. non-repetition) was counterbalanced across participants in each group. After an incorrect or belated response (latency longer than 1000 ms) a brief tone was presented to signal a mistake. Both the 2-back and the 3-back conditions consisted of two blocks of 51 + n trials. For example, a 2-back block consisted of 53 trials. Regardless of the current load condition, each block comprised 21 targets and 30 non-targets. All participants performed the 2-back condition first and then the 3-back condition, and each n-back condition was preceded by 17+n practice trials (7 targets and 10 targets).

Aside from parameters such as hit rates and correct rejections, we were mainly interested in target sensitivity, indexed by d' prime derived from signal detection theory (Swets, Tanner, & Birdsall, 1961). This informative measure combines hit and false alarm rates and thus provides an index of the ability to discriminate targets from non-targets, with higher scores signaling selective, correct reporting of targets, and thus better WM performance. Hence we

expected higher d' prime scores after Tyr administration. d' prime was calculated, and perfect scores were corrected for, as described earlier (Colzato, Jongkees, et al., 2013). One participant had a perfect hit rate and another had zero false alarms.

Tyrosine administration

To induce a moderate increase in DA we administered DA's precursor Tyr. Upon consumption Tyr is converted into L-dopa, which is subsequently converted into DA. Consistent with the hypothesis that Tyr administration increases DA, animal studies showed increased levels of prefrontal DA and homovanillic acid (HVA), the main metabolite of DA, after Tyr intake (Tam et al., 1990; Tam & Roth, 1997). Although in vivo, direct assessment of DA in humans is difficult, Tyr administration has been shown to significantly elevate levels of HVA in the spinal fluid of Parkinson's patients, suggesting an increase in DA (Growdon et al., 1982).

Previous studies showed doses of Tyr as low as 2.0 g have positive effects on WM performance as measured using the *N*-back task (Colzato, Jongkees, et al., 2013) and a variety of other cognitive functions (for a review, see Jongkees et al., 2015), suggesting this dose is sufficient for inducing a modest but functionally relevant increase in DA level. With the aim of replicating these findings, we administered 2.0 g of Tyr in the present study. The neutral substance microcrystalline cellulose was used as placebo (Thomas et al., 1999). Tyr or placebo was dissolved in 400 mL of orange juice.

As we did not adjust the dosage of Tyr to the individual participant's weight and BMI, this might have led to variation in response to the administration due to different substance concentration levels. To control for this source of variance we included BMI as covariate in our analyses.

Procedure

Participants came to the lab in the morning, having fasted since 10 o'clock in the evening prior to participation (Cuche et al., 1985; Glaeser et al., 1979). Informed consent was obtained and BMI was measured. Subsequently

participants consumed 2.0 g of Tyr or placebo dissolved in 400 mL orange juice. Afterwards they were offered apples and oranges, which contain negligible amounts of Tyr, to prevent strong hunger. 1 h after finishing the juice participants started the *N*-back task, which took approximately 20 min. Lastly, participants were debriefed and compensated for their participation with course credit or €10.

Statistical analysis

To assess the effect of Tyr intake on WM performance we conducted repeated measures ANCOVA with administration (placebo vs. Tyr) as between-subjects factor, WM load (2-back vs. 3-back condition) as within-subjects factor, BMI as covariate, and d' prime as the dependent measure. Similar analyses were performed using hit, false alarm, correct rejection, and miss rates, and reaction times (RT) as dependent measures.

Experiment 2

Participants

72 right-handed students were recruited from Leiden University. Participants were randomly assigned to one of four groups: AL-CR stimulation plus placebo, AL-CR stimulation plus Tyr, CL-AR stimulation plus placebo, or CL-AR stimulation plus Tyr. Each group consisted of 18 participants, which is comparable with—if not more than—previous tDCS studies on WM (Fregni et al., 2005; Hoy et al., 2013; Jeon & Han, 2012; Marshall et al., 2005; Mulquiney et al., 2011; Mylius et al., 2012; Ohn et al., 2008; Teo et al., 2011) and previous studies investigating the effect of a DA manipulation on the electrophysiological effects of tDCS (Fresnoza, Paulus, et al., 2014; Fresnoza, Stiksrud, et al., 2014; Kuo et al., 2008; Monte-Silva et al., 2009, 2010; Nitsche et al., 2006; Nitsche, Kuo, Grosch, et al., 2009). The four groups were comparable with respect to age, $F(3, 68) = 1.59$, $p = .201$, weight, $F(3, 68) = 1.89$, $p = .140$, BMI, $F(3, 68) = 1.00$, $p = .400$, and gender distribution, $X^2(3) = .32$, $p = .956$. Group demographics are shown in Table 1. Participants were again selected using the M.I.N.I (Sheehan et al., 1998) and underwent the same

health screening as described in experiment 1. Additionally, individuals with implants such as pacemakers, any kind of metal in their body, or skin conditions were excluded for safety reasons concerning the tDCS. None of the participants reported any health problems.

Task design

The same letter-based, verbal *N*-back task used in experiment 1 was used to assess WM performance. In line with our hypotheses in the Introduction, after placebo intake, we expected higher d' prime scores when stimulating with a typically performance-enhancing AL-CR, as compared to a typically impairing CL-AR montage. Given the hypothesized role of DA in the effects of tDCS, we expected these results to be modulated by Tyr.

One participant in the CL-AR plus Tyr group achieved a perfect hit rate in the 2-back condition and hence this score was corrected (Colzato, Jongkees, et al., 2013). No participant achieved zero false alarm rates.

Transcranial direct current stimulation

Two electrodes of 35 cm^2 (5 cm x 7 cm) were placed over DLPFC in a bilateral bipolar-balanced montage (Nasseri, Nitsche, & Ekhtiari, 2015), i.e. in symmetrical positions. Although many tDCS studies on WM have previously placed the return electrode over the contralateral supraorbital region, we opted for symmetrical positioning of electrodes to avoid uncertainty over where in the supraorbital region the electrode was placed. Additionally, and more importantly, this positioning served to maximize the likelihood current direction through the brain would be comparable across participants, thus reducing potential variability in response to the stimulation. For each individual participant the DLPFC was located using the international 10/20 system for placing electrodes on the scalp (Jasper, 1958). Accordingly, for the AL-CR montage the anode and cathode were placed over F3 and F4, respectively. For the CL-AR montage this placement was reversed.

Stimulation consisted of a current of 1000 μA delivered by a DC Brain Stimulator Plus (NeuroConn, Ilmenau, Germany), a device complying with the

Medical Device Directive of the European Union (CE-certified). The current was built up during a fade-in of 10 s, after which stimulation lasted for precisely 15 min and then ended with a 10 s fade-out. Impedance was below 15 kΩ throughout the stimulation. The after-effects of 15 min of tDCS typically last 1 h (Nitsche et al., 2008). We assessed WM performance off-line, that is after stimulation had finished. This mirrors the design of previous studies investigating the effect of DA manipulations on tDCS-induced cortical excitability. For example, Nitsche et al (2006) and Kuo et al. (2008) administered DA agents to participants, then stimulated the motor cortex and afterwards measured cortical excitability. Additionally, it is important to consider the effects of online tDCS may be particularly state-dependent, i.e. there may be variation in response due to differences in task-induced activity or baseline performance across participants (Antal et al., 2007; Bortoletto et al., 2015). To avoid minimize such a confound in our results we opted for offline assessment of WM performance.

The experience of side-effects due to tDCS was assessed through self-report ratings for the following symptoms: head ache, neck pain, nausea, muscle contractions in the face or neck, stinging sensation under the electrodes, burning sensation under the electrodes, and a nonspecific, uncomfortable feeling. Consistent with previous studies the most prominent side-effects were stinging and burning sensations under the electrodes (Bikson, Datta, & Elwassif, 2009), although none of the participants voiced major complaints.

Tyrosine administration

As in experiment 1, we administered 2.0 g of Tyr or a placebo dissolved in 400 mL orange juice.

Procedure

The experimental procedure was similar to experiment 1. Participants came to the lab in the morning, having fasted since 10 o'clock in the evening prior to participation (Cuche et al., 1985; Glaeser et al., 1979). Informed consent was obtained and BMI was measured. Subsequently participants consumed 2.0 g

of Tyr or placebo dissolved in 400 mL orange juice. Afterwards they were offered apples and oranges to prevent strong hunger. 45 min after finishing the juice the tDCS montage was applied. After mounting the electrodes on the head, which took approximately 10 to 15 min, at precisely 1 h after Tyr or placebo administration the stimulation was started. Once the 15 min stimulation had finished the montage was removed. The participants then started the *N*-back task, which took approximately 20 min. Afterwards, participants' experience of any side-effects due to tDCS was rated. Lastly, participants were debriefed and compensated for their participation with course credit or €15.

Statistical analysis

To assess the effect of tDCS combined with Tyr we performed repeated measures ANCOVA with montage (AL-CR vs. CL-AR) and administration (placebo vs. Tyr), as between-subjects factors, WM load (2-back vs. 3-back condition) as within-subjects factor, d' prime as the dependent measure, and BMI as covariate. Similar analyses were performed for hit, false alarm, correct rejection, and miss rates, and RT as dependent measures.

A significant interaction between montage and administration was further investigated with additional ANCOVAs to disentangle this effect on WM performance according to our main hypothesis. Control comparisons between experiment 1 and 2 were performed to further clarify the effect of combined tDCS and Tyr relative to administration of only Tyr.

Results experiment 1

Target sensitivity

To replicate the positive effect of Tyr intake on WM performance, participants completed a letter-based *N*-back task after they consumed either placebo or Tyr. For d' prime scores, there was a significant effect of WM load, $F(1, 33) = 10.45$, $p = .003$, partial $\eta^2 = .241$. This indicates significantly higher d' prime scores in the 2-back condition ($M = 2.74$) than in the 3-back condition ($M = 1.99$). More importantly, there was a main effect of administration, $F(1, 33) =$

6.94, p = .013, partial η^2 = .174. Target sensitivity was significantly higher after intake of Tyr (M = 2.69) than after placebo (M = 2.05). There was no significant interaction between WM load and administration, $F(1, 33) = 1.01$, p = .321, suggesting the effect of Tyr was comparable in the 2-back and 3-back conditions.

Other N-back parameters

Results for hits, misses, correct rejections, false alarms, and RT were similar to d' prime. Means are listed in Table 2.

For hits and misses there was again a main effect of administration, $F(1, 33) = 5.30$, p = .028, partial η^2 = .138. This suggests significantly higher hit rates and less misses after intake of Tyr (M = .86 and .14, respectively) than after placebo (M = .78 and .22, respectively). Similarly, for correct rejections and false alarms there was also a main effect of administration, $F(1, 33) = 6.81$, p = .014, partial η^2 = .171. This indicates significantly higher correct rejection and lower false alarm rates after Tyr intake (M = .92 and .08, respectively) than after placebo (M = .84 and .16, respectively).

For RT on target trials, there was no significant effect of administration, $F(1, 33) = 3.00$, p = .093, partial η^2 = .083. However, for RT on non-target trials there was a significant effect of administration, $F(1, 33) = 4.97$, p = .033, partial η^2 = .131, indicating faster responses on non-target trials after Tyr intake (M = 559) than after intake of placebo (M = 609).

BMI

For the sake of clarity we discuss the BMI results in this separate section. There were no significant main effects of BMI, all $p > .05$. Interestingly, there was a significant interaction between BMI and load when analyzing the d' prime scores, p = .015. However separate regression analyses for the two load conditions (2-back and 3-back) with BMI as predictor and d' prime as dependent measure revealed no significant effect of BMI on d' prime in either condition, ps > .05. Plotting the data revealed the interaction was likely driven by a tendency for d' prime scores to be lower in the 2-back condition with

increasing BMI, whereas this tendency was not observed in the 3-back condition. Lastly, in separate analyses we confirmed the effect of BMI did not differ in our two groups as indicated by a nonsignificant BMI and group interaction, *p*s > .05.

Table 2. Parameters of the N-back task in Experiment 1

	Placebo		Tyrosine	
	Mean	SD	Mean	SD
Hits*				
2-back	.83	.15	.91	.07
3-back	.72	.15	.82	.12
Misses*				
2-back	.17	.15	.09	.07
3-back	.28	.15	.18	.12
Correct rejections*				
2-back	.88	.12	.93	.05
3-back	.81	.11	.90	.06
False alarms*				
2-back	.12	.12	.07	.05
3-back	.19	.11	.10	.06
Reaction times 2-back				
Target	541	83	506	71
Non-target*	568	90	528	65
Reaction times 3 back				
Target	606	86	544	68
Non-target*	608	78	579	60

* $p < .05$

Results experiment 2

Target sensitivity

To investigate whether Tyr modulates the effect of tDCS on WM, participants completed a letter-based *N*-back task after being administered either placebo or Tyr and having been stimulated for 15 min with an AL-CR or CL-AR montage. For d' prime, there was a significant effect of WM load, $F(1, 67) = 5.23$, $p = .025$, partial $\eta^2 = .072$. Specifically, scores in the 2-back condition ($M = 2.35$) were significantly higher than in the 3-back condition ($M = 1.67$). There were no significant interactions between WM load and montage, $F(1, 67) = .06$, $p = .811$, between WM load and Tyr administration, $F(1, 67) = .42$, $p = .520$, or between WM load and both montage and Tyr administration, $F(1, 67) = .01$, $p = .921$, suggesting the difference in difficulty between the 2-back and 3-back conditions was experienced similarly across the four groups.

More importantly, although we did not find a main effect of montage $F(1, 67) = 2.79$, $p = .099$, or Tyr administration, $F(1, 67) = .09$, $p = .771$, we did find a significant interaction between montage and Tyr administration, $F(1, 67) = 4.81$, $p = .032$, partial $\eta^2 = .067$. This indicates target sensitivity was modulated by the combination of tDCS and Tyr. The interaction is illustrated in Figure 1. To disentangle this interaction, we ran separate ANCOVAs for the placebo and Tyr groups. After placebo intake there was only a small, non-significant difference between the AL-CR and CL-AR montages in terms of d' prime ($M = 2.06$ vs. 1.99), $F(1, 33) = .10$, $p = .749$. However, after Tyr intake there was a larger, significant difference between the AL-CR and CL-AR montages in the opposite direction of typical results on tDCS and WM, $F(1, 33) = 6.50$, $p = .016$, partial $\eta^2 = .165$. That is, d' prime scores were higher after CL-AR stimulation was combined with Tyr ($M = 2.23$) than when the typically WM-enhancing AL-CR stimulation was combined with Tyr ($M = 1.75$), see Figure 1. This finding suggests inducing a moderately higher DA level in participants modulates the effect of tDCS on WM and, strikingly, leads a typically-enhancing stimulation montage to impair performance. This finding provides first evidence in favor of a causal role for DA in the cognitive-behavioral effects of prefrontal tDCS.

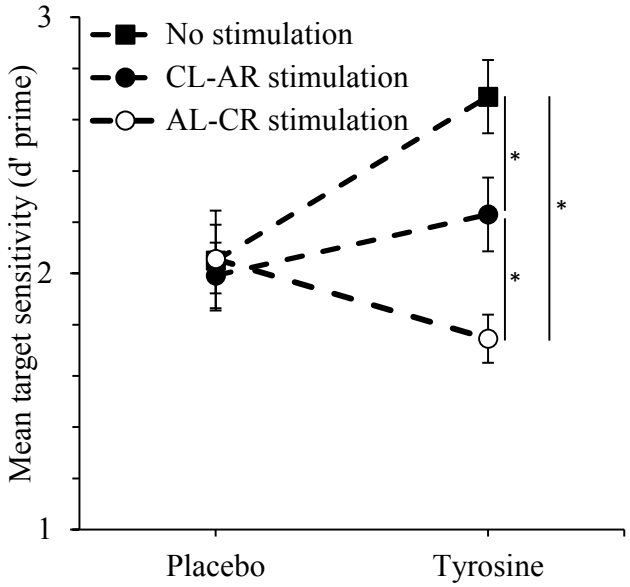

Figure 1. Mean d' prime scores in the *N*-back task for each group in experiment 1 (no stimulation plus placebo or Tyr) and experiment 2 (CL-AR stimulation plus placebo or Tyr and AL-CR stimulation plus placebo or Tyr). Scores are averaged across the two WM load conditions (2-back and 3-back). * $p < .05$

Other N-back parameters

Results for hits and misses showed similar patterns to d' prime. Means are listed in Table 3.

For hits and misses there was no main effect of montage, $F(1, 67) = 1.82$, $p = .182$, nor Tyr administration, $F(1, 67) > .001$, $p = .987$, but again there was a significant interaction between montage and Tyr administration, $F(1, 67) = 5.46$, $p = .022$, partial $\eta^2 = .075$. Additional ANCOVAs revealed a significant difference between the montages after Tyr intake, $F(1, 67) = 7.31$, $p = .011$, partial $\eta^2 = .181$. As with d' prime scores, the CL-AR group showed higher performance (i.e., more hits and less misses) than the AL-CR group when combined with Tyr, whereas no difference was observed when combined with placebo, $F(1, 33) = .40$, $p = .529$. There were no significant 2 or 3-way interactions between WM load, montage and Tyr administration, all ps > .398.

For correct rejections and false alarms there were no main effects of montage, $F(1, 67) = 2.25$, $p = .138$, or Tyr administration, $F(1, 67) = .41$, $p = .523$, nor an interaction between montage and Tyr administration, $F(1, 67) = 1.73$, $p = .193$. The only significant interaction involved WM load and montage, $F(1, 67) = 4.14$, $p = .046$, partial $\eta^2 = .058$, indicating responses to non-targets were modulated by tDCS montage and this modulation differed between the 2-back and 3-back conditions. A post hoc pairwise comparison of the two montages for each level of WM load revealed no significant difference between the two montages in the 2-back condition, $p = .909$, whereas the two montages did differ significantly in the 3-back condition, $p = .034$, partial $\eta^2 = .065$. Specifically, participants who were stimulated with an AL-CR montage showed overall less correct rejections and, correspondingly, more false alarms ($M = .81$ and .19, respectively) as compared to those stimulated with a CL-AR montage ($M = .85$ and .15, respectively), but only in the 3-back condition. It should be noted this interaction was independent of whether participants were given placebo or Tyr.

Table 3. Parameters of the N-back task in Experiment 2

	tDCS montage							
	AL-CR				CL-AR			
	Placebo		Tyrosine		Placebo		Tyrosine	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Hits*								
2-back	.84	.08	.80	.10	.83	.11	.87	.08
3-back	.75	.11	.71	.10	.71	.13	.76	.11
Misses*								
2-back	.16	.08	.20	.10	.17	.11	.13	.08
3-back	.25	.11	.29	.10	.29	.13	.24	.11
Correct rejections								
2-back	.89	.06	.89	.06	.88	.07	.89	.05
3-back^o	.83	.07	.79	.07	.85	.06	.85	.09
False alarms								
2-back	.11	.06	.11	.06	.12	.07	.11	.05
3-back^o	.17	.07	.21	.07	.15	.06	.15	.09
Reaction times 2-back								
Target	534	55	542	80	539	81	560	87
Non-target	602	61	578	62	581	69	595	71
Reaction times 3 back								
Target	555	67	566	64	562	67	567	84
Non-target	607	69	592	41	602	74	609	67

** $p < .05$ for difference AL-CR plus Tyr versus CL-AR plus Tyr*

^{o} $p < .05$ for difference AL-CR versus CL-AR, regardless of Tyr administration

For RT on target and non-target trials there were no significant effects of montage or Tyr administration, nor any 2 or 3-way interactions between WM load, montage, and Tyr administration, all *p*s > .327.

Control comparisons

We performed control comparisons to gain insight in how stimulation (AL-CR and CL-AR) combined with placebo or Tyr affected performance relative to administering placebo or Tyr without tDCS. To this end we performed two additional ANCOVA's in which we separately compared performance of the groups receiving placebo or Tyr in experiment 2 to the group that received placebo or Tyr in experiment 1, respectively. Since hits and misses followed the same pattern of results as d' prime, we only performed comparisons for the latter.

For the placebo groups, a repeated measures ANCOVA was performed with group (placebo-only vs. AL-CR stimulation plus placebo vs. CL-AR stimulation plus placebo) as between-subjects factor, WM load (2-back and 3-back condition) as within-subjects factor, BMI as covariate, and d' prime as dependent measure. There was no main effect of group, $F(1, 50) = .04$, $p = .960$, suggesting placebo plus tDCS did not affect performance as compared to administration of only placebo. Subsequently we performed the same analysis, but now with the Tyr-only group vs. AL-CR stimulation plus Tyr vs. CL-AR stimulation plus Tyr as between-subjects factor. This analysis did show a significant effect of group, $F(1, 50) = 11.84$, $p > .001$, partial $\eta^2 = .321$. All three groups differed significantly from each other, with participants in the Tyr-only condition having significantly higher d' prime scores than participants in the CL-AR stimulation plus Tyr condition, $p = .018$, and the AL-CR stimulation plus Tyr condition, $p < .001$, and with the CL-AR stimulation plus Tyr condition also showing higher scores than the AL-CR stimulation plus Tyr condition, $p = .022$.

BMI

For the sake of clarity we discuss the BMI results in this separate section. There were significant main effects of BMI only when comparing d' prime scores after CL-AR plus placebo versus CL-AR plus Tyr, p = .034, and when comparing d' prime scores for all three placebo conditions (AL-CR plus placebo, CL-AR plus placebo, and placebo-only), p = .032. In both analyses a higher BMI was associated with worse performance and we revisit this point in the discussion. Importantly, all other main effects of BMI were not significant nor did BMI interact significantly with load, all ps > .05. In separate analyses we confirmed BMI did not interact with tDCS montage and Tyr supplementation, indicating the effect of BMI was comparable in all groups, all ps > .05.

Discussion

The present study reports two major findings. First, we show Tyr administration, which presumably induces a modest increase in DA, enhances verbal WM performance as assessed in a letter-based *N*-back task. This finding replicates previous studies showing beneficial effects of Tyr on WM (for a review, see Jongkees et al., 2015). Second, we show that Tyr, and therewith presumably DA, modulates the effect of tDCS on verbal WM in a current direction-dependent manner. Whereas previous studies show AL-CR stimulation of DLPFC benefits WM performance (Fregni et al., 2005; Hoy et al., 2013; Jeon & Han, 2012; Mulquiney et al., 2011; Ohn et al., 2008; Oliveira et al., 2013; Teo et al., 2011) and CL-AR stimulation may impair performance (Marshall et al., 2005), in our study tDCS combined with Tyr led to a different pattern: CL-AR stimulation of the DLPFC led to higher WM performance than AL-CR stimulation when both are combined with Tyr. This finding is in line with previous genetic studies showing higher prefrontal DA is associated with differential responses to tDCS over DLPFC (Nieratschker et al., 2015; Plewnia et al., 2013) and also fits the finding that combining a DA agonist with anodal stimulation of the motor cortex inverts tDCS after-effects, leading to excitability diminishment rather than enhancement (Kuo et al., 2008).

These results provide first tentative evidence for a causal role of DA in modulating the cognitive-behavioral effect of tDCS over the prefrontal cortex. In doing so, this study supports the hypothesis that DA function may be one factor that contributes to variability in results of tDCS studies. This idea could have relevance for our null-finding in experiment 2: after placebo intake we found no difference in performance after AL-CR as compared to CL-AR stimulation, although the former typically enhances and the latter typically impairs WM. Many reasons may account for this null-finding. However, based on our finding that DA modulates the effects of tDCS, we speculate inter-individual variability in DA function might be one factor, either alone or in combination with others, that could explain this lack of difference. In order to prevent such difficult-to-interpret null-findings, we encourage future studies to take into account individual differences related to DA. Doing so would be an important step towards obtaining a clearer and consistent view of the efficacy of tDCS in modulating cognitive-behavioral performance.

As mentioned in the introduction, it remains unclear what the precise mechanism is that underlies the interaction between DA and tDCS. It might be DA, and by extension Tyr, affects excitability modulations by online tDCS via an influence on membrane potentials, as DA can enhance neural firing in neurons with high potentials while suppressing firing in neurons with low potentials (Frank, 2005; Hernández-López et al., 1997). Hence DA and Tyr may affect online tDCS, i.e. stimulation during the critical task, by modulating task-induced activity, which has been identified as a determinant of tDCS effects (Antal et al., 2007; Bortoletto et al., 2015). DA and Tyr may also affect neuroplasticity induced by tDCS, which may have occurred in the present study, by affecting the NMDA-receptors that are believed to underlie the after-effects of tDCS (Liebetanz et al., 2002; Nitsche et al., 2004; Nitsche, Fricke, et al., 2003). Indeed, D1 and D2 receptors have been shown to affect NMDA-receptor-mediated LTP and LTD, although results for D2 receptors have been inconsistent (Gurden et al., 2000; Huang et al., 2004; Spencer & Murphy, 2000).

Additionally, there are alternative but related hypotheses on the interaction between DA and tDCS and these are not necessarily mutually-exclusive. (i) Tanaka et al. (2013) showed cathodal tDCS increased DA in the rat striatum. As the relationship between DA and cognitive performance typically follows an inverted-U curve (Cools & D'Esposito, 2011), it has been argued tDCS might shift an individual's DA level towards an optimum associated with peak performance (Li et al., 2015). Thus, response to tDCS would depend in part on an individual's initial position relative to the optimal level of DA. This would be particularly relevant for the large body of tDCS studies in aging (Perceval, Flöel, & Meinzer, 2016), as the interaction between DA and tDCS might be magnified by the interplay between genes, DA function, and aging (Li, Lindenberger, & Bäckman, 2010; Lindenberger et al., 2008). (ii) One hypothesis, which might explain why cathodal tDCS of the left DLPFC in conjunction with Tyr led to higher WM scores than the same stimulation without Tyr, is the combination of high DA levels and cathodal stimulation may improve the signal-to-noise ratio in the brain (Kuo et al., 2008), allowing for more efficient neural function. Future studies may be able to validate these speculations, for example by showing enhanced cortical efficiency as indicated by reduced BOLD response during task engagement. (iii) An alternative mechanism relates to the calcium-dependence of tDCS-induced excitability alterations, DA activity, and task-related cortical activity alterations, probably transmitted via the glutamatergic system. Hereby it is important to acknowledge the effect of calcium enhancement on long-lasting cortical excitability alterations follows a non-linear rule. Low calcium enhancement results in reduced, whereas high calcium results in enhanced activity and excitability, but even larger calcium enhancement again reduces respective activity and excitability, possibly due to counter-regulative activation of potassium channels (Lisman, 2001; Misonou et al., 2004). Accordingly, it was shown recently that enhancing stimulation duration of anodal tDCS or combination of anodal tDCS with nicotine in non-smokers results in reduced cortical excitability, and that this process is calcium dependent (Lugon et al., 2015; Monte-Silva et al., 2013). Likewise, enhancing

stimulation intensity of cathodal tDCS switched the effects from excitability diminution to enhancement (Batsikadze, Moliadze, Paulus, Kuo, & Nitsche, 2013). One could speculate that in the present experiment task-dependent activation of neurons in combination with Tyr and anodal tDCS over left DLPFC led to a calcium overflow resulting in cortical activity reduction, and thus reduced performance, whereas the slight calcium increase probably caused by cathodal tDCS over left DLPFC in conjunction with Tyr optimized task-relevant calcium enhancement, and thus improved performance. Notably, performance after CL-AR stimulation combined with Tyr was still lower than after Tyr intake without tDCS, suggesting the effects of tDCS and a DA manipulation might not be additive and their combination might not be an enhancing method for all individuals. (iv) Lastly and particularly relevant for online assessment of performance, the effect of tDCS seems to strongly depend on task-induced activity, at least in the motor cortex (Antal et al., 2007; Bortoletto et al., 2015). Hence the same stimulation might have different effects depending on the extent to which neural activity is increased during task performance. On the other hand, DA is known to modulate task-induced activity and, indeed, the functional polymorphism in the COMT gene, which affects prefrontal DA degradation, is associated with different levels of prefrontal activation during WM engagement (Egan et al., 2001; Mattay et al., 2003). In line with this reasoning, the same COMT polymorphism modulates the effect of tDCS on executive function (Nieratschker et al., 2015; Plewnia et al., 2013). Hence we speculate individual differences related to genetically predetermined DA levels might influence the extent of neural activation during WM engagement and, in turn, this variability could lead to different effects of online tDCS.

Related to the topic of individual differences, we recommend future research to employ more adaptive WM tasks instead of 'static' tasks with the same conditions for each participant. For example, instead of having each participant perform a 2 and 3-back task, adaptive versions of the N-back task exist wherein *N* increases when participants perform well on the task and *N* decreases when they perform worse (e.g. Jaeggi, Buschkuehl, Shah, & Jonides,

2014). Such tasks can prove useful in future research that wishes to examine individual differences (and gain) in WM performance, as it can prevent ceiling effects from obscuring individual differences and practice effects from confounding effects of experimental manipulations in within-subjects designs. Also, it is interesting to note some of our analyses revealed a tendency for WM to be worse with increasing BMI, which is consistent with the idea obesity is related to impaired cognitive function (Prickett, Brennan, & Stolwyk, 2015). With respect to this finding it is important to note our experimental groups did not differ in terms of BMI. Future research might wish to further investigate the potential relationship between BMI and WM without having the results confounded by administration of Tyr or tDCS.

Future research may also wish to examine one notable difference between our and previous tDCS studies, which is that in the current experiment participants fasted overnight because of the Tyr administration. As of yet it is unclear if and how this might have affected the effects of tDCS and whether this fasting, perhaps in combination with Tyr and tDCS, may have contributed to the lack of an effect of tDCS on WM in the placebo conditions. Hence, it would be interesting for future studies to assess whether fasting can affect tDCS outcome.

Furthermore, it is important to acknowledge DA is probably one of many neurotransmitters relevant to tDCS effects, and thus future research should explore other neurotransmitter systems as well. As reviewed by (Stagg & Nitsche, 2011), a number of neurotransmitters are known to modulate the physiological effects of tDCS, but these investigations are restricted to the motor cortex and thus it remains unclear if and how these neurotransmitter determine cognitive-behavioral effects of tDCS. Important neurotransmitters that have been identified are glutamate and GABA, and their concentration levels change following anodal or cathodal stimulation over the motor cortex (Bachtiar, Near, Johansen-Berg, & Stagg, 2015; Soyoung Kim, Stephenson, Morris, & Jackson, 2014; Stagg et al., 2009). The consequent change in cortical excitation may facilitate or impair induction of LTP and LTD (Ziemann, Muellbacher, Hallett, & Cohen, 2001), and as such these neurotransmitters

may play a crucial role in the neuroplastic after-effects of tDCS (Stagg & Nitsche, 2011). Interestingly, individuals are known to differ in the balance between cortical glutamate and GABA, and this balance has been shown to predict response selection (de la Vega et al., 2014). Hence it would be interesting to see whether response to prefrontal tDCS can also be predicted based on individual differences in the glutamate/GABA balance. Other relevant neurotransmitters include acetylcholine, an increase of which abolished the after-effects anodal but prolonged the effects of cathodal tDCS (Kuo, Grosch, Fregni, Paulus, & Nitsche, 2007), and serotonin, an increase of which prolonged the after-effects of anodal tDCS but reversed the after-effects of cathodal tDCS from excitability diminishment into enhancement (Nitsche, Kuo, Karrasch, et al., 2009). Recently it was also shown noradrenaline modulates tDCS-induced plasticity, with different effects for acute and chronic pharmacological enhancement of noradrenaline activity (Kuo et al., 2016). This is particularly interesting when considering effects of Tyr administration may be mediated by DA and/or noradrenaline (Jongkees et al., 2015), as mentioned below. As such, future studies may wish to examine individual differences in terms of these neurotransmitters as well.

Before concluding, we wish to elaborate on some limitations of this study. First, in experiment 2 we did not include a sham-tDCS condition. Hence we cannot be sure the observed effects of tDCS are due to modulation of neural physiology or rather due to expectations of the participants evoked by the experience of mounting electrodes on the head and the accompanying tingling, burning sensations. However, we argue that an explanation of our results in terms of participants' expectations is unlikely as the placebo groups from both experiments were comparable in performance. If participants' expectations due to tDCS had indeed influenced our results, it would have likely resulted in differences between these groups.

The second limitation is we did not assess inter-individual variability in DA function, for example in terms of genetic polymorphisms. Although the present study aimed at finding proof-of-principle for the idea DA modulates prefrontal tDCS, not considering individual differences between our

participants may have led to the inability to replicate a beneficial effect of tDCS on WM. For this reason it would be valuable to replicate and validate our findings in future studies in which individual differences are taken into account.

The third limitation relates to the presumed effect of Tyr on catecholamine synthesis. Tyr is the precursor of both DA and noradrenaline (NA) and beneficial effects of Tyr on cognition may be mediated by increases in either DA or NA, or both (Jongkees et al., 2015). For this reason we cannot definitively conclude that the findings reported in the present study are mediated solely by DA and not by NA, in particular as pharmacological enhancement of NA has recently been shown to modulate tDCS-induced plasticity (Kuo et al., 2016). There is evidence that in particular DA modulates the effect of tDCS, as DA antagonists can abolish the effect of tDCS on cortical excitability (Nitsche et al., 2006), but more research is necessary before we could conclude the effects of Tyr on tDCS are mediated solely by DA.

Lastly, it is important to consider that our results may have depended significantly on our choice of stimulation parameters, such as location of the return electrode, size of the electrodes, applying stimulation before or during the task of interest, the current intensity, and stimulation duration. tDCS studies so far have used many different parameters, which may play an under-investigated role in determining the efficacy of the stimulation (see Woods et al., 2016). This highlights the possibility our results could have been different had we chosen different parameters, and future research should aim to systematically investigate whether the influence of DA on tDCS might depend on these parameters to produce different cognitive-behavioral outcomes.

Although more research is needed, the present study provides first evidence that Tyr administration modulates the cognitive-behavioral effects of tDCS and, in doing so, tentatively supports the hypothesis that DA plays a causal role in prefrontal tDCS. Despite probably being only one of many important factors, we recommend future studies to consider the effects of DA on tDCS in order to achieve more consistent and replicable results.

Chapter Seven

The COMT Val^{158}Met polymorphism does not modulate the after-effect of tDCS on working memory

Jongkees, B. J., Loseva, A. A., Nitsche, M. A., & Colzato, L. S. (Submitted). The COMT Val^{158}Met polymorphism does not modulate the after-effect of tDCS on working memory.

Abstract

Although transcranial direct current stimulation (tDCS) can alter cortical excitability, neural plasticity and cognitive-behavioral performance, its effects are known to vary across studies. A partial account of this variability relates to individual differences in dopamine function. Indeed, dopaminergic manipulations alter the physiological and cognitive-behavioral effects of tDCS, and genetic polymorphisms related to dopamine have predicted individual response to online tDCS (i.e., stimulation overlapping with the critical task). Notably, the role of individual differences in dopamine has not yet been properly assessed in the effect of offline tDCS (i.e., stimulation prior to the critical task). Therefore, we investigated if and how the COMT Val^{158}Met polymorphism (rs4680) modulates the after-effect of prefrontal tDCS on verbal working memory (WM). 139 participants were genotyped for the COMT Val^{158}Met polymorphism and received anodal-over-left, cathodal-over-right (AL-CR) dorsolateral prefrontal cortex stimulation, cathodal-over-left, anodal-over-right (CL-AR) or sham stimulation in a between-subjects, pretest-posttest study design. WM was assessed using the N-back task. The results provide no evidence that the COMT polymorphism impacts the after-effect of prefrontal tDCS on WM. Taken together with previous findings on the interaction between dopamine and tDCS effects, the results of the present study suggest that (i) dopamine might differentially impact online and offline effects of tDCS, and (ii) findings from studies including pharmacological manipulation should be generalized only with caution to findings of inter-individual differences. Specifically, state (i.e., a manipulation of) and trait (i.e., baseline) differences in dopamine appear to exert different effects on tDCS.

Introduction

Current research has increasingly focused on the idea that non-invasive brain stimulation can serve as an effective tool to investigate and possibly enhance the neuromodulation of cognitive-behavioral performance. Of the available techniques, transcranial direct current stimulation (tDCS) is a popular method of transiently enhancing performance or augmenting the gains from extended training. tDCS alters cortical excitability (Nitsche & Paulus, 2000) and, at longer stimulation periods, affects neural plasticity (Nitsche & Paulus, 2001; Nitsche, Nitsche, et al., 2003) by inducing a polarity-dependent shift in the resting membrane potential of cortical neurons. It has been questioned whether these physiological changes translate to reliable effects on cognition (Horvath et al., 2015a, 2015b; Mancuso, Ilieva, Hamilton, & Farah, 2016), but reviews on this issue often suffer many limitations that prevent an unequivocal answer (Antal et al., 2015). Notwithstanding the variability in results that might be explained by methodological differences across studies, it has been suggested that individual differences in dopamine (DA) function within and across studies might partially account for variable effects of tDCS (Li et al., 2015; Wiegand et al., 2016). In the present study we explore this idea by investigating whether a genetic predisposition towards higher or lower prefrontal DA activity predicts individual differences in the effect of tDCS on verbal working memory (WM).

There is converging evidence DA indeed has an important impact on tDCS effects. Pharmacological stimulation of DA receptors has non-linear effects on tDCS-induced neuroplasticity, and blockage of DA receptors can eliminate effects on plasticity entirely (Fresnoza, Paulus, et al., 2014; Fresnoza, Stiksrud, et al., 2014; Kuo et al., 2008; Monte-Silva et al., 2009, 2010; Nitsche et al., 2006; Nitsche, Kuo, Grosch, et al., 2009). These studies point to an inverted-U-shaped relationship between DA activity and tDCS effects (Wiegand et al., 2016), as low and high, but not moderate, stimulation of DA receptors abolished tDCS-induced changes in neuroplasticity (Fresnoza, Paulus, et al., 2014; Monte-Silva et al., 2010), whereas moderate DA enhancement strengthened long-term depression (LTD)-like effects of

cathodal tDCS, while it converted after-effects of anodal tDCS from long-term potentiation (LTP)- to LTD-like effects (Kuo et al., 2008; Monte-Silva et al., 2010). An inverted-U-shaped relationship is also observed in studies of pre-existing differences rather than artificially-induced changes in DA function, with results varying depending on the type of stimulation and experimental task conditions. Using the COMT Val^{158}Met polymorphism to estimate individual differences in prefrontal DA, it was shown tDCS impaired cognitive flexibility in individuals with high DA activity who received excitatory stimulation during task performance (Plewnia et al., 2013). In contrast, tDCS impaired response inhibition in individuals with low DA activity who received inhibitory stimulation (Nieratschker et al., 2015).

These results were mirrored in a recent study examining the effect of a modest dopaminergic manipulation on the cognitive-behavioral rather than the physiological effect of tDCS (Jongkees, Sellaro, et al., 2017). This was done by combining tDCS with administration of L-tyrosine, the biochemical precursor of L-dopa and DA, to transiently enhance DA activity. Specifically, it was shown that prefrontal tDCS impaired WM performance on the N-back task when L-tyrosine was combined with excitatory stimulation of the left dorsolateral prefrontal cortex (DLPFC), yet it trend-wise enhanced performance when L-tyrosine was combined with inhibitory stimulation of the left DLPFC. The authors speculated that DA and tDCS might interact on cortical excitability, with increased DA combined with excitatory stimulation resulting in overexcitability of the cortex whereas combined with inhibitory stimulation it might serve to promote cortical signal-to-noise ratio. Together with the studies on the COMT polymorphism, these findings highlight a state-dependency of tDCS effects, with the type of stimulation interacting with the dopaminergic activity state.

To account for these behavioral findings, it has been proposed that tDCS might bring an individual closer to or further away from an optimal level of dopaminergic signaling (Nieratschker et al., 2015; Plewnia et al., 2013; Wiegand et al., 2016), which would be consistent with animal literature demonstrating tDCS can enhance DA release (Tanaka et al., 2013).

Specifically, individuals with an already optimal level of signaling, such as those with high prefrontal DA activity due to genetic predisposition or L-tyrosine administration, might be pushed towards a suboptimal, too high level of activity that results in impaired performance when receiving excitatory stimulation. Conversely, individuals with a lower-than-optimal level of signaling due to low prefrontal DA activity might show impaired performance when that activity is further reduced by inhibitory stimulation. In brief, an individual's initial position on the inverted-U curve relating DA and performance would determine whether a shift toward the right or left on the curve (due to excitatory or inhibitory stimulation, respectively) enhances or impairs performance. It should be noted that this interaction between tDCS and DA might not necessarily reflect a direct impact of the former on the latter, but instead be mediated by tDCS-induced changes in levels of glutamate and GABA (Bachtiar et al., 2015; Soyoung Kim et al., 2014; Stagg et al., 2009).

The present study

The line of reasoning presented above has been primarily applied to online effects of tDCS, i.e., stimulation *overlapping* with the critical task. In the present study we investigated whether this hypothesis extends to offline tDCS as well, i.e., stimulation *prior* to the critical task. Whereas online effects of tDCS are attributed mainly to a modulation of cortical excitability, offline effects of tDCS reflect changes in neural plasticity (Nitsche & Paulus, 2000; Nitsche, Nitsche, et al., 2003). Both can be sensitive to DA, with the interaction between DA and online tDCS being mediated partially by interacting effects on task-induced activity (Bortoletto et al., 2015; Mattay et al., 2003), whereas the interaction with offline tDCS might be mediated by effects on N-methyl-D-aspartate (NMDA) receptors which drive neuroplasticity via long-term potentiation and depression (Gurden et al., 2000; Huang et al., 2004; Spencer & Murphy, 2000). Considering a DA manipulation altered the cognitive-behavioral after-effect of tDCS (Jongkees, Sellaro, et al., 2017) and individual baseline differences in DA have predicted online effects of tDCS (Nieratschker et al., 2015; Plewnia et al., 2013), it is conceivable these individual differences

predict the after-effects of offline tDCS as well. We were interested in the effects on WM in particular, because this cognitive function is the most-often investigated function in tDCS studies. Hence a demonstration or a lack of an impact of individual differences in DA on tDCS after-effects on WM would have implications for a majority of the existing tDCS literature.

Following the only two available studies on individual differences in DA and cognitive-behavioral effects of prefrontal tDCS (Nieratschker et al., 2015; Plewnia et al., 2013), we assessed genetic predisposition toward higher or lower dopaminergic signaling in the prefrontal cortex using the COMT Val^{158}Met polymorphism. The COMT enzyme is responsible for degradation of extracellular DA, and differences in thermolability of the enzyme determined by different COMT polymorphisms affect the rate at which DA is degraded (Weinshilboum, Otterness, & Szumlanski, 1999). Carriers of the Val allele have a less thermolabile enzyme that results in faster degradation and, hence, lower concentrations of DA, whereas carriers of the Met allele have a more thermolabile enzyme that results in slower degradation and, hence, higher concentrations of DA. The COMT polymorphism relates to prefrontal DA activity in particular (Karoum, Chrapusta, & Egan, 1994) due to a relative lack of DA transporters in the prefrontal cortex (PFC) as compared to their abundance in the striatum (Lewis et al., 2001). Consistent with a lower prefrontal DA concentration, Val carriers demonstrate less efficient cortical processing (Egan et al., 2001; Mattay et al., 2003) and worse behavioral performance during WM tasks (Goldberg et al., 2003), but also better task-switching performance as compared to Met carriers (Colzato, Waszak, Nieuwenhuis, Posthuma, & Hommel, 2010). Most important for our purposes, this polymorphism has previously predicted the effect of prefrontal tDCS on cognitive-behavioral performance (Nieratschker et al., 2015; Plewnia et al., 2013), making it the most obvious marker of individual differences in DA for the present purpose.

Considering tDCS effects likely vary depending on experimental parameters such as electrode placement and stimulation duration, we opted for a stimulation montage and duration of which the after-effects are sensitive to

a mild DA manipulation (Jongkees, Sellaro, et al., 2017). Electrodes were placed over DLPFC in a bilateral bipolar-balanced montage (Nasseri et al., 2015). This montage enhanced WM in antidepressant-free patients with major depressive disorder (Oliveira et al., 2013) and, more importantly, interacted in healthy adults with a dopaminergic manipulation on WM (Jongkees, Sellaro, et al., 2017) in a manner similar to studies on individual differences in DA and cognitive-behavioral effects of tDCS (Nieratschker et al., 2015; Plewnia et al., 2013).

In brief, 139 participants were genotyped for the COMT Val^{158}Met polymorphism and received either anodal-over-left, cathodal-over-right (AL-CR) DLPFC stimulation, cathodal-over-left, anodal-over-right (CL-AR) or sham stimulation in a between-subjects, sham-controlled, pretest-posttest study design. Based on previous findings (Jongkees, Sellaro, et al., 2017; Nieratschker et al., 2015; Plewnia et al., 2013), as compared to sham stimulation, we expected individuals with high dopaminergic signaling, i.e., Met carriers, to demonstrate worse WM performance after receiving excitatory stimulation (AL-CR) over the left DLPFC, whereas individuals with low dopaminergic signaling, i.e., Val carriers, were expected to demonstrate worse WM performance after receiving inhibitory stimulation (CL-AR) over the left DLPFC. The inverted-U-curve proposed by (Wiegand et al., 2016) also suggests that Val carriers potentially benefit behaviorally from a slight increase in dopaminergic signaling due to excitatory stimulation (i.e., being shifted right and upwards on the inverted-U-curve). Notwithstanding these hypothesized findings, it is important to consider that pharmacological manipulations do not necessarily mimic the effects of natural variation in a neurotransmitter system (Boy et al., 2011), pointing to the possibility that COMT-tDCS interactions do not necessarily mirror the interaction between dopaminergic manipulations and tDCS. This is a significant possibility in light of the fact that no published study has yet demonstrated a role for individual differences in DA in the after-effects of tDCS on WM. This suggests DA-tDCS interactions might vary or not apply to every type of stimulation and/or experimental task, as our results will indeed indicate.

Material and methods

Participants

139 right-handed undergraduate students participated in a study on tDCS and memory. Participants were randomly assigned to one of the three stimulation types (AL-CR, CL-AR, or sham). 9 participants were identified as performance outliers as described in the Results section, leaving a total of 130 participants for further analysis. The resulting groups did not differ with respect to age, $F(4,121) = .61$, $p = .656$, or gender distribution, $X^2(4, N = 130) = 1.06$, $p = .901$, see Table 1 for group demographics. All participants were screened individually using the Mini International Neuropsychiatric Interview (MINI), a short, structured interview of approximately 15 min that screens for several psychiatric disorders and drug use (Sheehan et al., 1998), and has been used previously in neuromodulation research, including research on L-tyrosine and tDCS (Jongkees, Immink, & Colzato, 2017; Jongkees, Sellaro, et al., 2017). Participants were included if they met the following criteria: (i) between 18 and 30 years; (ii) no history of neurological or psychiatric disorders; (iii) no history of substance abuse or dependence; (iv) no chronic or acute medication; (v) no implants such as pacemakers or any kind of metal in the body, nor any skin conditions, for safety reasons concerning tDCS. One exception was hormonal contraceptive use in females, which was required to limit fluctuations in hormone levels that can influence DA function and confound group differences (Colzato & Hommel, 2014; Czoty et al., 2009; Jacobs & Esposito, 2011). All participants met these criteria. Before the study, participants were informed of the procedure and potential side-effects of tDCS (i.e., itching, stinging or burning sensation from the electrodes, reddening of the skin and headache). None of the participants reported major side-effects. The study conformed to the ethical standards of the declaration of Helsinki and the protocol was approved by the local ethical committee (Leiden University, Institute for Psychological Research).

Table 1. Group demographics

	AL-CR	CL-AR	Sham
N			
Met/Met	16	11	13
Val/Met	20	21	19
Val/Val	12	8	10
Gender F:M			
Met/Met	9:7	7:4	9:4
Val/Met	15:5	11:10	15:4
Val/Val	8:4	6:2	8:2
Age in years			
Met/Met	21.1 (3.1)	22.1 (2.3)	21.5 (2.8)
Val/Met	22.4 (2.9)	21.3 (2.7)	21.4 (2.5)
Val/Val	22.5 (2.9)	23.1 (3.9)	22.7 (3.2)

Genotyping

Genetic material to determine COMT genotype was collected using buccal swabs, which were analyzed by the company BaseClear (The Netherlands). The SNP Val158Met of the COMT gene (rs4680) was genotyped using Applied Biosystems (AB) TaqMan technology. All genotypes were scored by two independent readers by comparison to sequence-verified standards. For COMT Val158Met three genotype groups were established: Val/Val homozygotes, Val/Met heterozygotes and Met/Met homozygotes. COMT genotype was available in all participants.

Genotype distribution for COMT Val^{158}Met polymorphism in our Dutch healthy population was 30 Val/Val homozygous subjects (23.08%), 60 Val/Met heterozygous subjects (46.15%) and 40 Met/Met homozygous subjects (30.77%). All resulting genotype frequencies from our cohort of participants did not deviate from Hardy-Weinberg equilibrium (p = .415). No significant differences were found among genotype frequencies with respect to age, $F(2,127) = 1.85$, $p = .161$ or gender distribution, $X^2(2, N = 130) = .94$, $p = .625$.

N-back task

WM performance was assessed using the N-back task (Kane et al., 2007), which is predominantly used in tDCS studies on WM (Au et al., 2016; Fregni et al., 2005; Hoy et al., 2013; Mylius et al., 2012; Ohn et al., 2008; Oliveira et

al., 2013; Teo et al., 2011; Zaehle et al., 2011). As in the study on L-tyrosine and tDCS (Jongkees, Sellaro, et al., 2017), a letter-based N-back task was used to assess verbal WM (Colzato, Jongkees, et al., 2013). To prevent potential ceiling-effects induced by repeated practice in a pretest-posttest design, a 2-back and 4-back condition was included in each pretest and posttest.

Stimuli were presented in the middle of a computer screen with a refresh rate of 60 Hz and a 800 x 600 resolution using E-Prime 2.0 software. Participants were comfortably seated approximately 50 cm from the screen while wearing headphones. Responses were given using the 'z' and 'm' buttons of a QWERTY keyboard for targets (i.e., repetition) and non-targets (i.e., non-repetition), respectively. Mapping of response buttons to targets and non-targets was not counterbalanced across participants to prevent differences in response mapping across genotypes. After an incorrect or belated response (latency longer than 1000 ms) a brief tone was presented to signal the error. Both the 2-back and the 4-back conditions consisted of two blocks of 51 + N trials. For example, a 2-back block consisted of 53 trials. Regardless of the load condition, each block comprised 21 targets and 30 non-targets. All participants performed the 2-back condition first and then the 4-back condition, and each N-back condition was preceded by 17 + N practice trials (7 targets and 10 targets).

Transcranial direct current stimulation

In line with (Jongkees, Sellaro, et al., 2017), two electrodes of 35 cm^2 (5 cm x 7 cm) were placed over DLPFC in a bilateral bipolar-balanced montage (Nasseri et al., 2015), i.e., in symmetrical positions. For each individual participant the DLPFC was located using the international 10/20 system for placing electrodes on the scalp (Jasper, 1958). As such, for the AL-CR montage the anode and cathode were placed over F3 and F4, respectively, whereas this placement was reversed for the CL-AR montage. In the sham condition, half of participants received the AL-CR montage and the other half received the CL-AR montage.

Stimulation consisted of a current of 1000 μA delivered by a DC Brain Stimulator Plus (NeuroConn, Ilmenau, Germany), a device complying with the Medical Device Directive of the European Union (CE-certified). The current was built up during a fade-in of 10 s, after which stimulation lasted for precisely 15 min and then ended with a 10 s fade-out. Sham stimulation was exactly the same but lasted for 15 s instead of 15 min, thus providing a similar initial sensation as active stimulation. The after-effects of 15 min of tDCS typically last 30 to 60 min, whereas stimulation of only a few seconds produces no changes in cortical excitability or plasticity (Nitsche et al., 2008).

The experience of side-effects due to tDCS was assessed through self-report ratings for the following symptoms: (i) headache, (ii) neck pain, (iii) nausea, (iv) muscle contractions in the face or neck, (v) stinging sensation under the electrodes, (vi) burning sensation under the electrodes, and (vii) a nonspecific, uncomfortable feeling. Consistent with previous studies, the most prominent side-effects were stinging and burning sensations under the electrodes (Bikson et al., 2009), although no participants voiced major complaints.

Procedure

Participants gave written consent upon entering the lab. After filling in a questionnaire assessing their general health, they completed a pretest of the N-back task, which took on average 20 min. Subsequently the tDCS montage was mounted on the participants' scalp and stimulation was started. During the 15 min of stimulation, participants gave buccal swabs to determine COMT genotype. Following stimulation, the tDCS electrodes were removed and participants completed the posttest of the N-back, which was identical in structure to the pretest and took on average 20 min. In total the procedure took approximately 90 min.

Statistical analysis

Aside from parameters such as hit rate and correct rejections, we were interested in target sensitivity, indexed by d' prime derived from signal

detection theory (Swets et al., 1961). This measure combines hit rate and false alarms to provide an index of the ability to discriminate targets from non-targets, with higher scores indicating more selective and correct reporting of targets. d' prime was calculated, and perfect scores were corrected for, as described earlier (Colzato, Jongkees, et al., 2013).

First, each group was checked for outlier performance (below or above 3 standard deviations of the group mean) on d' prime, hit rate, correct rejections and reaction time (RT). Subsequently, a repeated measures analysis of variance (rmANOVA) was conducted with time (pretest vs posttest) and WM load (2-back vs 4-back) as within-subject factors and type of stimulation (AL-CR vs CL-AR vs sham) and COMT genotype (Val/Val vs Val/Met vs Met/Met) as between-subject factors. Separate analyses were performed for d' prime, hit rate, correct rejections and RT for targets and non-targets.

Results

4 participants were identified as outliers based on either pretest or posttest d' prime scores, additional 3 participants were identified as outliers based on hit rate or correct rejections, and another 2 participants were identified as outliers based on RT. This left a total of 130 participants for subsequent analyses. See Table 2 for an overview of group scores on the N-back, and see Figure 1 for a depiction of the d' prime scores.

None of the dependent variables (d' prime, hit rate, correct rejections and RT) demonstrated a main effect of stimulation ($ps \geq .406$), an interaction between time and stimulation ($ps \geq .494$), nor a three-way interaction involving load ($ps \geq .252$), suggesting that tDCS did not modulate N-back performance when disregarding COMT genotype. Only RT to non-targets revealed a main effect of COMT, $F(2,121) = 3.43$, $p = .036$, partial $\eta^2 = .054$, with Val homozygotes demonstrating higher RT than Met homozygotes ($M = 591$ vs 557 ms, $p = .012$) but not Val/Met heterozygotes ($M = 577$ ms, $p = .286$), nor was there a significant difference between Met homozygotes and heterozygotes ($p = .068$). All other measures revealed no main effect of COMT

(*p*s ≥ .140), nor an interaction with time (*p*s ≥ .465) or a three-way interaction involving load (*p*s ≥ .211).

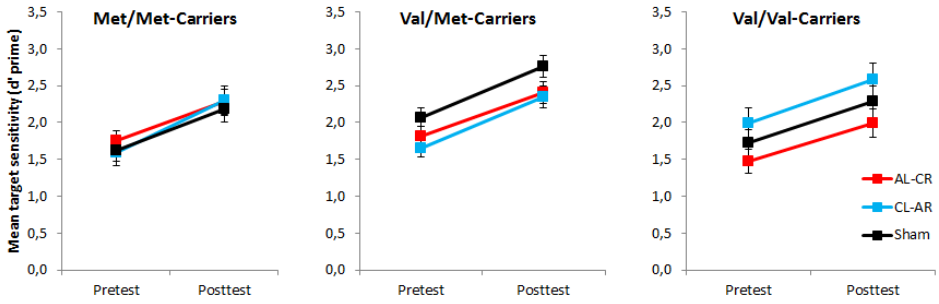

Figure 1. D' prime scores as a function of time (pretest vs posttest), stimulation (AL-CR vs CL-AR vs Sham), and COMT genotype (Met/Met vs Val/Met vs Val/Val).

Most important to the present study, no dependent measures demonstrated a significant three-way interaction between time, stimulation and COMT (*p*s ≥ .476), nor a four-way interaction involving load (*p*s ≥ .505) except for RT to targets $F(4, 121) = 2.67$, $p = .036$, partial $\eta^2 = .054$. To disentangle this four-way interaction we first computed individual difference scores for pretest and posttest RT and then separately submitted 2-back and 4-back scores to the ANOVA with stimulation and genotype as between-subject factors. This revealed no significant interaction between stimulation and COMT for either the 2-back, $F(4,121) = 1.53$, $p = .198$, or the 4-back, $F(4,121) = 1.03$, $p = .394$.

To obtain further evidence for a lack of an impact of COMT on tDCS effects and WM performance, we performed post-hoc comparisons using non-parametric Mann-Whitney's U tests for the 2 main hypotheses. Specifically, previous studies predicted Met homozygotes would demonstrate impaired performance following AL-CR stimulation as compared to sham, whereas Val homozygotes would become impaired following CL-AR stimulation as compared to sham. Difference scores for pretest and posttest for each dependent variable were computed separately for the 2-back and 4-back, but none of the comparisons demonstrated significant stimulation group

differences, *p*s ≥ .326. As such, the results do not point towards a modulation of tDCS after-effects on WM by the COMT genotype.

Table 2. N-back scores

	AL-CR		CL-AR		Sham	
	Pretest	Posttest	Pretest	Posttest	Pretest	Posttest
2-back						
d' prime						
Met/Met	1.96 (.51)	2.39 (.49)	1.81 (1.14)	2.65 (.97)	1.73 (.38)	2.36 (.68)
Val/Met	1.98 (.61)	2.55 (.71)	1.78 (.59)	2.42 (.79)	2.24 (.80)	2.88 (.98)
Val/Val	1.72 (.62)	2.30 (.55)	2.26 (.72)	2.87 (.53)	1.83 (.50)	2.30 (.81)
Hit rate in %						
Met/Met	84.1 (9.4)	89.9 (6.1)	79.9 (13.4)	91.1 (8.8)	83.2 (8.5)	91.2 (5.6)
Val/Met	86.1 (8.4)	91.8 (8.9)	83.8 (7.8)	90.4 (7.1)	88.5 (10.0)	92.6 (7.4)
Val/Val	80.6 (9.7)	88.7 (3.8)	89.9 (6.1)	96.7 (2.2)	86.2 (7.1)	89.5 (8.5)
Correct reject. in %						
Met/Met	80.5 (5.2)	83.7 (6.6)	76.2 (15.3)	83.8 (10.7)	75.5 (6.3)	79.5 (11.4)
Val/Met	78.6 (8.4)	82.5 (7.9)	75.5 (11.1)	82.0 (12.0)	78.7 (12.3)	86.0 (11.8)
Val/Val	77.8 (11.3)	83.2 (9.9)	80.0 (9.4)	81.9 (11.2)	74.2 (8.6)	80.3 (11.4)
RT_{Target} *in ms*						
Met/Met	598 (75)	554 (76)	583 (48)	550 (57)	589 (53)	548 (52)
Val/Met	610 (51)	589 (57)	593 (73)	568 (67)	613 (73)	593 (54)
Val/Val	615 (50)	591 (73)	626 (73)	601 (69)	618 (55)	600 (75)
$RT_{Non\text{-}target}$ *in ms*						
Met/Met	558 (85)	502 (71)	560 (95)	495 (72)	526 (75)	482 (79)
Val/Met	543 (94)	480 (81)	545 (73)	499 (64)	506 (51)	458 (61)
Val/Val	522 (59)	495 (73)	535 (82)	461 (60)	540 (86)	486 (77)
4-back						
d' prime						
Met/Met	1.55 (.87)	2.17 (.70)	1.37 (.88)	1.96 (.91)	1.53 (.61)	2.02 (.63)
Val/Met	1.65 (.58)	2.26 (.60)	1.52 (.54)	2.27 (.81)	1.90 (.58)	2.64 (.80)
Val/Val	1.22 (.37)	1.69 (.55)	1.72 (.28)	2.28 (.42)	1.62 (.56)	2.26 (.62)
Hit rate in %						
Met/Met	57.9 (17.0)	65.6 (14.8)	54.8 (16.6)	57.6 (18.8)	57.3 (14.0)	64.1 (15.2)
Val/Met	58.5 (11.4)	64.3 (12.9)	60.8 (12.7)	65.4 (17.9)	62.8 (11.9)	71.8 (14.2)
Val/Val	54.0 (13.2)	63.3 (19.1)	57.4 (11.8)	63.7 (9.8)	56.4 (12.1)	63.1 (14.7)
Correct reject. in %						
Met/Met	89.1 (7.6)	94.5 (5.3)	86.4 (11.5)	93.9 (7.0)	89.0 (7.9)	94.0 (4.1)
Val/Met	90.3 (7.1)	95.8 (4.7)	87.5 (7.1)	95.6 (3.3)	92.5 (5.5)	96.5 (5.0)
Val/Val	85.4 (8.4)	89.0 (5.4)	92.5 (4.9)	96.7 (3.1)	91.7 (4.7)	96.7 (2.2)
RT_{Target} *in ms*						
Met/Met	595 (72)	573 (43)	616 (81)	589 (94)	605 (79)	567 (64)
Val/Met	601 (70)	572 (91)	623 (100)	563 (108)	575 (53)	531 (62)
Val/Val	593 (61)	524 (63)	583 (61)	541 (47)	600 (43)	542 (66)
$RT_{Non\text{-}target}$ *in ms*						
Met/Met	588 (83)	528 (78)	566 (69)	524 (76)	551 (74)	504 (74)
Val/Met	578 (62)	533 (56)	583 (51)	529 (61)	596 (69)	548 (67)
Val/Val	570 (79)	524 (72)	622 (23)	563 (68)	610 (72)	552 (78)

Average N-back scores with standard deviation in parentheses

Discussion

The present study investigated whether the after-effect of prefrontal tDCS is modulated by individual differences in DA function. To this end participants were genotyped for the COMT Val^{158}Met polymorphism to estimate prefrontal DA activity and completed tests of WM performance before and after tDCS over the DLPFC. Although a mild DA manipulation previously modulated the after-effect of tDCS on WM (Jongkees, Sellaro, et al., 2017), the current results indicate this effect does not extend to pre-existing differences in, rather than a manipulation of DA activity. Although the result contrasts with two previous studies on COMT genotype and online effects of prefrontal tDCS on behavioral performance (Nieratschker et al., 2015; Plewnia et al., 2013), this does not undermine the results from previous studies. Instead, our results add to them by suggesting two important implications for future studies on tDCS.

First, whereas previous studies looked at an interaction between COMT and *online* effects of tDCS (i.e., stimulation overlapping with the critical task), the present study examined *offline* effects of tDCS (i.e., stimulation *prior* to the critical task). Online effects of tDCS are likely to reflect transient changes in cortical excitability (Nitsche & Paulus, 2000), whereas offline effects of tDCS are related to changes in synaptic plasticity (Nitsche & Paulus, 2001; Nitsche, Nitsche, et al., 2003). As such, the current results combined with previous findings indicate that the COMT genotype might differentially affect tDCS-induced changes in cortical excitability and neural plasticity. Although the present study implies this distinction exclusively at a behavioral level of results, future studies might investigate whether online and offline effects on physiology are also differentially affected by COMT genotype. Such a distinction would notably contrast with the glutamatergic and GABAergic systems, which instead have been shown to be relevant for the offline but not online effects of tDCS (Nitsche, Fricke, et al., 2003).

Second, the results underscore a need for caution when generalizing results from pharmacological manipulation of a neurotransmitter system to results from pre-existing baseline differences in that system. Whereas administration of DA's precursor L-tyrosine did modulate the after-effect of

prefrontal tDCS on WM (Jongkees, Sellaro, et al., 2017), this pattern of results was not mirrored by the COMT genotype as shown in the present study. Although it is possible that similar effects are observable on a physiological level, e.g., the directionality of change in cortical excitability, the impact of genetic predisposition might not have been large enough to immediately produce detectable differences at the behavioral level. On the one hand, this might be explained by the possibility that pharmacological manipulation induces larger changes in a neurotransmitter system that more easily cross a threshold at which behavioral changes are observed. As such, it might be that the smaller effect of COMT genotype requires longer periods of stimulation, repeated stimulation and large sample sizes to become apparent. On the other hand, it is possible that manipulation of a neurotransmitter system exerts different physiological and behavioral effects than naturally-occurring variation in that system (Boy et al., 2011), leading to different interactions with the psychophysiology of tDCS.

Notably, in neither this study nor the study on L-tyrosine (Jongkees, Sellaro, et al., 2017) did tDCS have a main effect on WM. Although this might be taken as evidence against the efficacy of tDCS in enhancing cognition, it is important to consider the possibility that tDCS effects can require several sessions to become behaviorally observable, possibly strengthening the consolidation of practice between sessions (Au et al., 2016; Au, Karsten, Buschkuehl, & Jaeggi, 2017). More importantly for the interpretation of the present study, L-tyrosine was shown to modulate the effect of single-session tDCS whereas COMT genotype did not as shown here. In light of the possibility that COMT effects might be smaller than pharmacological manipulation of DA, future studies could examine whether COMT genotype does predict effects of tDCS following multiple sessions of stimulation, and as mentioned before, whether these effects are different for online and offline tDCS (Mancuso et al., 2016).

Regardless of the exact underlying mechanism, the differential effect of L-tyrosine and COMT on tDCS after-effects on WM cannot be attributed to methodological differences between studies such as type of montage or

duration of stimulation, which were identical in both studies (Jongkees, Sellaro, et al., 2017). One notable difference is that the present study includes a pretest of WM performance, which might have produced a learning effect that obscured tDCS-induced changes in performance and its interaction with COMT. Although a pretest was necessary to exclude the possibility that results were driven by baseline differences due to COMT genotype, the present study cannot definitively rule out that a learning effect accounts for the different results across studies. One method of alleviating this issue in future studies might be to use adaptive N-back tasks (Au et al., 2016; Jaeggi et al., 2014), which potentially lessen the obscuring effect of practice in static N-back tasks.

To conclude, the present study demonstrates no impact of COMT genotype on the impact of the after-effect of single-session prefrontal tDCS on WM. In doing so, this study indicates that (i) DA might differentially modulate the effects of online and offline tDCS, and (ii) more generally, tDCS results obtained in pharmacological studies should be generalized with caution to studies of individual differences in neurotransmitter function.

Chapter Eight

Influences of glutamine administration on response selection and sequence learning: a randomized-controlled trial

Jongkees, B. J., Immink, M. A., & Colzato, L. S. (2017). Influences of glutamine administration on response selection and sequence learning: a randomized-controlled trial. *Scientific Reports, 7:2693.*

Abstract

Precursors of neurotransmitters are increasingly often investigated as potential, easily-accessible methods of neuromodulation. However, the amino-acid glutamine, precursor to the brain's main excitatory and inhibitory neurotransmitters glutamate and GABA, remains notably little investigated. The current double-blind, randomized, placebo-controlled study provides first evidence 2.0 g glutamine administration in healthy adults affects response selection but not motor sequence learning in a serial reaction time task. Specifically, glutamine increased response selection errors when the current target response required a different hand than the directly preceding target response, which might indicate enhanced cortical excitability via a presumed increase in glutamate levels. These results suggest glutamine can alter cortical excitability but, despite the critical roles of glutamate and GABA in motor learning, at its current dose Gln does not affect sequence learning.

Introduction

There is growing research interest in evaluating the neuromodulatory effects of exogenous administration of neurotransmitter precursors on cognition. Upon administration, precursors are assumed to be converted into their end-products, thus increasing neurotransmitter levels and consequently, influencing cognitive function. For example, tyrosine and tryptophan are two amino acid precursors of monoamine neurotransmitters that have been demonstrated to provide neuromodulatory effects. Tyrosine is a precursor of dopamine and norepinephrine, and its administration has been shown to modulate, amongst others, working memory (Colzato, Jongkees, et al., 2013; Thomas et al., 1999) and Stroop performance (Deijen & Orlebeke, 1994) (for a review, see Jongkees et al., 2015). Tryptophan is a precursor of serotonin (5-HT) and its administration has been shown to affect social behaviour and mood (for reviews, see Silber & Schmitt, 2010; Steenbergen, Jongkees, Sellaro, & Colzato, 2016; Young, 2013), as well as improve or impair cognitive function depending on the individual's mood and stress level due to mild sedation (Silber & Schmitt, 2010). While neuromodulatory effects of tyrosine and tryptophan have attracted substantial research attention, the amino acid precursor glutamine (Gln) has not been well researched as a potential neuromodulator of cognitive function despite being the precursor of glutamate (Glu) and γ-aminobutyric acid (GABA) (Walls, Waagepetersen, Bak, Schousboe, & Sonnewald, 2015), which are the main excitatory and inhibitory neurotransmitters, respectively, within the brain (Petroff, 2002). After absorption into the circulatory system, Gln is able to pass through the blood-brain barrier (Lee, Hawkins, Viña, & Peterson, 1998) upon which it then increases Glu and GABA levels in the brain (Bowyer, Lipe, Matthews, Scallet, & Davies, 1995). Glu and GABA play critical roles in shaping cortical excitability and synaptic plasticity (Boy et al., 2010; Floyer-Lea, Wylezinska, Kincses, & Matthews, 2006; Nakamura, Kitagawa, Kawaguchi, & Tsuji, 1997; Stagg, Bachtiar, & Johansen-Berg, 2011; Werhahn, Kunesch, Noachtar, Benecke, & Classen, 1999; Ziemann et al., 2015). Because of their effects on cortical excitability, levels of Glu and GABA are implicated in, amongst

others, response selection and inhibition (de la Vega et al., 2014; Munakata et al., 2011; Snyder et al., 2010), impulsivity (Boy et al., 2011), error detection and response conflict monitoring (van Veen & Carter, 2006). Notably, cortical excitation facilitates the adjustment of synaptic strength via NMDA-receptor-driven long-term potentiation (LTP) (Ziemann & Siebner, 2008), thereby implicating Glu and GABA in learning as well. Given that Gln administration, via central changes in Glu and GABA levels, has the potential to alter cortical excitability and thus response selection and inhibition behaviour, it seems appropriate to consider the effects of Gln administration on sequence learning. Sequenced actions heavily rely on response selection processes (Deroost & Soetens, 2006) and the acquisition of sequence patterns is associated with increases in cortical excitability (Lin et al., 2011). Because sequenced actions are fundamental to most everyday tasks in humans (Clegg, DiGirolamo, & Keele, 1998), it is important to investigate the potential neuromodulatory effects provided by Gln administration.

Glutamate and GABA

As the primary excitatory and inhibitory neurotransmitters in the brain, higher levels of Glu and GABA respectively increase and decrease cortical excitability. It is hypothesized (de la Vega et al., 2014; Munakata et al., 2011; Snyder et al., 2010) that increased cortical inhibition due to high GABA levels can sharpen task-relevant representations in the cortex and inhibit competing responses, thereby facilitating response selection and inhibition processes. It could then be argued that increased cortical excitation due to high Glu levels might have the opposite effect by facilitating activation of competing responses, thus increasing the time necessary to resolve response selection processes and impairing accuracy of selection processes (de la Vega et al., 2014). This model of the roles of Glu and GABA in response selection is supported by studies that directly assessed brain neurotransmitter levels using magnetic resonance spectroscopy (MRS), albeit with a particular focus on GABA. For example, individual differences in regionally-specific GABA concentration have been shown to predict motor decision speed in a saccade

distractor task (Sumner, Edden, Bompas, Evans, & Singh, 2010), with higher levels predicting faster response initiation to a target in the face of distractors. Conversely, higher striatal GABA concentration has been associated with overall faster responses (Dharmadhikari et al., 2015) and higher accuracy (Haag et al., 2015) in the Simon task. Furthermore, higher GABA concentration, in particular in airplane pilot trainees, has been associated with a more serial as opposed to parallel action cascading strategy, which has been argued to indicate more efficient action control (Yildiz et al., 2014). Lastly, one study using MRS to assess the balance between Glu and GABA, rather than their individual levels, indicated a higher Glu-to-GABA ratio is associated with increased selection costs and slower reaction times in language production tasks (de la Vega et al., 2014). In sum, there is converging support for the idea that increased GABA facilitates response selection via reduced cortical excitability, whereas increased Glu impairs response selection via heightened cortical excitability.

With respect to learning, studies have indirectly examined GABA and Glu by assessing the behavioural effects of a history of concussive injuries, which is thought to lead to accumulation of brain GABA and consequently stronger intracortical inhibition. This has important implications for learning, as excitation of the cortex facilitates LTP-driven learning via activation of NMDA receptors (Ziemann & Siebner, 2008). Consistent with reduced LTP due to higher GABA levels, previously-concussed athletes demonstrated reduced synaptic plasticity and less implicit motor sequence learning in a serial reaction time (SRT) task when compared to unconcussed teammates (de Beaumont, Tremblay, Poirier, Lassonde, & Théoret, 2012). A follow-up study demonstrated that older concussed athletes had greater age-related decreases of Glu and that Glu concentration was positively related to motor sequence learning in the SRT task (de Beaumont et al., 2013). These findings are consistent with the important role of the primary motor cortex (M1) in sequence learning (Wright et al., 2016) and the fact that M1 is sensitive to Glu and GABA (Hasan et al., 2013; Stagg, 2014; Ziemann et al., 2001). In sum,

they suggest sequence learning would benefit from increased excitation and suffer from increased inhibition of the cortex.

The present study

Despite the critical roles of Glu and GABA in response selection and cortical excitability, we are not aware of any studies with healthy adults that have focused on the effects of Gln administration on these processes. Because of this lack of previous studies and the fact that Gln is the precursor to both Glu and GABA, which are hypothesized to have opposite effects on response selection and learning, it is difficult to establish *a priori* the direction in which Gln administration modulates performance. This is further compounded by the aforementioned finding that not just individual Glu and GABA levels but also the relative balance between the two determines performance (de la Vega et al., 2014) and it remains unclear in favour of which neurotransmitter Gln would modulate this balance, if at all. Nevertheless, the direction of our results can tentatively suggest how the Glu-to-GABA ratio is modulated. As indicated by the previously discussed findings, increased GABA facilitates response selection but impairs motor sequence learning. Thus, improved response selection and/or impaired learning performance following Gln administration could be indicative of an increase in GABA level (see also, de Beaumont et al., 2012). The opposite results, i.e. decrements in response selection and/or enhanced learning performance, would then be consistent with an increased Glu level (see also, de Beaumont et al., 2013).

To investigate the effect of low dose Gln (2.0 g) on sequence learning we utilized the SRT task (Abrahamse & Noordzij, 2011; Nissen & Bullemer, 1987; Schwarb & Schumacher, 2012), performance on which has been related to Glu and GABA levels (e.g., de Beaumont et al., 2013, 2012). It represents a simple 4-choice reaction time task and thus involves response selection, inhibition and error detection processes that may be sensitive to a Gln-induced manipulation of Glu and/or GABA levels. The response sequence can be varied randomly, in which case participants can rely solely on the stimulus for selecting the appropriate response and have a 25% chance of guessing the

correct response. As such, random blocks are particularly stimulus-oriented. However, the task also includes blocks with an embedded, second-order conditional (SOC) sequence that allows (unconscious) anticipation of the correct response and potentially induces a shift from stimulus-based to plan-based control (Tubau et al., 2007). The implicit learning of the sequence is typically reflected in a gradual decrease in response latency and modulation of this decline in response latency would indicate a potential influence of Gln on motor sequence learning. Contrasting results from stimulus-oriented, random blocks with those from SOC blocks can shed light on a possible differential effect of Gln on stimulus-based versus plan-based action control.

Methods

Participants

A total of 91 students from Leiden University were recruited to participate for money or course credit. Using a double blind, placebo-controlled design, participants were randomly assigned to receive either Gln ($N = 48$) or a neutral placebo ($N = 43$). See Table 1 for group characteristics. The groups did not differ in terms of gender distribution, age, weight, BMI, or hours of sleep the night before the study but the Gln group did contain significantly more left-handed individuals.

Study participation eligibility criteria were based on previous studies on neuromodulation from our lab (Colzato, Jongkees, et al., 2013; Colzato, Pratt, & Hommel, 2010; Colzato, Zech, et al., 2012; Steenbergen, Sellaro, & Colzato, 2014). Specifically, interested individuals were screened for cardiac, hepatic, renal, neurologic or psychiatric disorders, and medication (except oral or implanted contraceptives) or recreational drug use and those who reported any of these conditions were not eligible to participate. Females were only eligible to participate if they used either oral or implanted hormonal contraception, to reduce the impact of fluctuating estrogen-dopamine interactions on our results (Colzato & Hommel, 2014; Czoty et al., 2009; E. Jacobs & Esposito, 2011).

Prior to participation informed consent was obtained from all participants. The study conformed to the ethical standards of the Declaration of Helsinki and the protocol was approved by the local ethical committee (Leiden University, Institute for Psychological Research).

Table 1. Group characteristics

	Gln	Placebo	*p*
N, Total	48	43	
N, Male:Female	14:34	13:30	.912
N, Right:Lefthanded	40:8	42:1	.022
Age, years *M (SD)*	20.5 (2.5)	20.6 (2.5)	.904
Weight, kg *M (SD)*	66.1 (7.7)	65.1 (7.6)	.531
BMI, kg/m^2 *M (SD)*	21.6 (2.5)	21.9 (2.2)	.632
Sleep, hours *M (SD)*	7.3 (1.3)	6.8 (1.2)	.086

Glutamine administration

All doses were prepared and coded by a researcher not involved in running the study, to blind the experiment leader to the administered dose. Participants received either 2.0 g Gln or 2.0 g microcrystalline cellulose, a neutral placebo, dissolved in 400 mL of orange juice. Given the lack of prior studies on Gln administration and cognition, this dose was based on previous studies with tyrosine in our lab that showed reliable effects (Colzato, Jongkees, et al., 2013, 2014; Steenbergen, Sellaro, Hommel, et al., 2015). The dose of 2.0 g is safe and less than the normal daily intake of approximately 3-6 g Gln from protein (Gleeson, 2008) and far less than in studies on Gln and gastrointestinal function that administered 10 g or more daily (Arwert, Deijen, & Drent, 2003; Lima et al., 2013; Mitter et al., 2012).

Serial reaction time task

To assess response selection and sequence learning participants performed a standard SRT task (Abrahamse & Noordzij, 2011; Nissen & Bullemer, 1987; Schwarb & Schumacher, 2012) presented using E-Prime 2.0 software (Psychology Software Tools, Inc., Pittsburgh, PA, USA). In this task four horizontally-aligned empty squares were presented in the centre of the screen. On each trial one of the squares turns red and the participant must press a corresponding button on the QWERTY keyboard (from left to right: V, B, N,

M) using the index and middle fingers of the left (V, B) and right (N, M) hand. An error sound is presented if the wrong button is pressed, along with the Dutch words "Verkeerde toets!" ("Wrong button!"). Reaction time (RT) is measured in milliseconds as the latency in the key press to the stimulus and if RT exceeds 3,000 ms, the Dutch words "Te langzaam!" ("Too slow!") are presented. Following the response, the four empty squares appear for a 50 ms response-stimulus interval before the next stimulus is presented. Participants were instructed that accuracy and response speed were equally important in the task. Participants first completed three random ordered blocks, then twelve sequence learning blocks in which responses followed a 12-item SOC sequence (VBVNMBNVMNBM, see Reed & Johnson, 1994), which was cycled through ten times in each block. To determine sequence dependence or the serial effect as opposed to general practice effects, a random-ordered block was inserted followed by a final block that re-introduced the SOC sequence. The sudden introduction of a random response sequence likely interferes with the anticipation of responses in a plan-based action control style, requiring an abrupt shift to stimulus-oriented control. Hence RT and response errors are expected to sharply increase in the random block (Willingham, Nissen, & Bullemer, 1989) but performance is expected to recover when the sequence is reintroduced in the final SOC block. All blocks contained 120 trials and after completion of each block performance feedback indicating number of errors and mean RT was presented followed by a 30 s rest interval. Following completion of the final block, participants were asked to respond "Yes" or "No" to a question that asked if they noticed a pattern in the responses at any point of the task to determine explicit awareness of the serial sequence. When answering "Yes", participants were then asked to use the response keys to produce one cycle of the 12-item sequence as a recall test.

Procedure

Participants entered the lab to be tested individually between 09:00-10:00, having fasted overnight (compare Colzato, Jongkees, et al., 2013; Steenbergen et al., 2014). Informed consent was obtained, after which they consumed 2.0 g

of either Gln or placebo dissolved in 400 mL orange juice. Afterwards, apples and oranges were offered to prevent strong hunger. Although Gln has been shown to increase *in vitro* rat brain Glu and GABA levels as soon as 15 minutes (Bowyer et al., 1995), in line with previous studies on precursors of neurotransmitters, testing commenced precisely one hour after Gln or placebo administration to allow time for plasma and brain Gln levels to increase (compare Colzato, Jongkees, et al., 2013; Steenbergen et al., 2014; Steenbergen, Sellaro, Hommel, et al., 2015). Participants then performed the SRT task, which took approximately 30 minutes. Lastly, participants were debriefed on the nature and hypotheses of the study and were compensated and thanked for their participation.

Analysis

For each participant, REP was determined based on the number of error trials (incorrect key press) as a percentage of the total number of trials in each block. MRT was then calculated for each participant and each block, after removing error trials as well as trials with outlier RT (1.2%) based on RT that was more than 3 standard deviations above the individual's overall mean for correct trials. Participant REP and MRT were then submitted to separate repeated measures ANOVA that i) compared performance between Gln and placebo Groups in the first three stimulus-oriented Blocks, ii) compared sequence learning between Gln and placebo Groups in SOC Blocks 1-11, and iii) compared sequence-dependent learning in Gln and placebo Groups across Blocks 12 (SOC), 13 (random) and 14 (SOC). Although others (Abrahamse & Noordzij, 2011; Willingham et al., 1989) proposed averaging performance over SOC Blocks 12 and 14 before comparison with random Block 13, performance in Block 14 might still suffer from interference by the previous random block. As such, we argue that comparing all three blocks can provide a clearer picture of how performance is affected by the introduction of the random block. Explicit sequence awareness frequency was compared between Gln and placebo groups using X^2 analysis. Sequence recall was analysed based on a response chunking approach (Jiménez, 2008; Koch & Hoffmann, 2000;

Verwey & Abrahamse, 2012). The presence of chunks of the training SOC sequence was determined for each participant with chunk lengths ranging between 4 to 12 items. The probability of entering the smallest chunk length, a 4-item chunk, by chance was calculated as 11% (.33 x .33) given that there are no consecutive repetitions in the SOC sequence structure (Borragán, Slama, Destrebecqz, & Peigneux, 2016). To identify any matches between the participant's recalled sequence and the target sequence, the participant's sequence was divided into chunks made up of between 4 and 12 items. These chunks could commence with any sequence item, with the condition that the end of the sequence could not be extended to the initial sequence items since the participant's chunk needed to be contiguous. The target sequence was also divided into chunk lengths of between 4 and 12 items, however, here these chunks could start with any item in the sequence and continue on to include items at the beginning of the sequence. Continuing the chunks past the end of the SOC sequence reflects the repeating nature of the sequence, meaning the participant could have treated the commencement of a chunk at any point of the repeated sequence. Performance on chunk recall of the sequence was based on the number of matched chunks and mean length of the matched chunks for each participant. Only the longest chunk was recorded as a match and matched chunks were only recorded once in the event the participant repeated the same chunk. As the 12-item sequence recall allows for 9 possible 4-item chunks, a participant would be expected to recall approximately 1 valid 4-item chunk by chance (9 x .11). Participant's recalled chunk count and mean chunk length were separately submitted to ANOVA for Group comparisons. All repeated measures analyses use Greenhouse-Geisser correction when the sphericity assumption was violated and all post-hoc comparisons use Fisher's LSD adjustment. For all tests a significance threshold of .05 was adopted.

Random ordering of responses in the first three stimulus-oriented blocks and random Block 13 could have introduced group differences in the number of reversal trials resulting in MRT performance artefacts (Reed & Johnson, 1994; Vaquero, Jiménez, & Lupiáñez, 2006). A reversal trial occurs when the third trial of any three consecutive trails involves the same target

response as the first trial (Vaquero et al., 2006). With respect to the number of reversal trials in the first three stimulus-oriented blocks, there was a non-significant effect of Group (p = .21) and a non-significant Group x Block interaction (p = .13). In addition, the number of reversal trials in Block 13 did not significantly differ between Groups (p = .12). In stimulus-oriented blocks, MRT was significantly longer in reversal trials than in non-reversal trials, $F(1,89) = 145.8$, $p < .0001$, partial $\eta^2 = 0.62$, however, there was a non-significant Group x Reversal Trial interaction (p = .68) and a non-significant Group x Reversal Trial x Block interaction (p = .61). In SOC blocks, MRT was significantly longer in reversal trials than in non-reversal trials, $F(1,89) = 24.5$, $p < .0001$, partial $\eta^2 = 0.22$, however, there was again a non-significant Group x Reversal Trial interaction (p = .56) and a non-significant Group x Reversal Trial x Block interaction (p = .89). This indicates any group differences in these blocks are not confounded by differences in the number of reversal trials.

Results

To assess the effect of Gln on processes associated with response selection and inhibition, one group of participants was administered 2.0 g Gln (N = 48) and another group received a neutral placebo (N = 43). Groups were then compared on response error percentage (REP) and mean reaction time (MRT) in the SRT task.

Response error percentage

REP results are illustrated in Figure 1 (top panel). In the first three stimulus-oriented blocks, repeated measures ANOVA indicated a non-significant effect of Group, *(*p = .21), but a significant effect of Block, $F(2,178) = 20.6$, $p < .001$, partial $\eta^2 = .19$ and a significant Group x Block interaction, $F(2,178) = 4.1$, $p = .02$, partial $\eta^2 = .04$. REP for the Gln group in stimulus-oriented block 1 (M = 1.96 , SD = 1.53) and block 2 (M = 3.40, SD = 2.50) was not significantly different from REP for the placebo group in block 1 (M = 2.17 , SD = 2.06) and block 2 (M = 3.10, SD = 2.02) (p = .58, .53). However, in block 3 REP was significantly higher (p = .012) in the Gln group (M = 4.15 , SD = 2.37) than the

placebo group (M = 2.97 , SD = 1.97). To further explore the effect of Gln on REP we divided error trials in the first three stimulus-oriented blocks according to whether current error trial and the preceding trial required the same or a switched hand to make the response. REP was then submitted to repeated measures ANOVA with the factors Group and Hand Switch (same vs. switched hand). This revealed no significant interaction between Group and Hand Switch nor a three-way interaction involving Block, both $p > .490$. An additional analysis with the factors Group and Switch Direction (left-to-right vs. right-to-left) revealed no significant interaction between Group and Switch Direction nor a three-way interaction involving Block, both $p > .731$.

For SOC sequence blocks 1-11, repeated measures ANOVA indicated a significant effect of Group, $F(1, 89) = 4.7$, $p = 0.03$, partial $\eta^2 = .05$, and Block, $F(10,890) = 2.3$, $p = .01$, partial $\eta^2 = .03$, while there was no significant Group x Block interaction, *(*$p = .97$). Across these blocks, the Gln group had significantly higher REP (M = 3.67, SD = 2.55) than the placebo group (M = 2.91, SD = 2.04). REP in blocks 4 and 5 was significantly higher than in block 1 while REP in block 11 was significantly lower than block 3-5 (all $p < .05$).

To further explore the effect of Gln on REP we divided error trials in SOC blocks 1-11 again according to whether current error trial and the preceding trial required the same or a switched hand to make the response. The total number of errors per SOC block was then submitted to repeated measures ANOVA with the factors Group and Hand Switch. This revealed a significant effect of Group $F(1,89) = 4.34$, $p = .04$, $\eta^2 = .046$ and Hand Switch, $F(1,89) = 123.1$, $p < .001$, $\eta^2 = .58$, as well as a significant Group x Hand Switch interaction, $F(1,89) = 4.5$, $p = .037$, $\eta^2 = .048$. Post-hoc comparisons indicated a significantly higher total amount of errors on trials requiring a switch of hands in both the Gln (M = 43.9, SD = 22.5 vs. M = 18.9, SD = 10.0) and placebo (M = 34.0, SD = 17.4 vs. M = 17.0, SD = 11.2) groups, both ps < .001. Importantly, the Gln group demonstrated significantly more switch hand errors ($p = .023$) than the placebo group whereas the groups did not differ on same hand errors ($p = .406$). Exploring these results yet further by dividing switch trials in those requiring a left-to-right or right-to-left switch revealed

significant effects of Group, $F(1,89) = 5.4$, $p = .023$, $\eta^2 = .057$ and Switch Direction, $F(1,89) = 55.6$, $p < .001$, $\eta^2 = .384$, indicating significantly more errors on a left-to-right ($M = 23.1$, $SD = 12.7$) than right-to-left switch ($M = 16.3$, $SD = 9.7$), but this effect was not modulated by Gln as revealed by a nonsignificant Group x Switch Direction interaction, $p = .658$. In sum, Gln increased response errors when the hand required to carry out the target response on the current trial was not the hand used on the preceding trial and this effect was independent of the direction of the switch. To exclude the possibility this effect is driven by a group difference in amount of left-handed participants (see Table 1), we again analysed the effects of Group and Hand Switch on REP after excluding all left-handed participants. The Group x Hand Switch interaction remained significant and in the same direction, $F(1, 80) = 4.4$, $p = .039$, $\eta^2 = .052$, thereby excluding a potential confounding of the effect by group differences in handedness.

To assess sequence dependent learning we compared REP in the 12th SOC block to the 13th random block and the 14th SOC block. Repeated measures ANOVA indicated a significant effect of Block, $F(2,178) = 128.1$, $p < .001$, partial $\eta^2 = .59$, while the Group main effect ($p = .21$) and the Group x Block interaction, ($p = .16$) were not significant. REP in block 13 ($M = 6.25$, $SD = 3.02$) was significantly higher than block 12 ($M = 2.82$, $SD = 1.74$, $p < .0001$) and block 14 ($M = 2.11$, $SD = 1.90$, $p < .0001$). REP in block 12 was also significantly higher ($p < .01$) than in block 14. Additional analyses revealed no significant interaction between Group and Hand Switch, $F(1, 89) = .22$, $p = .642$, or between Group and Switch Direction (left-to-right vs. right-to-left), $F(1, 89) = .13$, $p = .722$.

Mean reaction time

MRT results are presented in Figure 1 (bottom panel). In each of three analyses, there was only a significant effect of Block in the three stimulus-oriented blocks, $F(2,178) = 89.2$, $p < .001$, partial $\eta^2 = 0.50$, in SOC blocks 1-11, $F(10,890) = 104.4$, $p < .001$, partial $\eta^2 = 0.54$, and when comparing SOC block 12 to random block 13 and SOC block 14, $F(2,178) = 321.4$, $p < .001$,

partial $\eta^2 = 0.78$. In contrast, no significant main effects of Group or Group x Block interactions were obtained (all $p > .53$). In the stimulus-oriented blocks, MRT was significantly higher in block 1 ($M = 454.51$, $SD = 59.02$) than block 2 ($M = 413.86$, $SD = 50.19$, $p < .0001$) and block 3 ($M = 413.99$, $SD = 51.82$, $p < .0001$) while MRT did not significantly differ between blocks 2 and 3 ($p = .99$). For SOC sequence learning blocks, MRT was significantly lower as learning progressed across blocks 1 to 11. For sequence-dependent learning assessment blocks, MRT in block 13 (M = 396.18, SD = 34.41) was significantly higher than block 12 (M = 299.69, SD = 56.10, p < .0001) and block 14 (M = 302.16, SD = 49.80, p < .0001). MRT in block 12 was not significantly different (p = .37) than MRT in block 14. None of the stimulus-oriented blocks, SOC sequence learning blocks or sequence-dependent learning blocks involved a significant Group x Hand Switch interaction, all $p > .31$, or a significant Group x Switch Direction interaction, all $p > .63$. In sum, Gln did not affect MRT in any of the SRT blocks.

Sequence recall

The frequency of participants reporting explicit awareness of a sequence pattern was not significantly different between Gln ("Yes" = 36, "No" = 12) and placebo ("Yes" = 34, "No" = 9) groups $X^2(91) = 0.21$, $p = 0.65$. The number of sequence chunks recalled did not significantly differ between Gln (M = 1.04, SD = 0.54) and placebo groups ($M = 1.00$, $SD = 0.56$, $p = 0.72$). Similarly, the average length of sequence chunks recalled did not significantly differ between Gln (M = 4.65, SD = 2.70) and placebo groups ($M = 4.91$, $SD = 2.87$, $p = 0.66$). In sum, Gln did not seem to affect measures of sequence recall.

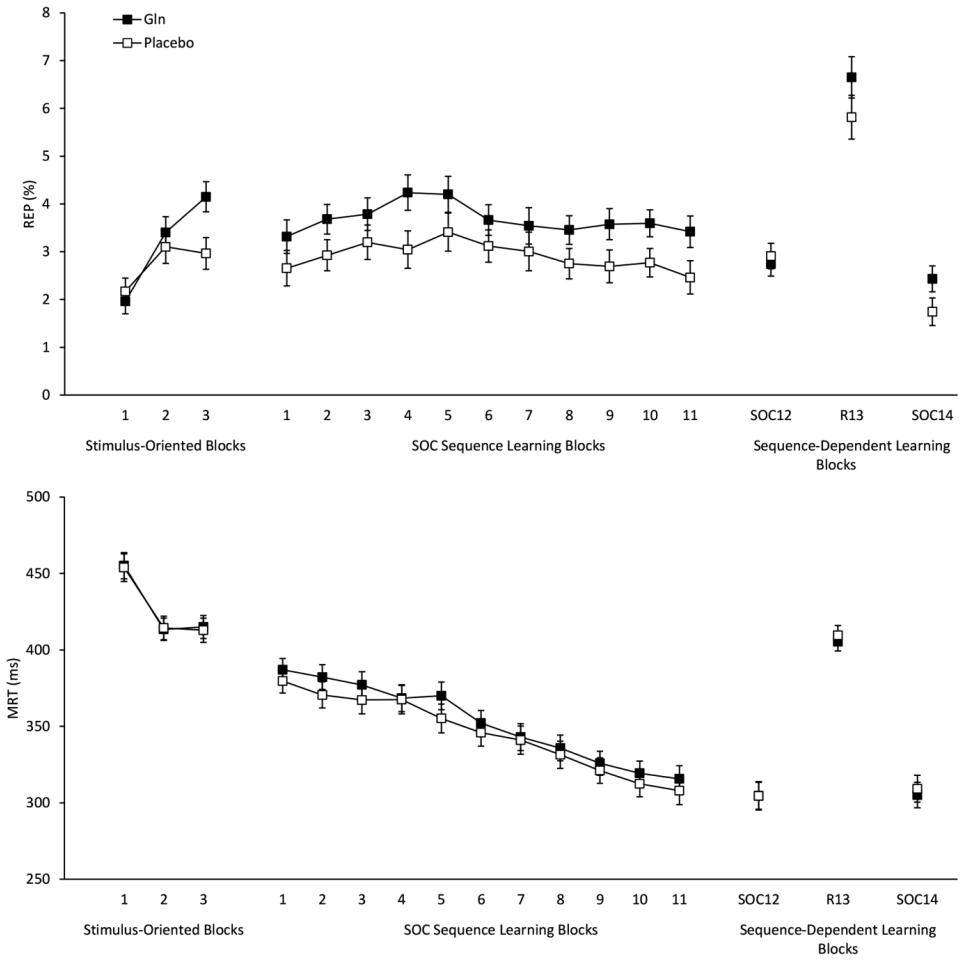

Figure 1. Mean REP (top panel) and MRT (bottom panel) as a function of block and group (Gln vs. placebo). 'SOC' refers to an SOC sequence block and 'R' refers to a random sequence block. Bars represent standard error of the means.

Discussion

The present paper is one of the first to report proof-of-principle that the amino acid Gln, the precursor of the brain's main excitatory neurotransmitter Glu and inhibitory neurotransmitter GABA (Petroff, 2002; Walls et al., 2015), modulates cognitive function related to response selection but not sequence learning. Specifically, Gln administration led to an overall increase in response

selection errors in both stimulus-oriented and SOC sequence-learning blocks of an SRT task without affecting sequence-dependent learning or sequence recall, suggesting Gln affected primarily stimulus-based rather than plan-based control (Tubau et al., 2007). More specifically, Gln impaired performance when the hand required to carry out the target response differed on the current and preceding trial, indicating Gln primarily affected the laterality of response selection processes. This raises the possibility that Gln, via a presumed increase in Glu, enhanced the lateral motor activation associated with the most recent target response, which presented conflict when the next trial required the other hand to press the correct key. This notion is indirectly supported by findings on GABA and the Simon task (Dharmadhikari et al., 2015; Haag et al., 2015) that suggest increased cortical inhibition benefits processing of laterality of responses. The opposite, that is in impairment in processing the laterality of responses, might then be expected when cortical inhibition is reduced by Glu. Consistent with this idea, heightened cortical excitability has been hypothesized to account for impaired response selection by facilitating activation of competing responses (de la Vega et al., 2014). Furthermore, it is interesting to note the increase in response selection errors emerged only after the first two (stimulus-oriented) blocks, indicating Gln might have affected performance only after initial task familiarization when participants might start responding with less deliberation and instead perform more automatically. On the other hand, the Gln effect seems to have worn off near the end of the task, suggesting that at this point Gln may have been metabolized down to levels that no longer influenced behaviour or participants in the Gln condition could overcome the effect on response selection with sufficient training. It is also important to note the Gln-induced increase in response errors was not due to a speed-accuracy trade-off, as response latencies were unaffected. In sum, the present study provides first evidence indicating Gln administration can modulate cognitive-behavioural performance by enhancing cortical excitability.

Whereas Gln seems to have affected response selection processes in both stimulus-oriented (random) and SOC blocks, its effects did not seem to

extend to sequence-dependent learning. This might tentatively indicate that LTP-driven plasticity was not altered despite the presumed increase in cortical excitation. For now we can only speculate on the reason for this selectivity. First, it might be our Gln dose of 2.0 g was too low to induce changes in synaptic plasticity. Previous studies that mainly focused on Gln's positive effects on gastrointestinal function administered daily doses of 10 g or more (Arwert et al., 2003; Lima et al., 2013; Mitter et al., 2012), which exceeds the average daily intake of 3-6 g Gln (Gleeson, 2008) and is 5 or more times our dose of 2.0 g. It remains unclear whether a higher dose could lead to a more pronounced and longer-lasting cognitive response, hence future studies might systematically vary Gln dose to clarify this issue. Second, Gln might have affected response selection but not sequence-dependent learning because of regional specificity, in line with reports of regionally-specific effects of GABA (Boy et al., 2010, 2011; Sumner et al., 2010). Although this remains speculative, perhaps Gln at a dose of 2.0 g primarily affected response conflict and impulsivity in the prefrontal cortex without affecting sequence-dependent learning mediated by motor area M1 (Wright et al., 2016). Future studies could employ MRS to assess whether Gln has dissociable effects on Glu and GABA levels in different regions. Third, in the present study three random, stimulus-oriented blocks were always presented before twelve SOC blocks. This order of presentation might have predisposed participants to stimulus-based rather than plan-based control and discouraged sequence learning in SOC blocks, limiting the possibility of finding an effect of Gln on plan-based control. Hence, whereas this study presented the random blocks *after* Gln administration and immediately before starting the SOC blocks, future studies may wish to present the first random blocks *before* Gln administration to render them more as task familiarization, or systematically vary the order in which the blocks are presented.

The lack of previous studies with Gln and cognitive-behavioural performance made it difficult to predict *a priori* the direction in which Gln would enhance performance. However, the present results may form a basis for novel hypotheses that can be tested in the future and thereby provide

converging evidence that Gln modulates performance via increased cortical excitability. For example response inhibition, i.e. the ability to withhold prepotent responses, seems to benefit from increased cortical inhibition due to increased GABA levels (Quetscher et al., 2015; Steenbergen, Sellaro, Stock, Beste, et al., 2015). Similarly, higher GABA levels have been associated with less impulsivity (Boy et al., 2011). This suggests the opposite, that is an impairment in response inhibition and increase in impulsivity, might occur when cortical excitability is enhanced due to Gln-induced increases in Glu levels. Hence a study showing Gln reduces response inhibition efficiency and/or increases impulsivity would converge on the idea Gln enhances cortical excitability.

The present study employed a between-subjects design because asking participants whether they noticed a response sequence in the SRT task is likely to affect subsequent task performance. Because of the between-subjects design, one might argue our results are simply due to baseline group differences in response selection efficiency rather than due to the Gln administration. Although we argue this is unlikely with our sample size that is larger than in most amino-acid precursor studies, this is not to say such alternative explanations of our data are impossible. Therefore, future studies may wish to exclude the possibility of pre-existing group differences by i) using tasks that allow for a within-subjects design, ii) assessing baseline response selection performance and iii) taking pre and post-administration measurements of cortical excitability, for example by using motor-evoked potentials (MEP) to assess both pre-existing differences and Gln-induced changes in cortical excitability. If MEP measurements confirm enhanced excitability of the cortex after Gln administration and if this change would correlate with the individual frequency of response selection errors, that would provide strong support for the mechanism of action hypothesized in the present study.

It may also be interesting for future studies to consider the role of individual differences in the balance between cortical Glu and GABA levels, as variability in this balance rather than the individual neurotransmitter levels

has previously been shown to predict individual differences in response selection efficiency (de la Vega et al., 2014). Although the present study suggests Gln administration at a group level enhanced this balance in favour of Glu, it seems plausible individual response to Gln might be predicted by the pre-existing Glu/GABA balance, with Gln perhaps having more pronounced or even opposite effects in individuals with a balance highly in favour of Glu or GABA.

Lastly, it is important to consider that the effect of Gln reported here seems to apply to sequential motor control but not sequence learning, as Gln affected performance similarly in the stimulus-oriented and SOC blocks of the SRT task. However, the present version of the SRT task includes relatively few stimulus-oriented blocks, which limits the assessment of sequential motor control separately from sequence learning. To disentangle and separately investigate these processes, future research should aim to balance the amount of stimulus-oriented and SOC blocks (Vaquero et al., 2006).

To conclude, the present study is the first to investigate Gln administration in healthy adults in relation to response selection and sequence learning performance. Results show Gln impairs response selection but does not alter sequence learning, suggesting an increase in cortical excitability without affecting synaptic plasticity. As such, despite the critical roles of Glu and GABA in motor learning, this study finds no evidence for an effect of their precursor on sequence learning performance but does present first evidence that Gln modulates sequential motor control processes.

Chapter Nine

Transcutaneous vagus nerve stimulation (tVNS) enhances response selection during sequential action

Jongkees, B. J., Immink, M. A., Finisguerra, A., & Colzato, L. S. (Submitted). Transcutaneous vagal nerve stimulation (tVNS) enhances response selection during sequential action.

Abstract

Transcutaneous vagus nerve stimulation (tVNS) is a non-invasive and safe technique that transiently enhances brain GABA and noradrenaline levels. Although tVNS has been used mainly to treat clinical disorders such as epilepsy, recent studies indicate it is also an effective tool to investigate and potentially enhance the neuromodulation of action control. Given the key role of GABA in neural plasticity and cortical excitability, we investigated whether tVNS, through a presumed increase in brain GABA concentration, modulates sequential behavior in terms of response selection and sequence learning components. To this end we assessed the effect of single-session tVNS in healthy young adults (N = 40) on performance on a serial reaction time task, using a single-blind, sham-controlled between-subject design. Active as compared to sham tVNS did not differ in terms of acquisition of an embedded response sequence and in terms of performance under randomized response schedules. However, active tVNS did enhance response selection processes. Specifically, the group receiving active tVNS did not exhibit inhibition of return during response reversals (i.e., when trial N requires the same response as trial N-2, e.g. 1-2-1) on trials with an embedded response sequence. This finding indicates that tVNS enhances response selection processes by increasing availability of response structure information to prevent disengagement from a recently performed response. More generally, these results add to converging evidence that tVNS enhances action control performance.

Introduction

Non-invasive methods of brain stimulation have become an increasingly popular approach to probing the relationship between neurochemistry and cognitive-behavioral performance. Although transcranial direct current stimulation (tDCS) is currently the subject of great scientific interest (Plewnia et al., 2015), it has recently been suggested that transcutaneous (through the skin) vagus nerve stimulation (tVNS) may be a novel technique to investigate and potentially enhance the neuromodulation of action control (van Leusden,

Sellaro, & Colzato, 2015). Converging evidence from animal and clinical studies suggests that tVNS increases levels of GABA (Ben-Menachem et al., 1995; Marrosu et al., 2003) and noradrenaline (NA) in the brain (Raedt et al., 2011; Roosevelt, Smith, Clough, Jensen, & Browning, 2006). Consistent with this literature, tVNS has been shown to increase intracortical inhibition in healthy adults (Capone et al., 2015), supporting the idea that tVNS might alter and potentially enhance performance related to the GABAergic and noradrenergic systems. Given the crucial role for GABA in the neuromodulation of response selection (Bar-Gad, Morris, & Bergman, 2003; de la Vega et al., 2014; Munakata et al., 2011) and motor learning (Floyer-Lea et al., 2006; Stagg et al., 2011), we investigated the effects of tVNS on implicit sequence learning and response selection processes underlying sequential action.

The neurochemical effects of tVNS have the potential to alter cortical excitability and synaptic plasticity, which are shaped by brain GABA concentration (Boy et al., 2010; Floyer-Lea et al., 2006; Nakamura et al., 1997; Stagg et al., 2011; Werhahn et al., 1999; Ziemann et al., 2015). Consistent with this neuromodulatory role, individual differences in GABA level have been related to response selection and inhibition (de la Vega et al., 2014; Munakata et al., 2011; Snyder et al., 2010), impulsivity (Boy et al., 2011), error detection and conflict monitoring (van Veen & Carter, 2006), as well as implicit motor learning (de Beaumont et al., 2012; Stagg et al., 2011). These findings raise the possibility that tVNS, via a transient increase in GABA concentration, might modulate and potentially enhance such processes (van Leusden et al., 2015).

Recent studies confirm this hypothesis by showing that tVNS can indeed improve cognitive-behavioral performance. While the effect of tVNS on sequenced action, defined here as a sequence of movements that are serially ordered to achieve a task goal (Abrahamse, Ruitenberg, de Kleine, & Verwey, 2013; Sakai, Hikosaka, & Nakamura, 2004), has not been previously addressed, previous work has demonstrated that tVNS can enhance processes thought to underlie motor sequence performance and learning. For example,

Beste et al. (2016) demonstrated improved inhibitory control from tVNS. As robust response selection is crucial to sequenced actions (Deroost & Soetens, 2006), enhanced inhibition from tVNS might facilitate selection of the target response through suppression of competing non-target alternatives (Colzato, Ritter, & Steenbergen, 2018; de la Vega et al., 2014; Munakata et al., 2011). Consistent with this notion, Steenbergen et al. (2015) reported that tVNS enhanced response selection when two responses were executed in succession. In addition to response selection processes, tVNS has been reported to enhance processes that have been associated with the acquisition of sequenced movements. When responses follow an implicit sequential structure, associative memory allows for development of an integrated representation of the sequence or sequence elements based on formed associations between responses (Hommel, 1996). Interestingly, it has recently been shown that tVNS improves associative memory (Jacobs, Riphagen, Razat, Wiese, & Sack, 2015). Furthermore, increased post-error slowing is thought to be an important component of sequence learning (Ruitenberg, Abrahamse, de Kleine, & Verwey, 2014) as it reflects upon rule-based performance (Tam, Maddox, & Huang-Pollock, 2013). Sellaro et al. (2015) demonstrated increased post-error slowing from tVNS. In sum, these findings support the hypothesis that tVNS can enhance response selection processes during sequential action.

However, there is also the possibility that tVNS can result in suppression of sequential learning. Sequence acquisition is typically associated with an *increase* rather than a *decrease* in cortical excitability (Lin et al., 2011), and indeed, some have demonstrated that increased GABA predicts reduced implicit motor sequence learning (de Beaumont et al., 2012; Stagg et al., 2011). In light of these previous studies, the effect of tVNS on sequence acquisition remains uncertain. Therefore, the present study set out to clarify the effect of tVNS on sequence acquisition and response selection during sequential action.

The present study

In more general terms, with the present study we set out to extend the literature on tVNS enhancement of cognitive-behavioral performance by investigating

its potential to improve sequential action control. Given that tVNS increases brain GABA, which is crucial to the modulation of action control processes (Bar-Gad et al., 2003; de la Vega et al., 2014; Floyer-Lea et al., 2006; Munakata et al., 2011; Stagg et al., 2011), we tested the hypothesis that tVNS might enhance sequential action as assessed on a serial reaction time task (SRTT) (Nissen & Bullemer, 1987). The SRTT is a 4-choice reaction time task that involves response selection, inhibition of non-target responses and implicit formation of response sequence structures, each of which may be sensitive to GABA and NA changes from tVNS. Typically, a second-order conditional (SOC) response sequence is embedded in the SRTT unbeknownst to the participants. Implicit acquisition of the sequence structure results in increasingly shorter response latencies and less response errors as the task progresses (Abrahamse & Noordzij, 2011; Nissen & Bullemer, 1987; Schwarb & Schumacher, 2012). However, there is potential difficulty in disentangling the nature of these improvements (Jongkees, Immink, et al., 2017) as performance improvements might not necessarily be due to implicit learning processes but rather reflect general practice effects (Abrahamse & Noordzij, 2011). For this reason, a transfer approach is used to judge the extent by which performance improvements rely on the practiced sequence (Abrahamse & Noordzij, 2011; Robertson, 2007; Willingham, 1999). In the SRTT variation employed in the present experiment, each block of trials included both an embedded SOC sequence as well as a transfer sequence based on a pseudo-random stimulus presentation schedule. In addition to evaluating performance improvement across practice, this approach allowed for comparisons between sequenced trials and randomised trials as an index of sequence learning. Post-error slowing was also evaluated for trials under sequenced and random schedules to investigate the effects of tVNS on sequence learning processes. As tVNS might not enhance sequence learning but rather improve response selection processes, overall task accuracy and reaction time (RT) performance was assessed under the view that increased accuracy or reduced response latency under tVNS reflects efficiency of selecting the target response. To probe inhibitory processes that are relied upon to select target responses, we

applied the concept of inhibition of return (Posner & Cohen, 1984; see Klein, 2000; Lupiáñez, Tudela, & Rueda, 1999 for reviews) to the SRTT to further investigate response selection processes under tVNS. In the SRTT, inhibition of return is evaluated by comparing RT on reversal trials to non-reversal trials (Vaquero et al., 2006). A reversal trial is defined as occurring when the target response location for trial N is a repetition of the target response location for trial N-2 (e.g., 1-2-1; Vaquero et al., 2006). Longer response latencies for reversal trials as compared to non-reversal trials reflects inhibition of an action that has been recently performed (Klein, 2000). Increased GABA levels due to tVNS might result in suppression of inhibition of return, thereby allowing efficient selection of a response even when it has been recently performed.

Methods

Participants

Forty undergraduate students from Leiden University were offered partial course credit for participation in a study on tVNS. Participants were randomly assigned to either the active ($N = 20$) or sham ($N = 20$) tVNS group. The groups were comparable with respect to age ($M = 22.3$ vs 22.5 years, SD = 2.7 vs 2.5, respectively), t(38) = .244, $p = .809$, and gender distribution, ($F:M$ = 14:6 vs 18:2, respectively), $X^2(1, N = 40) = 2.50$, $p = .114$. Participants were screened individually using the Mini International Neuropsychiatric Interview (MINI), a short, structured interview of approximately 15 min that screens for several psychiatric disorders and drug use (Sheehan et al., 1998), and has been used previously in neuromodulation research (Jongkees, Immink, et al., 2017; Jongkees, Sellaro, et al., 2017). Participants were included if they met the following criteria: (i) between 18 and 30 years; (ii) no history of neurological or psychiatric disorders; (iii) no history of substance abuse or dependence; (iv) no chronic or acute medication; and (v) no implants or cardiac disorders for safety reasons concerning the tVNS. Before the start of the study, participants were informed of the procedure and potential side-effects of the tVNS (i.e., itching, stinging or burning sensation from the electrodes, reddening of the skin and head ache). None of the participants reported major side-effects. The study

conformed to the ethical standards of the declaration of Helsinki with written informed consent from all subjects and the protocol was approved by the local ethical committee (Leiden University, Institute for Psychological Research).

Transcutaneous vagus nerve stimulation

tVNS stimulates the afferent auricular branch of the vagus nerve, which is located medial of the tragus at the entry of the acoustic meatus (Kreuzer et al., 2012). In order to avoid stimulation of fibers to the heart, tVNS is safe to be applied to the left but not the right ear (Kreuzer et al., 2012; Sperling et al., 2010). The tVNS device consisted of two titan electrodes mounted on a gel frame and connected to a wired neurostimulating device (CMO2, Cerbomed, Erlangen, Germany), see Figure 1. Following the suggestions by Dietrich et al. (2008) for optimal stimulation, the tVNS® device was programmed to a stimulation intensity of .5 mA, delivered with a pulse width of 200-300 μs at 25 Hz. Both active and sham stimulation constantly alternated between active stimulation for 30 s, followed by a break of 30 s. Consistent with (Kraus, Kiess, Schanze, Kornhuber, & Forster, 2007), sham stimulation was applied by placing the electrodes over the center of the left ear lobe instead of the outer auditory canal, as the ear lobe is free of vagus innervation (Peuker & Filler, 2002) and its stimulation produces no activation in the cortex and brain stem (Kraus et al., 2013).

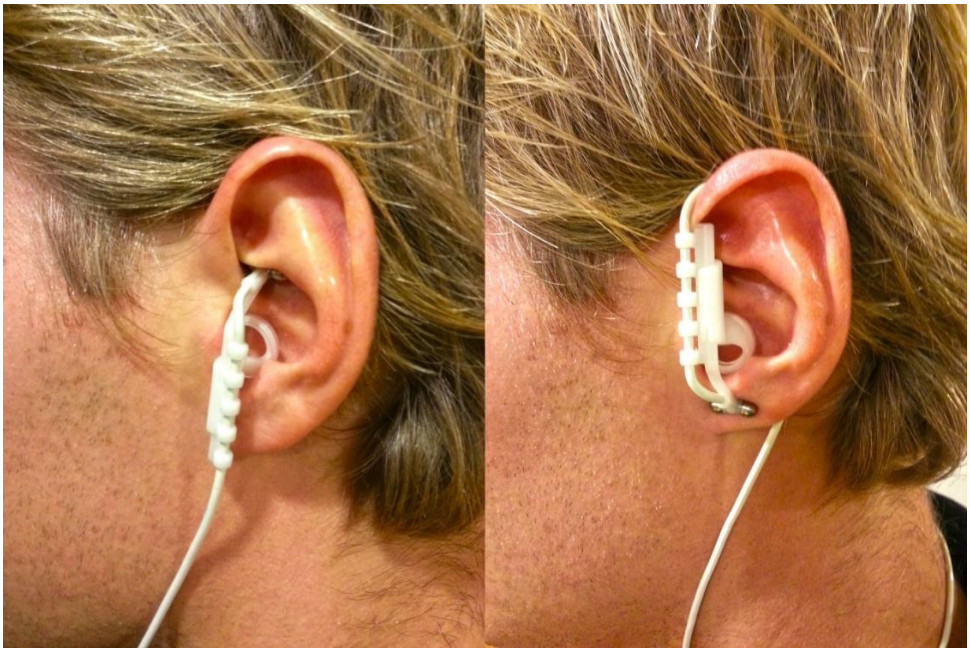

Figure 1. Positioning of the tVNS electrodes in the active (left) and in the sham (right) condition.

Serial reaction time task

To assess response selection and sequence learning, participants performed an adapted SRT task (Vaquero et al., 2006) presented using E-Prime 2.0 software (Psychology Software Tools, Inc., Pittsburgh, PA, USA). In this task four horizontally-aligned empty squares are presented in the centre of the screen. On each trial one of the squares turns red and the participant must press a corresponding button on the QWERTY keyboard (from left to right: V, B, N, M) using the index and middle fingers of the left (V, B) and right (N, M) hand. An error sound is presented if the wrong button is pressed, along with the Dutch words "Verkeerde toets!" ("Wrong button!"). Reaction time (RT) is measured in milliseconds as the latency in the key press to the stimulus and if RT exceeds 3,000 ms, the Dutch words "Te langzaam!" ("Too slow!") are presented. Following the response, the four empty squares appear for a 50 ms response-stimulus interval before the next stimulus is presented. Participants

were instructed that accuracy and response speed were equally important in the task.

Participants completed 3 task familiarization blocks of 120 randomly sequenced trials prior to stimulation, and then performed 15 experimental blocks each consisting of 10 cycles of 12 trials while stimulation was applied. Each experimental block alternated between a cycle of random trials and two cycles of SOC trials (R-SOC-SOC-R-SOC-SOC-R-SOC-SOC-R), with each SOC cycle containing the same 12-item response sequence (VBVNMBNVMNBM) (Reed & Johnson, 1994). Whereas performance gradually improves on SOC trials as the response sequence is implicitly learned, the random response sequence prevents anticipation of responses and thus requires stimulus-oriented control. Hence RT and response errors are expected to be higher on random cycles (Willingham et al., 1989) but performance is expected to recover on SOC trials. After completion of each block, performance feedback indicated the number of errors and mean RT followed by a 30 s rest interval.

The random response sequences were generated prior to the study and held constant across all participants, to avoid chance-based group differences in the structure of the random cycles. For example, performance artefacts may occur due to differences in the number of reversal trials (Reed & Johnson, 1994; Vaquero et al., 2006). A reversal trial occurs when the third trial of any three consecutive trials involves the same target response as the first trial (e.g., V-B-V). Random cycles were generated to match SOC cycles on the number of reversals and hand switches (left-to-right and right-to-left) across trials (Jongkees, Immink, et al., 2017) and immediate response repetitions were not allowed within a random cycle nor at the transition between a random and SOC cycle. As such, any group difference in performance is not confounded by chance-based differences in the structure of random cycles.

Procedure

Upon entering the lab, informed consent was obtained and participants practiced the SRT to familiarize themselves with the task. Subsequently tVNS

was applied and after 15 min of stimulation the experimental SRT task was started. Stimulation was applied throughout the entire task, which took on average 30 minutes. After the task participants were asked to rate, on a five-point (1-5) scale, to what extent they experienced (i) headache, (ii) neck pain, (iii) nausea, (iv) muscle contraction in the face and/or neck, (v) stinging sensation under the electrodes, (vi) burning sensation under the electrodes, (vii) uncomfortable (non-specific) feelings, and (viii) other sensations or adverse effects. None of the participants reported major side-effects.

Statistical analyses

The percentage of response accuracy (PACC) and mean reaction time (MRT) for SRTT familiarization performance was calculated for each individual participant. MRT calculation was based on correct trials only. PACC and MRT for task familiarization were submitted separately to univariate analysis to test for any Group performance differences prior to stimulation conditions.

For performance in SRTT experimental blocks, PACC was calculated for each individual according to Sequence Type (SOC or random) and Trial Type (non-reversal, reversal) factors and submitted to a 2 (Group: active, sham) x 2 (Sequence Type: SOC, random) x 2 (Trial Type: non-reversal, reversal) analysis of variance (ANOVA) with repeated measures on the last two factors. MRT was calculated based on correct trials according to Sequence Type, Trial Type and Block (1-15) factors. MRT was then submitted to a 2 (Group) x 2 (Sequence Type) x 2 (Trial Type) x 15 (Block) ANOVA with repeated measures on the last three factors. For the purpose of the present experiment, a significant Group x Sequence Type x Block interaction was identified as being a critical test of enhanced sequence learning during active stimulation. A significant main effect of Group or a significant Group x Sequence Type interaction represented key identifiers of response selection efficacy. Enhanced response selection during active stimulation based on suppression of inhibition of return was expected to be revealed either as a significant Group x Trial Type interaction or a Group x Sequence Type x Trial Type interaction. Analysis for inspection of post-error slowing involved

aggregating correct trial MRT separately for post-error trials (a correct trial that was preceded by an error trial), post-correct trials (a correct trial succeeding a correct trial) under SOC and random sequence types. MRT was then submitted to a 2 (Group) x 2 (Preceding Error) x 2 (Sequence Type) ANOVA with repeated measures on the last two factors. A significant Group x Preceding Error or Group x Preceding Error x Sequence Type interaction was identified as reflecting active and sham stimulation differences on post-error slowing.

Mauchly's test was used to test the sphericity assumption for repeated measures ANOVA. Where sphericity was violated, a Huynh-Feldt correction was applied to the p value. Significant interactions were further analyzed using Fisher's LSD post-hoc comparisons. For all analyses, a criterion of $p < .05$ was used to infer significant effects, interactions and differences.

Results

PACC and MRT performance during familiarization of the SRTT did not significantly differ between active and sham stimulation groups; $p = .12$ and $p = .64$, respectively. PACC performance during experimental blocks did not significantly differ between stimulation groups ($p = .37$) and there were no significant interactions between the Group factor and Sequence Type and Trial Type factors (p's > .39).

For experimental block MRT performance, a significant Sequence Type x Block interaction ($F[14, 532] = 5.45$, $p < .0001$, $\eta^2_p = .125$) provides support for sequence learning within the SRT task, see Figure 2. With the exception of Block 2 ($p = .19$), MRT was significantly lower on SOC sequence trials than random trials (p's < .05). However, the Group x Block interaction ($p = .89$) was not significant. Important for the evaluation of sequence learning differences between stimulation groups, the Group x Sequence Type x Block interaction was not significant ($p = .76$). Further inspection of sequence learning based on assessment of post-error slowing did not reveal significant Group x Preceding Error ($p = .27$) or Group x Preceding Error x Sequence

Type (p = .64) interactions. Thus, these results do not indicate that active tVNS stimulation enhanced sequence learning.

With respect to the evaluation of response selection enhancement, neither the Group effect (p = .93) or the Group x Sequence Type interaction (p = .07) for MRT were significant. In terms of inhibition of return as an index of response selection efficacy, the stimulation groups did not significantly differ between non-reversal trials and reversal trials (p = .16). However, a significant Group x Sequence Type x Trial type interaction (F[1, 38] = 5.05, $p < .05$, η^2_p = .117) indicated that enhancement of response selection through suppression of inhibition of return depended on the nature of the sequence structure that the reversal trial was performed in, see Figure 3. Specifically, under active stimulation and in SOC sequence trials, MRT was not significantly different between non-reversal and reversal trials (p = .10). In contrast, under sham stimulation, MRT for SOC sequence trials was significantly longer for reversal trials than non-reversal trials ($p < .0001$). For random trials, both active and sham stimulation groups demonstrated significantly longer MRT for reversal trials and non-reversal trials (both comparisons, $p < .001$). Nevertheless, under active stimulation, there were no significant differences between SOC sequence reversal trials and random sequence non-reversal trials (p = .42). In sum, these results indicate active tVNS eliminated inhibition of return during SOC sequenced response schedules.

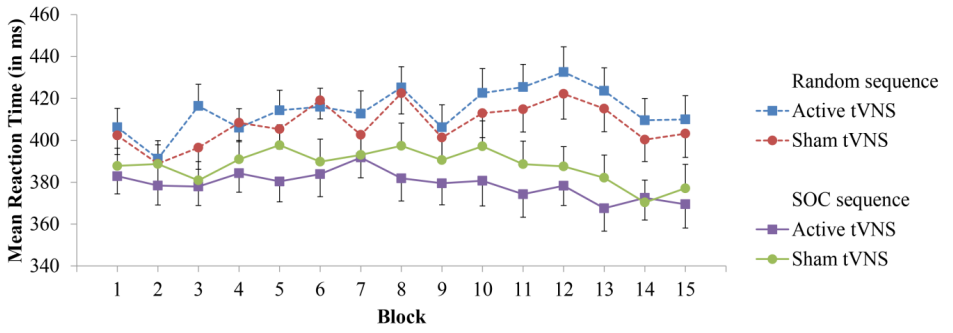

Figure 2. Mean reaction time in the serial reaction time task as a function of block, sequence type, and tVNS group.

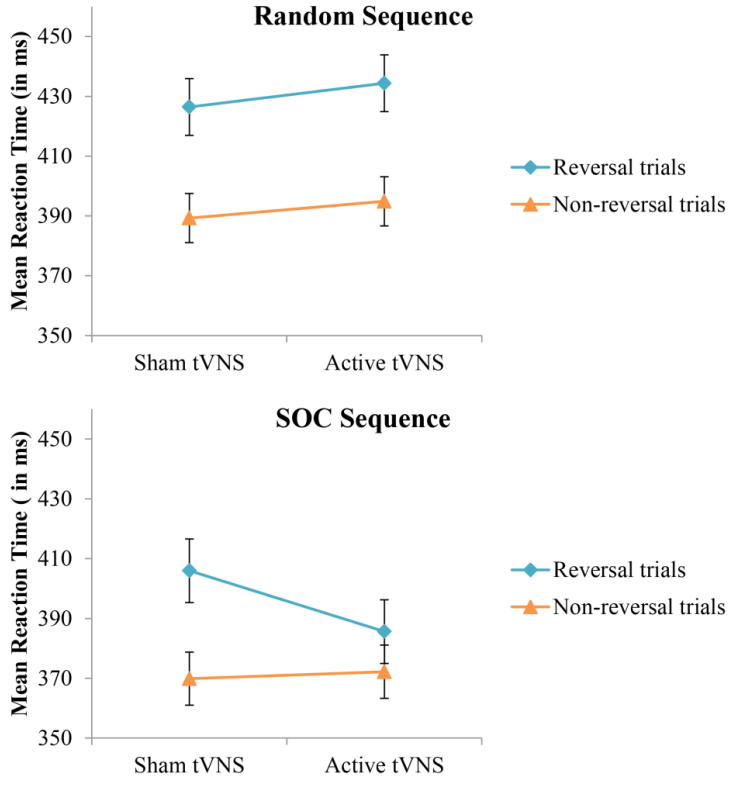

Figure 3. Mean reaction time in the serial reaction time task as a function of trial type, sequence type and tVNS group. Whereas both groups demonstrate a typical increase in reaction time on reversal trials during random response sequences, this increase is eliminated in the active tVNS group on trials with an embedded (SOC) response sequence.

Discussion

The present study demonstrates that single-session tVNS improves response selection during sequential action. Whereas individuals tend to slow their responses when a response sequence contains an immediate reversal (e.g., 1-2-1 instead of 1-2-3) (Vaquero et al., 2006), this inhibition-of-return-like effect was eliminated under active tVNS while participants carried out an implicitly-learned response sequence. The effect of tVNS was exclusive to response latency and did not extend to response errors, suggesting that the results are not attributable to a change in the speed-accuracy trade-off. This finding provides convergent evidence for the potential of tVNS to enhance action control in healthy adults.

In particular, this beneficial effect of tVNS on response selection is consistent with a wide range of studies demonstrating that increased GABA concentration facilitates action control. Via a modulation of intracortical inhibition and cortical signal-to-noise ratio, a higher GABA concentration is likely to reduce competition between behavioral alternatives and thereby facilitate the selection of the correct response while withholding an inappropriate alternative (de la Vega et al., 2014; Munakata et al., 2011). In light of the inhibition of return effect, tVNS-induced enhancement of GABA could have served to disinhibit the response on trial N-2, thereby facilitating its selection.

tVNS did not enhance or diminish implicit motor sequence learning. Of note was the low rate of implicit learning in both groups. The task structure might have limited the opportunity to acquire the SOC sequence due to alternation of random and SOC response cycles within each block. Although this structure served to offer a more balanced inspection of performance on randomly sequenced versus SOC sequenced trials, the high prevalence of and frequent switching towards random response sequences might have interfered with participants' ability to acquire the SOC sequence by predisposing them to a stimulus-based rather than a plan-based action control style (c.f. Tubau, Hommel, & López-Moliner, 2007). A reduced tendency for plan-based control might have then limited the potential for implicit learning to be modulated by

tVNS. Therefore, we recommend the null-finding regarding tVNS and motor sequence learning to be examined in future studies that employ a more classic SRTT in which experimental blocks are strongly dominated by SOC cycles.

Notwithstanding the observed null-findings for sequence learning, the fact that tVNS enhanced performance under increased response selection demands, where there is tendency to inhibit the target response, is of potential theoretical interest and is reminiscent of a previous finding that tVNS enhanced inhibitory control only when working memory was also involved (Beste et al., 2016). In the present study tVNS selectively enhanced response selection on reversal trials during SOC cycles. From a neurobiological perspective, it is plausible that GABA's disinhibitory effects on response selection have greatest behavioral impact, and are more sensitive to manipulation, during conditions of response conflict when several response alternatives are strongly activated or inhibited, such as on reversal trials. This might also explain the lack of an effect of tVNS on the majority of SOC trials (i.e., non-reversal trials), as these trials might have led to insufficient activation or inhibition of responses alternatives for a manipulation of GABA to be behaviorally detectable.

Lastly, although the observed effects of tVNS on SRTT performance are consistent with a modulation of the GABAergic system, it is important to acknowledge that the noradrenergic system can also be affected by tVNS (Raedt et al., 2011; Roosevelt et al., 2006). A shortcoming of the present study is that its behavioral findings cannot distinguish between effects on these different neurotransmitter systems. Although the results are in line with an enhancement of GABA, future studies should provide clarity on this issue by for example including physiological markers of GABAergic and noradrenergic activity in an attempt to relate baseline differences and changes in these markers to tVNS-induced changes in SRTT performance.

To conclude, the present study extends the previous literature on tVNS and action control performance by showing that tVNS enhanced response selection processes during sequential action.

Chapter Ten

The effect of cerebellar tDCS on sequential response selection processes

Jongkees, B. J., Immink, M.A., Boer, O. D., Yavari, F. B., Nitsche, M. A., & Colzato, L. S. (Submitted). The effect of cerebellar tDCS on sequential motor control and learning.

Abstract

In recent years, transcranial direct current stimulation (tDCS) has received considerable attention as a means to transiently alter cortical excitability and synaptic plasticity. So far, only few studies have investigated the cognitive-behavioral effects of applying tDCS to the cerebellum. Given the role of the cerebellum in fine motor control and motor coordination, we investigated whether cerebellar tDCS modulates response selection processes. Seventy-two participants received either anodal (excitatory), cathodal (inhibitory) or sham (placebo) tDCS while performing a serial reaction time task (SRTT). To compare acute and long-term effects of tDCS on response selection, participants came back for follow-up 24 hours after stimulation. Results indicate that the three groups did not differ in performance prior to tDCS. Although tDCS did not affect implicit motor learning, anodal as compared to cathodal and sham stimulation did modulate response selection processes as evidenced by overall increased response latencies both during stimulation and at 24 hours follow-up. These results are consistent with the notion that the cerebellum exerts an inhibitory effect on primary motor cortex (M1), which results in delayed movement when this inhibition is strengthened by tDCS.

Introduction

Recent years have seen a substantially growing interest in non-invasive methods of brain stimulation. In particular, transcranial direct current stimulation (tDCS) has received considerable attention as a means to transiently alter cortical excitability and synaptic plasticity (Nitsche & Paulus, 2000, 2001; Nitsche, Nitsche, et al., 2003; Plewnia et al., 2015). Although many studies have examined the cognitive-behavioral effects of stimulating cortical areas such as dorsolateral prefrontal cortex and primary motor area (M1), very recent studies have begun to investigate the cerebellum as a potential site of stimulation (van Dun, Bodranghien, Mariën, & Manto, 2016). The cerebellum plays a critical role in sensorimotor control, such as planning, initiation and organization of movement (Manto et al., 2012). This raises the question whether cerebellar tDCS can modulate response selection processes. Investigating this issue has the potential to further our knowledge of the cerebellum's involvement in sensorimotor control and offer rehabilitation strategies for patients with cerebellar dysfunction. Therefore, in the present study we set out to clarify the effects of cerebellar tDCS by assessing response selection and motor sequence acquisition in the serial reaction time task (SRTT) both during stimulation and at 24 h follow-up.

tDCS is typically applied by mounting two electrodes on the scalp, with a current of 1-2 mA running between the electrodes. This is thought to alter the resting membrane potential of neurons in a polarity-dependent manner: neurons beneath the anode are slightly depolarized and thus have an increased likelihood of firing, whereas neurons beneath the cathode are slightly hyperpolarized and thus have a reduced likelihood of firing (Nitsche & Paulus, 2000). At longer stimulation periods, tDCS can also affect neural plasticity for minutes or hours following stimulation (Nitsche & Paulus, 2001; Nitsche et al., 2008; Nitsche, Nitsche, et al., 2003) by producing changes in levels of glutamate and GABA (Bachtiar et al., 2015; Soyoung Kim et al., 2014; Nitsche, Fricke, et al., 2003; Stagg et al., 2009).

Of relevance to the present study's objective, previous research has demonstrated that cerebellar tDCS modulates a phenomenon referred to as

cerebello-brain inhibition (CBI) in a polarity-dependent manner. That is, Purkinje cells in the cerebellum exert an inhibitory tone over M1 via the dentate-thalamo-cortical pathway (Kelly & Strick, 2003; Middleton & Strick, 2000), and this inhibition is strengthened by anodal tDCS and weakened by cathodal tDCS relative to sham stimulation (Galea, Jayaram, Ajagbe, & Celnik, 2009). Combined with the fact that motor sequence acquisition is typically associated with an increase in excitability of M1 (Lin et al., 2011), this suggests that anodal tDCS could hinder the initiation of movement and impair acquisition of motor sequences, whereas cathodal tDCS could facilitate these processes.

Studies on cerebellar tDCS have not yet unequivocally confirmed or falsified these hypotheses. In support of these expectations, anodal tDCS has previously produced a delay in the initiation of muscle activity (Dutta, Paulus, & Nitsche, 2014) and impaired handwriting legibility with the non-dominant hand (Foerster et al., 2013). However, these findings contrast with two reports that anodal tDCS enhanced implicit motor sequence learning (Ehsani, Bakhtiary, Jaberzadeh, Talimkhani, & Hajihasani, 2016; Ferrucci et al., 2013). Unfortunately, these studies report only that the stimulation produced a larger difference in reaction time (RT) between random and sequenced response blocks, but do not clarify whether this difference is driven by an increase in RT for random responses, a decrease in RT for sequenced responses, or both. Furthermore, one of these studies used a symbolic rather than spatial stimulus-response mapping (Ehsani et al., 2016), which further complicates the interpretation of the results, whereas the other study observed no sequence learning in the group receiving sham stimulation (Ferrucci et al., 2013). Considering also the fact that these studies have primarily focused on anodal rather than cathodal tDCS, there is still much uncertainty about the effects of cerebellar tDCS on sensorimotor control.

In the present study we set out to clarify this issue by examining the effects of anodal, cathodal and sham tDCS of the cerebellum on response selection and motor sequence acquisition in a SRTT with a spatial stimulus-response mapping. The SRTT is a 4-choice RT task (Nissen & Bullemer, 1987)

that involves response selection, inhibition of non-target responses and implicit formation of response sequence structures, each of which may be sensitive to a modulation of cerebellar excitability (and indirectly, M1 excitability) via tDCS. Typically, a second-order conditional (SOC) response sequence is embedded in the SRTT unbeknownst to the participants. Implicit acquisition of this sequence structure results in increasingly shorter RT and less response errors as the task progresses (Abrahamse & Noordzij, 2011; Nissen & Bullemer, 1987; Schwarb & Schumacher, 2012). However, there is potential difficulty in disentangling the nature of these improvements (Jongkees, Immink, et al., 2017) as performance improvements might not necessarily be due to implicit learning processes but rather reflect general practice effects (Abrahamse & Noordzij, 2011). For this reason, a transfer approach is commonly used to judge the extent to which performance improvements rely on the practiced sequence (Abrahamse & Noordzij, 2011; Robertson, 2007; Willingham, 1999). This was implemented in the present experiment by presenting 10 out of 13 SRTT blocks that exclusively contained the same repeating SOC response sequence. The remaining three blocks (1, 7 and 13) were probe blocks that consisted predominantly of the trained SOC sequence, but also an untrained SOC sequence in order to disentangle sequence-specific learning from general practice effects. In light of the effects of cerebellar tDCS on CBI, we expected anodal relative to sham tDCS to impair overall RT and sequence acquisition, whereas cathodal relative to sham tDCS was expected to produce the opposite behavioral results. Furthermore, to investigate the effect of tDCS on consolidation processes following training, we assessed SRTT performance not only during stimulation but also at 24 h follow-up.

Materials and methods

Participants

Seventy-two right-handed, healthy undergraduate students from Leiden University were offered partial course credit for participation in a study on brain stimulation. Participants were randomly assigned to receive either anodal ($N = 24$), cathodal ($N = 24$), or sham ($N = 24$) stimulation. Group demographics

are presented in Table 1. The groups were comparable with respect to age, $F(2,69) = .675$, $p = .512$, gender distribution, $X^2(2, N = 72) = .572$, $p = .751$, and hours of sleep, $F(2, 69) = .118$, $p = .888$. Participants were screened individually using the Mini International Neuropsychiatric Interview (MINI), a short, structured interview of approximately 15 min that screens for several psychiatric disorders and drug use (Sheehan et al., 1998), and has been used previously in research on tDCS (Jongkees, Sellaro, et al., 2017) and the SRTT (Jongkees, Immink, et al., 2017). Participants were included if they met the following criteria: (i) between 18 and 30 years; (ii) no history of neurological or psychiatric disorders; (iii) no history of substance abuse or dependence; (iv) no chronic or acute medication; and (v) no implants or cardiac disorders for safety reasons concerning the tDCS. Before the start of the study, participants were informed of the procedure and potential side-effects of the tDCS (i.e., itching, stinging or burning sensation from the electrodes, reddening of the skin and head ache). None of the participants reported major side-effects. The study conformed to the ethical standards of the declaration of Helsinki with written informed consent from all subjects and the protocol was approved by the local ethical committee (Leiden University, Institute for Psychological Research).

Table 1. Group demographics

	Stimulation		
	Anodal	Cathodal	Sham
Male-to-female ratio	7:17	7:17	5:19
Age in years	19.8 (1.6)	19.5 (1.5)	19.3 (1.8)
Sleep session #1 in h	7.3 (1.8)	7.3 (1.1)	7.6 (1.0)
Sleep session #2 in h	7.4 (1.5)	7.1 (1.2)	7.2 (1.2)

Standard deviation in parentheses

Cerebellar transcranial direction current stimulation

Cerebellar tDCS was applied using three electrodes of 35 cm^2 (5 cm x 7 cm), with the target electrode centered over the inion and the two reference electrodes placed over bilateral mastoid to limit the effects of the reference electrodes on cortical activity. Whereas previous studies typically placed the target electrode lateral to the inion to investigate effects on unimanual performance (Ehsani et al., 2016; Ferrucci et al., 2013), others have centered the target electrode over the inion for bilateral stimulation of the cerebellum

(Ho et al., 2014; Martin et al., 2015; Panouillères, Miall, & Jenkinson, 2015). As the SRTT in the present study required bimanual performance, we also opted to center the target electrode over the inion. Stimulation consisted of a current of 1 mA delivered by a DC Brain Stimulator Plus (NeuroConn, Ilmenau, Germany), a device complying with the Medical Device Directive of the European Union (CE-certified). The current was built up during a fade-in of 10 s, after which stimulation lasted for precisely 20 min and then ended with a 10 s fade-out. All participants finished the SRTT task within the 20 min of stimulation. Impedance was below 15 kΩ throughout the stimulation.

SimNIBS, a freely available software software (www.simnibs.org), was used to develop the head model for finite element modeling (Thielscher, Antunes, & Saturnino, 2015; Windhoff, Opitz, & Thielscher, 2013). SimNIBS uses FreeSurfer and FSL BET to segment the head. SimNIBS pipeline was applied on a realistic head model which has been provided by SimNIBS as the example dataset (http://simnibs.de/version2/documentation). Five tissue segments are considered in the model: scalp, skull, cerebrospinal fluid (CSF), gray matter (GM), and white matter (WM). Electrodes, modeled as saline-soaked 5×7 cm^2 rectangular sponges, were positioned over the inion and mastoids. Current intensity was set to 1mA for the electrode over inion and 0.5 mA for each of the electrodes over mastoids. Finite Element Method (FEM) in SimNIBS pipeline was employed to calculat electric field (EF) distribution. Spatial distribution of the normalized EF values calculated by the computational analysis of the head model are shown in Figure 1. Electric field strength exhibits high values in the surface and deep layers of the cerebellum.

The experience of side-effects due to tDCS was assessed through self-report ratings on a five-point scale for the following symptoms: head ache, neck pain, nausea, muscle contractions in the face or neck, stinging sensation under the electrodes, burning sensation under the electrodes, and a nonspecific, uncomfortable feeling. Consistent with previous studies the most prominent side-effects were stinging and burning sensations under the electrodes (Bikson et al., 2009), although none of the participants voiced major complaints.

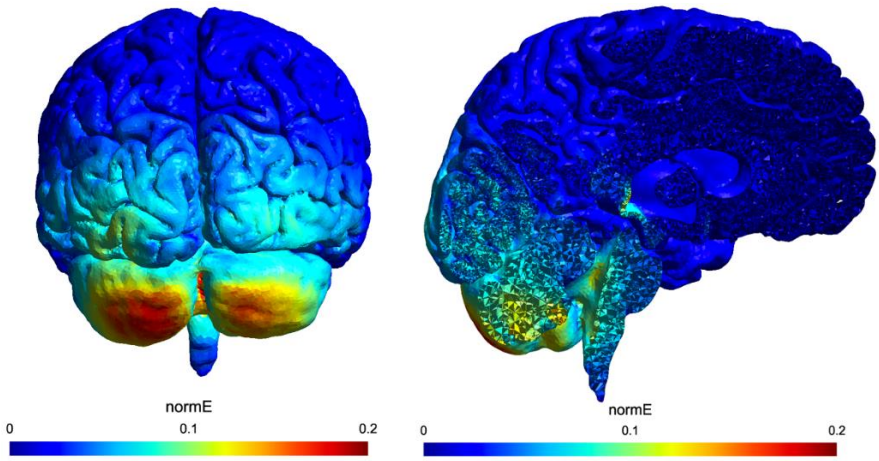

Figure 1. Spatial distribution of the normalized electric field calculated using SimNIBS pipeline; Anode: 5cmx7cm, centred over the Inion, 1mA current, two cathodes over mastoids, 5cmx7cm, 0.5mA current each.

Serial reaction time task

To assess response selection and sequence learning, participants performed a SRTT (Vaquero et al., 2006) presented using E-Prime 2.0 software (Psychology Software Tools, Inc., Pittsburgh, PA, USA). In this task four horizontally-aligned empty squares are presented in the centre of the screen. On each trial one of the squares turns red and the participant must press a corresponding button on the QWERTY keyboard (from left to right: V, B, N, M) using the index and middle fingers of the left (V, B) and right (N, M) hand. An error sound is presented if the wrong button is pressed, along with the Dutch words “Verkeerde toets!” (“Wrong button!”). RT is measured in ms as the latency in the key press to the stimulus and if RT exceeds 3,000 ms, the Dutch words “Te langzaam!” (“Too slow!”) are presented. Following the response, the four empty squares appear for a 50 ms response-stimulus interval before the next stimulus is presented. Participants were instructed that accuracy and response speed were equally important in the task.

All participants completed one task familiarization block of 120 randomly sequenced trials prior to stimulation to check for pre-existing group differences in response selection efficiency. Subsequently, participants

performed 13 training blocks that each consisted of 10 cycles of 12 trials while stimulation was applied. Blocks 2-6 and 8-12 consisted of 10 cycles of the same repeating 12-item SOC response sequence (VBVNMBNVMNBM) (Reed & Johnson, 1994). In order to disentangle sequence-specific performance from general practice effects, blocks 1, 7 and 13 were probe blocks. These blocks always started and ended with two cycles of the same SOC sequence in training blocks. Randomly inserted in the remaining six cycles were two consecutive cycles of an untrained transfer SOC sequence. This transfer sequence limits anticipation of responses and thus RT and response errors are expected to be higher for transfer sequences, but performance is expected to recover on the trained SOC trials. After completion of each block, performance feedback indicated the number of errors and mean RT followed by a 30 s rest interval.

At 24 h follow-up, participants completed the test phase of the SRTT consisting of 3 blocks, the first and third being probe blocks while the second exclusively contained the trained SOC, to investigate whether cerebellar tDCS affected overnight consolidation processes.

Procedure

Upon entering the lab, informed consent was obtained and participants completed the familiarization block of the SRTT. Subsequently, tDCS was applied for 20 min, during which participants completed 13 blocks of the SRTT. Stimulation was applied throughout the entire task, which took no more than 20 min to complete. After the task participants were asked to rate, on a five-point scale, to what extent they experienced adverse effects due to the stimulation. None of the participants reported major side-effects. All participants came back to the lab 24 h after the first session to complete 2 probe blocks and one block with the trained SOC without stimulation. The two sessions together took an approximate total of 60 min to complete.

Analysis

To compare SRTT performance between groups, percent accuracy (PAC) was calculated for each participant in familiarization, training and test phases of the SRTT. PAC for each phase was separately submitted to one-way analysis of variance (ANOVA) using the aov function.

For analysis of RT performance in SRTT phases, all incorrect trials were removed. RT data in familiarization, training and test SRTT phases were analysed using linear mixed-effects modelling (LMM) with the lme4 package in R (Bates, Mächler, Bolker, & Walker, 2015). The LMM approach does not require data averaging like traditional ANOVA analysis approaches and so LMM provides a more selective approach to investigating experimental effects and interactions (Lo & Andrews, 2015). This is because LMM allows for control of variance associated with random factors (Baayen, Davidson, & Bates, 2008). In the present LMM analyses, we treated participants and response stimuli as random factors. For fitted LMM models, we used the car package in R (Fox & Weisberg, 2011) to conduct type III Wald *F* tests with Satterthwaite degrees of freedom approximation (Luke, 2017).

LMM for RT in familiarization included Group (Sham, Anodal, Cathodal stimulation) as a fixed factor. Training RT data was first analysed with LMM on the 10 training blocks that involved only the target SOC sequence (blocks 2-6 and 8-12) to evaluate overall performance improvements with the training sequence. For this, we included Group and Block as fixed factors. We then conducted separate LMM on training RT data from the three probe blocks (blocks 1, 7 and 13) to evaluate sequence-specific learning by comparing performance on the target SOC sequence and the transfer SOC sequence. Here, we included Group, Block and Sequence Type (trained and transfer SOC) as fixed factors. To evaluate sequence-specific learning outcomes at test (24 h follow-up) relative to the end of training, we conducted LMM on RT data for the three Groups across the third and final probe block of training (training block 13) and the two probe blocks at test (test blocks 1 and 3) with Group, Block and Sequence Type as fixed factors. Finally, to evaluate test RT performance when only the SOC trained sequence was

present, we conducted LMM on the second test block with Group as a fixed factor. Significant effects from LMM were graphed using the effects (Fox, 2013) and ggplot2 (Wickham, 2009) R packages.

Results

We observed no significant group differences for PAC in familiarization (M = 97.20%, SD = 2.50, p = .71), training (M = 96.94%, SD = 1.65, p = .58) and test (M = 97.31%, SD = 1.75, p = .34).

Familiarization and training (day 1)

At the outset of the experiment, the groups did not differ in RT performance, as the Group effect was not significant for familiarization RT (p = .48). For RT in training blocks, in which only the target SOC sequence was performed, there was a significant Group x Block interaction, $F(18,83831) = 3.06$, $p < .001$. The source of this interaction was based on the anodal stimulation group demonstrating longer RT than sham and cathodal stimulation groups. The difference in RT for the anodal group compared to sham and cathodal groups was larger in the initial training blocks but decreased as training progressed, see Figure 2. Analysis of RT in probe blocks revealed a significant Block x Sequence Type interaction, $F(2, 24936.9) = 204.10$, $p < .001$, and a significant Group x Block interaction, $F(4, 24936.8) = 6.71$, $p < .001$. The significant Block x Sequence Type interaction (see Figure 3) follows a typical sequence learning pattern: in the first probe block, RT is equivalent between training and transfer sequences, but then RT decreases across probe blocks for the training sequence while RT for transfer sequence remains relatively unchanged. The significant Group x Block interaction (see Figure 4) follows a similar pattern as that observed for training blocks as depicted in Figure 2. Specifically, RT in probe blocks 1 and 2 is longer under anodal stimulation than sham and cathodal stimulation, but RT in the last probe block is equivalent between stimulation groups. The anodal group demonstrated a larger decrease in RT between probe blocks 2 and 3 than sham and cathodal groups across both training and transfer sequences. It does not appear that cerebellar tDCS influenced sequence

specific learning as in probe blocks, neither the Group x Sequence Type or Group x Block x Sequence Type interactions were significant (p = .64 & .58, respectively).

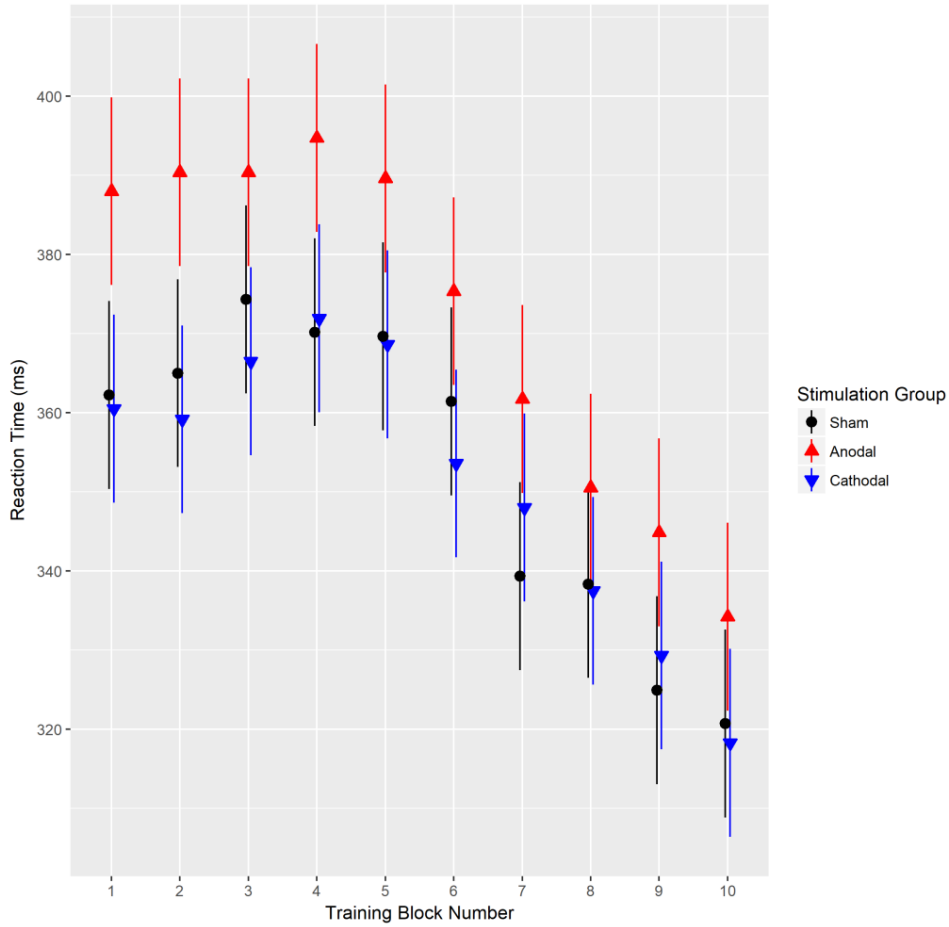

Figure 2. Mean RT in ms as a function of stimulation group and training blocks that only include the trained SOC (blocks 2-6 and 8-12). The anodal stimulation group demonstrates longer RT in early training blocks but no longer differs from the other groups at the end of training.

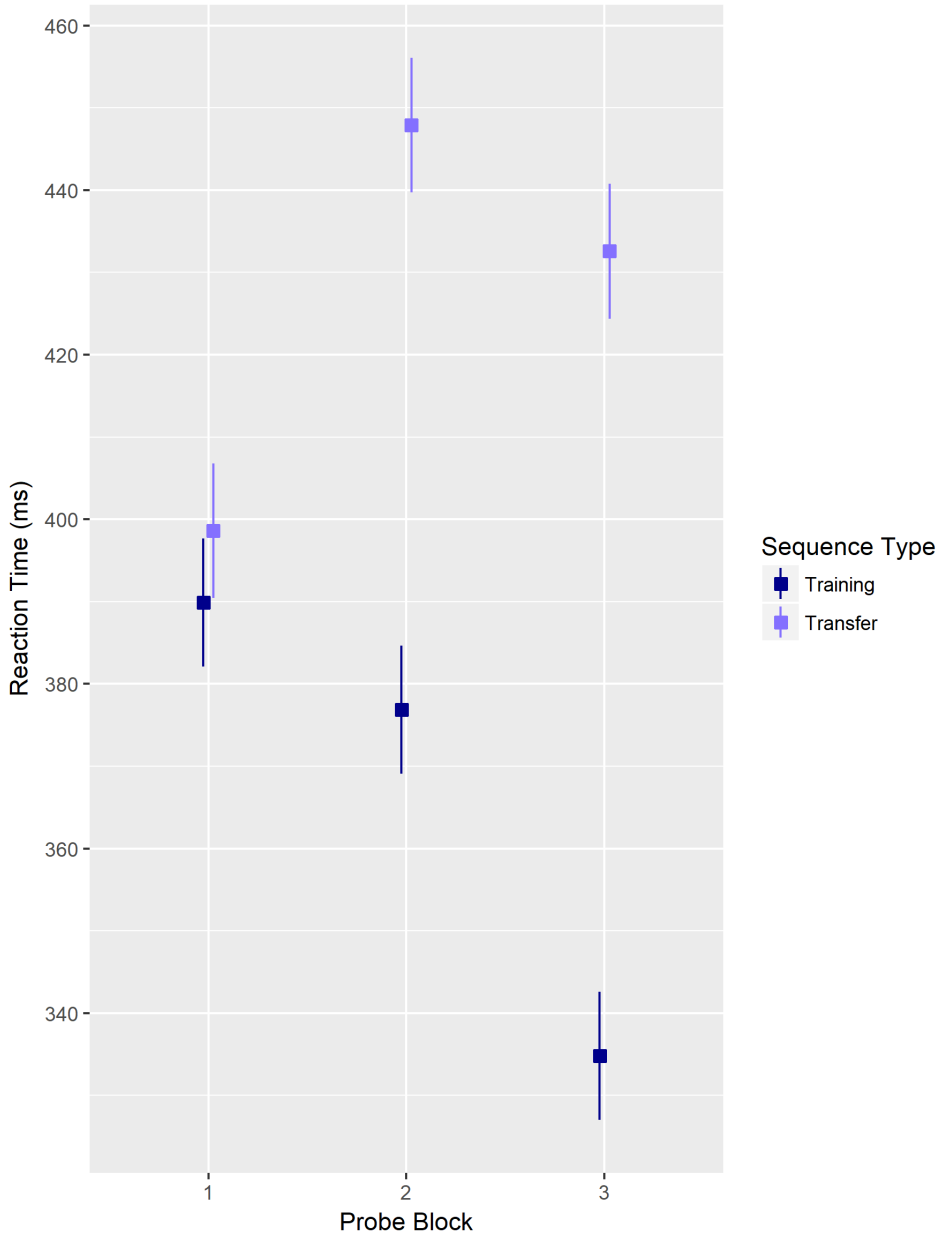

Figure 3. Mean RT in ms as a function of sequence type in the three probe blocks during training (blocks 1, 7 and 13). Performance on both sequences is comparable in the first block but diverges in the second and third probe block, demonstrating a typical sequence learning pattern.

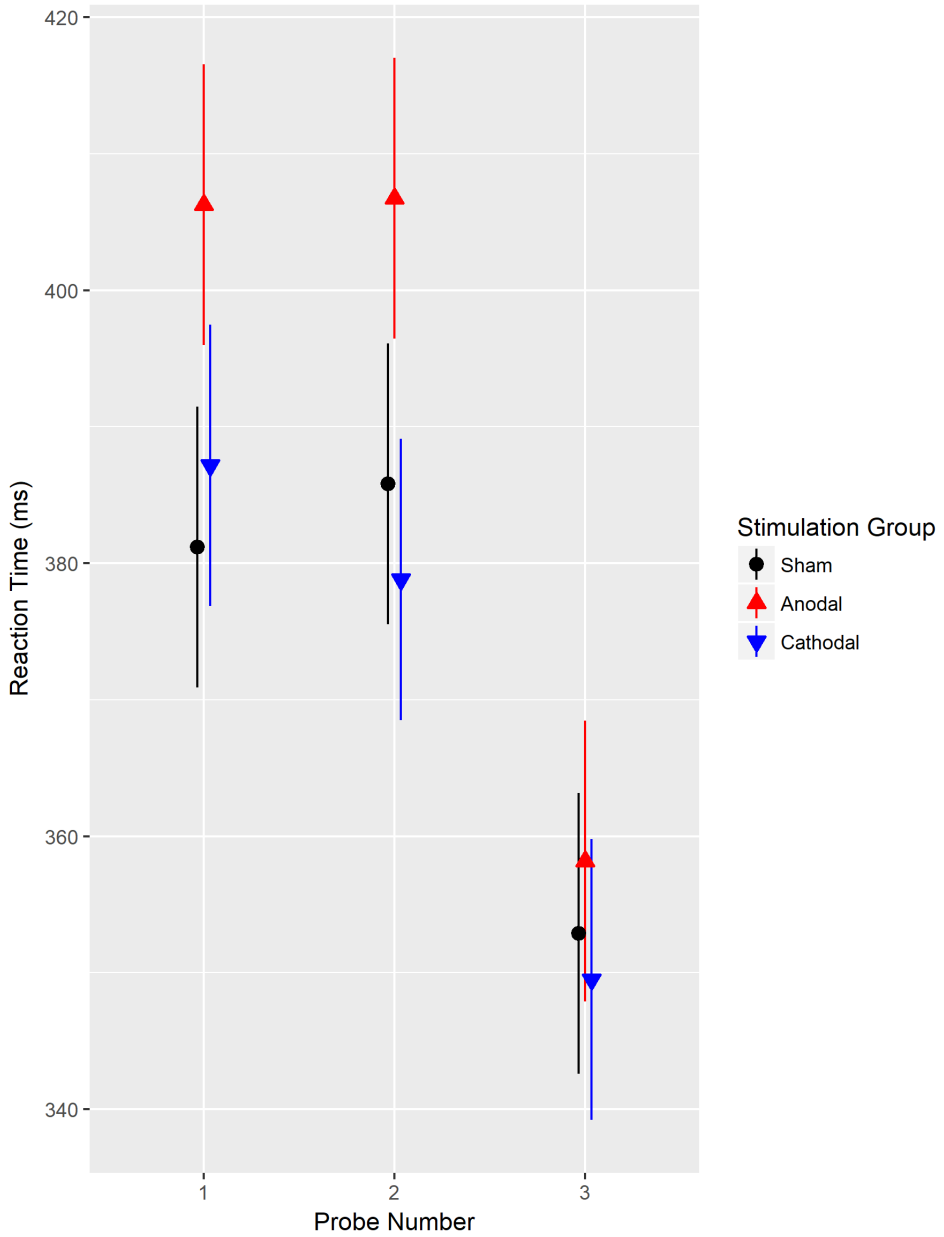

Figure 4. Mean RT in ms as a function of stimulation group and probe blocks during training (blocks 1, 7 and 13). As in the training blocks containing only the trained SOC (see Figure 1), in probe blocks 1 and 2 but not 3 the anodal stimulation group demonstrates longer RT.

Test (24 h follow-up)

Analysis of RT in the final training probe block (i.e., end of training) and the two test probe blocks (i.e., at 24 h follow-up) revealed a significant Block x Sequence Type interaction, $F(2,24944.4) = 41.88$, $p < .001$. RT for both sequence types decreased from training probe 3 to the test probe 1, suggesting a general practice effect. From test probe 1 to 2, RT further decreased for the trained sequence but increased for the transfer sequence, see Figure 5. In addition, there were significant interactions between Group and Block, $F(4,24944.4) = 5.37$, $p < .001$, and Sequence Type, $F(4,24944.4) = 6.40$, $p < .001$. The Group x Block interaction, illustrated in Figure 6, is based on all three groups demonstrating decreased RT between training probe block 3 and test probe block 1 while only the cathodal group demonstrated significantly shorter RT in test probe block 2 than test probe block 1 ($p < .01$). Underlying the Group x Sequence Type interaction was the anodal group demonstrating longer RT for trained and transfer sequences than the sham and cathodal groups, with this difference being larger for trained sequences than transfer sequences, see Figure 7. No significant group differences were observed for RT in test block 2, which involved only the trained sequence ($p = .14$).

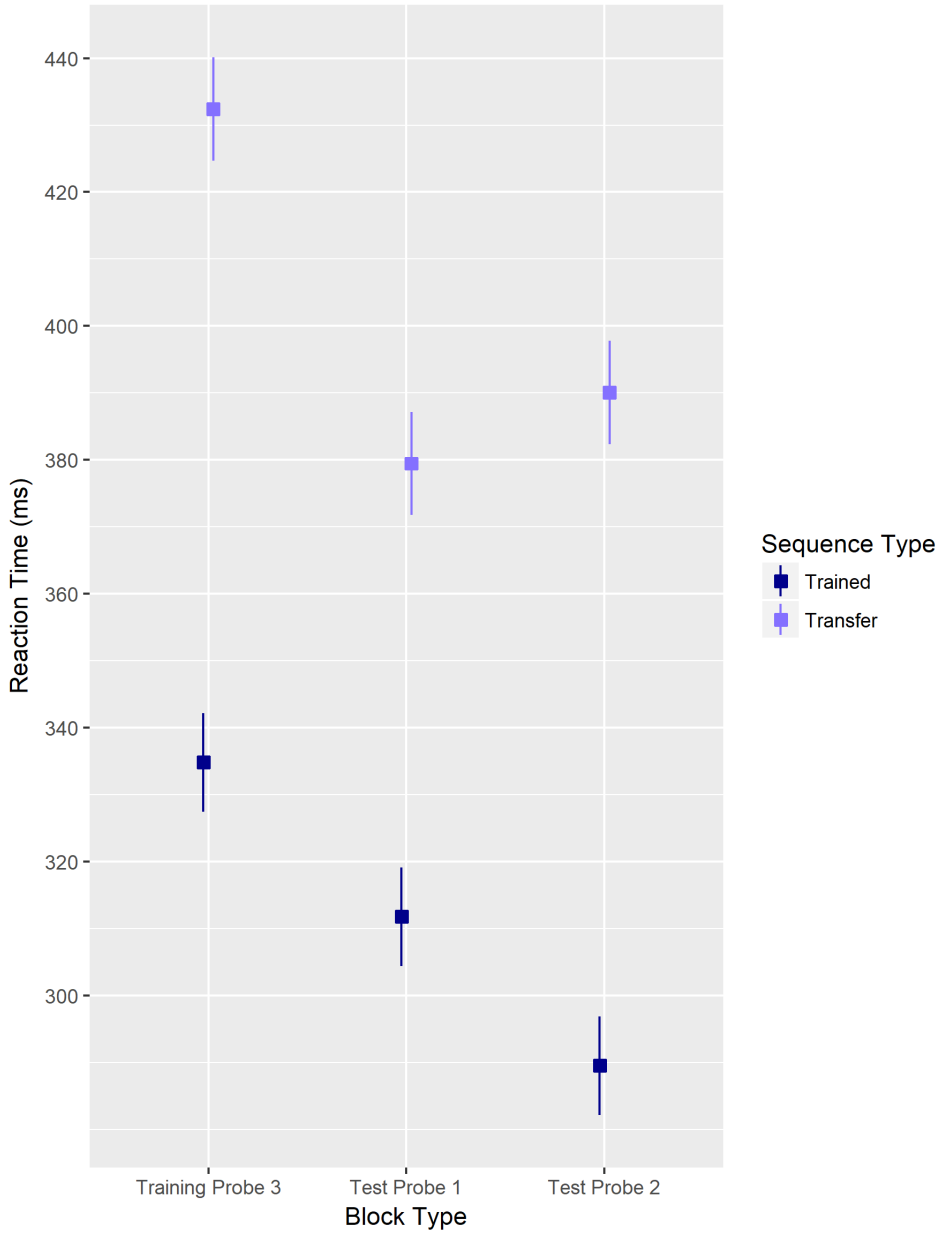

Figure 5. Mean RT in ms as a function of sequence type in the third and final probe block during training (block 13) and the probe blocks during test (at 24 h follow-up). Performance on both sequences benefits from overnight sleep, but further expose to the trained SOC facilitates performance on this sequence whereas it interferes with performance on the transfer sequence.

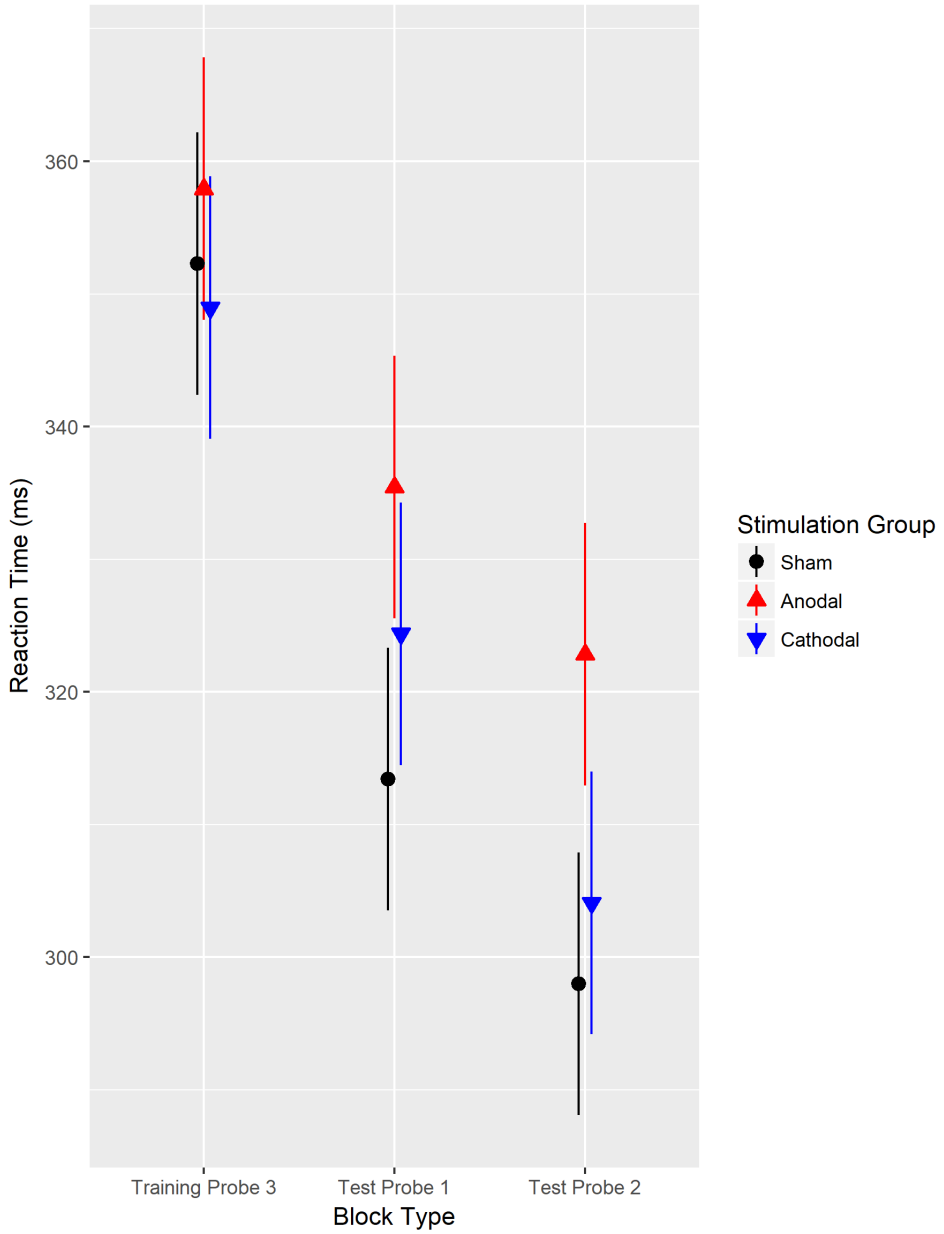

Figure 6. Mean RT in ms as a function of stimulation group in the third and final probe block during training (block 13) and the probe blocks during test (at 24 h follow-up). The groups no longer differed at the end of training on day 1, but the anodal stimulation group again demonstrated longer RT in probe blocks at 24 h follow-up.

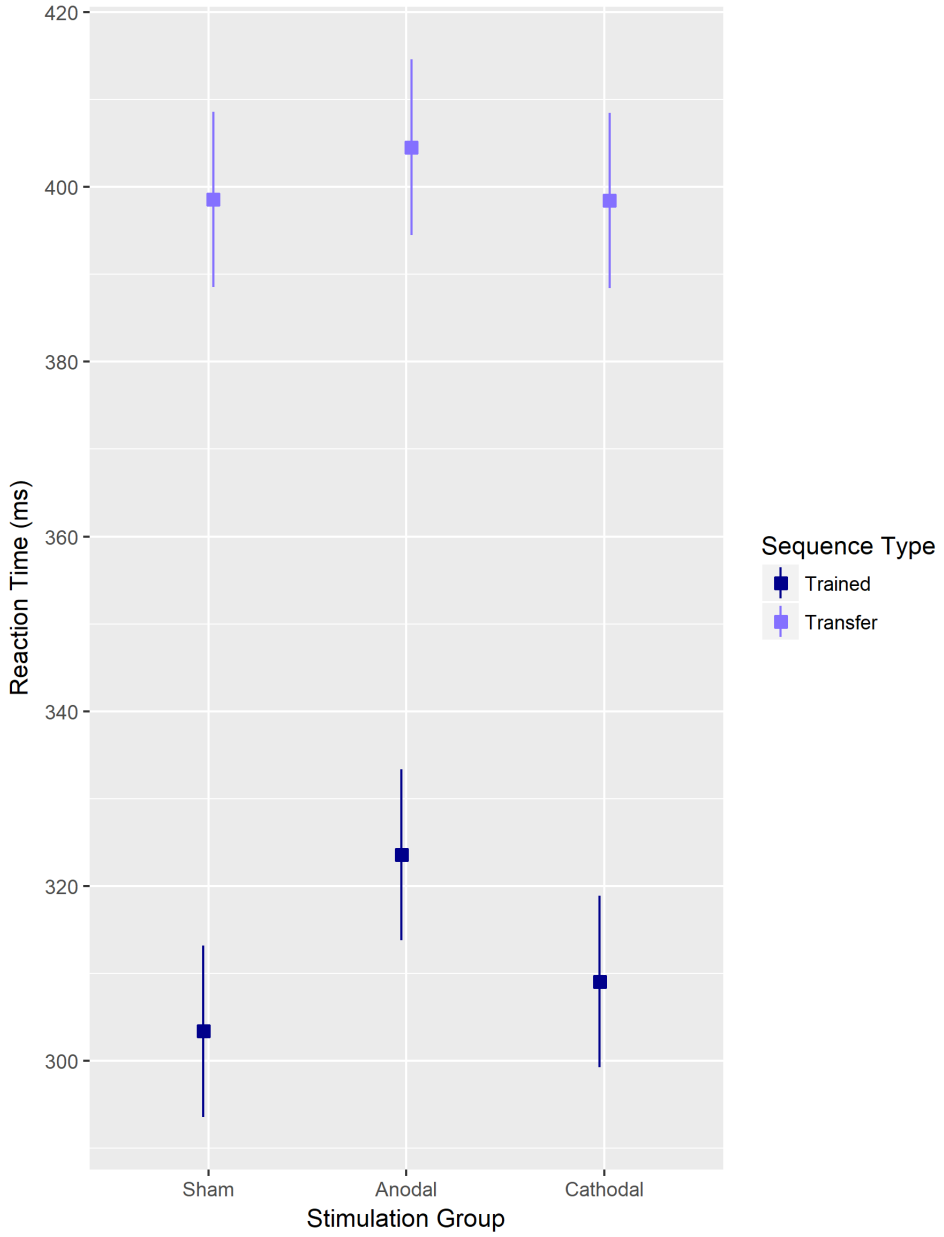

Figure 7. Mean RT in ms as a function of sequence type and stimulation group collapsed across the two probe blocks during test (at 24 h follow-up). The anodal stimulation group demonstrates longer RT than cathodal and sham groups, and this difference is greater for the trained SOC.

Discussion

The present study investigated the effects of tDCS of the cerebellum on response selection and motor sequence acquisition. In brief, the results demonstrated suppressed task performance, evidenced by overall longer RT, under anodal as compared to cathodal and sham tDCS, whereas there were no differences between cathodal and sham stimulation. This pattern persisted at 24 h follow-up as indicated by longer RT under anodal tDCS, in particular for the trained as compared to a transfer SOC sequence. Crucially, this group difference was not a pre-existing one, as RT performance before stimulation did not differ between the groups. The finding that anodal tDCS of the cerebellum delayed initiation of responses is consistent with a previous study demonstrating that anodal tDCS over the cerebellum delays initiation of muscle activity (Dutta et al., 2014), and it supports the idea that excitatory stimulation of this region strengthens the inhibitory tone exerted by the cerebellum over M1 (Galea et al., 2009; Kelly & Strick, 2003; Middleton & Strick, 2000). As such, the present study provides convergent evidence that tDCS over the cerebellum can affect response selection processes.

In more detail, all groups demonstrated motor sequence acquisition during training, as evidenced by decreased RT as the task progressed and an increasing difference in RT for trained and transfer response sequences. Notably, anodal tDCS was associated with an overall increased RT during training that did not depend on the specific SOC sequence (trained or transfer) being performed. As such, anodal tDCS did not appear to selectively affect sequence acquisition but instead produced an overall delay in initiation of responses. This delay decreased across training, suggesting that participants were able to compensate for their impairment with sufficient practice. At 24 h follow-up the group that previously received anodal tDCS again demonstrated increased RT in probe blocks. This tentatively suggests that, on the long term, anodal tDCS impaired the use of the trained sequence structure when exposed to an interfering transfer sequence.

This finding that anodal tDCS effects persisted at 24 h follow-up is of particular interest, as the effect of a single 20 min bout of tDCS on cortical

excitability is supposedly of short duration (approximately 1 h) (Nitsche et al., 2008). As such, it is unlikely that this long-term impact is due to a persisting change in cerebellar or M1 excitability. Of potential relevance is the fact that the anodal group only differed in performance at follow-up when the trained and transfer sequence were presented in the same block, but not when the block contained only the trained sequence. This implies that the selective impairment of performance is related to increased interference between the trained and transfer sequences when the two are performed in close temporal succession. Hence, the detrimental effect of anodal tDCS on sequence acquisition was not immediately apparent (i.e., during training on day 1) but did render performance of the trained sequence more vulnerable to interference later on (i.e., at 24 h follow-up).

The present findings are in line with previous studies applying anodal tDCS directly over M1, which was associated with enhanced response selection as evidenced by faster responses in an SRTT (Ehsani et al., 2016; Kantak, Mummidisetty, & Stinear, 2012; Nitsche, Schauenburg, et al., 2003). Although these studies varied in their methods of analysis, they indicate that increasing excitability of M1 can facilitate overall response selection and implicit motor sequence learning. Taken together with the findings from the present study, we argue that increasing excitability of M1 by directly applying anodal tDCS to this region facilitates response selection, whereas indirectly decreasing its excitability by applying anodal tDCS to the cerebellum produces the opposite behavioral result.

However, it should be mentioned that the present findings contrast with previous reports on anodal tDCS over the cerebellum and SRTT performance (Ehsani et al., 2016; Ferrucci et al., 2013), which demonstrated a facilitation rather than impairment of response selection. Although it remains speculative what accounts for this difference in results, it should be noted that one of the studies used a symbolic stimulus-response mapping rather than a spatial one (Ehsani et al., 2016). It is therefore possible that anodal tDCS facilitated the use of such a mapping rather than response selection processes per se, which is a question that future studies should investigate systematically. Curiously,

the other study demonstrating enhanced response selection with anodal tDCS over the cerebellum based this conclusion on the comparison with a sham stimulation group that did not demonstrate sequence learning at all (Ferrucci et al., 2013). As such, this particular finding should be interpreted with caution, as SRTT performance typically does demonstrate a sequence learning pattern (Abrahamse & Noordzij, 2011; Nissen & Bullemer, 1987; Schwarb & Schumacher, 2012). In light of this heterogeneity in results, there is a strong need for systematic and independent replication of these previous and current findings.

Interestingly, the present study found behavioral effects of tDCS over the cerebellum exclusively for anodal stimulation, whereas it was previously shown that cathodal tDCS over cerebellum also affects CBI (Galea et al., 2009) and therefore could potentially produce opposite behavioral results. Notably, the previously-reported effect of cathodal tDCS on CBI was obtained with a current intensity of 2 mA, whereas in the present study we used the lower intensity of 1 mA. Hence we speculate that the stimulation intensity used in the present study was not sufficient for behavioral effects of cathodal stimulation to become apparent. As such, future studies should investigate whether the effect of cathodal tDCS over the cerebellum is dose-dependent and if at a higher current intensity it indeed produces opposite behavioral effects as those obtained with anodal tDCS.

Additionally, follow-up studies might incorporate magnetic resonance spectroscopy (MRS) to measure individual differences and changes in glutamate and GABA levels. tDCS is known to directly affect levels of glutamate and GABA in a polarity-dependent manner (Bachtiar et al., 2015; Soyoung Kim et al., 2014; Nitsche, Fricke, et al., 2003; Stagg et al., 2009), and individual differences in (the ratio between) glutamate and GABA are related to response selection efficiency (de la Vega et al., 2014; Munakata et al., 2011; Snyder et al., 2010). As such, future studies employing MRS could establish whether changes in glutamate and GABA level contribute to the behavioral effects observed in the present study, and determine whether individual

differences in baseline levels of these neurotransmitters predict behavioral responsivity to cerebellar tDCS.

To conclude, the present study adds to a very recently-established body of literature by reporting on the effects of cerebellar tDCS on response selection and motor sequence acquisition. In brief, the results are consistent with the idea that cerebellar tDCS affects CBI, thereby modulating M1 excitability and the efficiency of response selection processes.

Discussion

The research included in this dissertation investigated the biological underpinnings of cognitive-behavioral control—both to gain further insight into its underlying mechanisms and to evaluate the efficacy of potential enhancement techniques. Rather than repeating the conclusions of each chapter, which are already summarized in the Introduction's Overview section, this Discussion will instead highlight three important lessons that can be gathered by comparing and contrasting some of the chapters' findings.

First, the study on color vision and cognitive control presented in Chapter Two suggested that better color vision is associated with processing goals in a parallel, overlapping rather than serial, step-by-step manner. This was assessed using an action-cascading (also known as multitasking) paradigm, in which participants are given either no time, i.e., 0 ms, to prepare for a task-switch (the SCD0 condition) or are given 300 ms to prepare for a task-switch (the SCD300 condition). It is traditionally thought (Stock et al., 2014; Verbruggen et al., 2008) that the former condition gives participants a choice between a serial or parallel strategy, because they must decide whether to first finish fully processing the previous goal (i.e., serial processing) or already start processing the next goal simultaneously (i.e., parallel processing). In contrast, the 300 ms interval in the SCD300 condition is thought to enforce a serial, step-by-step manner of goal-activation by offering ample time to finish processing of the first goal before indicating the nature of the second goal. The traditional interpretation of the paradigm's results, then, is that longer reaction times in the SCD0 as compared to SCD300 condition indicate a more parallel goal-activation strategy. That is, the relatively worse performance in the SCD0 condition is thought to result from parallel processing that allows different goals to interfere with each other, accounting for longer reaction times. In other words, longer reaction times in the SCD0 condition as compared to the SCD300 condition are thought to reflect a parallel processing strategy that is associated with a more flexible but interference-prone cognitive control mode.

However, a different interpretation is also conceivable. Consider that the 300 ms interval in the SCD300 condition gives participants 'a lot' of time (in the context of neural processing) to switch between different goals. In contrast, the SCD0 condition gives very little time to switch between goals. As such, rather than indicating parallel and interference-prone processing, longer reaction times in the SCD0 condition might reflect an inability to switch under time pressure—meaning that longer reaction times in this condition are actually diagnostic of greater cognitive stability rather than flexibility. This alternative interpretation converges on a study relating color vision to response conflict (Colzato, Sellaro, et al., 2014). There it is suggested that individuals with good color vision (who had longer SCD0 than SCD300 reaction times in Chapter Two) actually have a more stable and interference-resistant cognitive control mode. This is concluded based on the finding that they demonstrate a smaller congruency effect in the Simon paradigm, which indicates a superior ability to ignore task-irrelevant information. Taking together this report and Chapter Two, these findings highlight that it is crucial to consider alternative interpretations of results. An important step in doing so is to use and contrast different experimental paradigms, as in this particular example the action-cascading and Simon paradigms.

The second important lesson from this dissertation lies in the contrasting results of Chapters Six and Seven. In both cases, studies are presented that aim to investigate the role of dopamine in a brain stimulation technique called transcranial direct current stimulation (tDCS). Previous studies have demonstrated that a genetic predisposition toward higher or lower prefrontal dopaminergic signaling determines the cognitive-behavioral response to tDCS when stimulation is applied *during* task performance (Nieratschker et al., 2015; Plewnia et al., 2013). Chapter Six aimed to extend this finding by investigating whether dopamine also plays a role in tDCS when stimulation is applied *before* task performance. Indeed, this chapter reports that individuals who received L-tyrosine supplementation—which modestly enhances dopamine activity—responded differently to tDCS in terms of working memory performance than those who were supplemented with a

placebo. Subsequently, Chapter Seven investigated whether this pattern of results can be replicated not using a dopaminergic manipulation but instead using baseline, pre-existing individual differences in dopamine activity. To investigate this, participants were genotyped to estimate prefrontal dopaminergic signaling (as in previous studies on tDCS), and underwent the same stimulation protocol of Chapter Six. Contrary to the previous chapter and previous studies, these individual differences did not predict different responses to the tDCS for individuals with higher as compared to lower prefrontal dopaminergic signaling. Considering this and previous findings, Chapters Six and Seven suggest that (i) dopamine might differentially affect tDCS depending on whether stimulation is applied during or before task performance, and (ii) tDCS is affected in different ways by a manipulation of dopamine activity (as in Chapter Six) and baseline differences in dopamine (as in Chapter Seven). The latter point has the broader implication that researchers should be careful when generalizing results from manipulation of a neurotransmitter system to naturally-occurring differences in activity of that system—something that is a common practice in cognitive neuroscience research.

The third and final lesson lies in the apparent contrast between Chapters Nine and Ten. Both chapters investigate the effect of a presumed increase in neural inhibition on response selection. In Chapter Nine neural inhibition is enhanced using a technique called transcutaneous (through the skin) vagus nerve stimulation (tVNS). This technique is often used to treat epilepsy patients, as it can enhance the release of the inhibitory neurotransmitters GABA and noradrenaline. Because of the increase in these neurotransmitters, intracortical inhibition is stronger, presumably making it easier for the brain to select the appropriate response among competing response alternatives. Consistent with this idea, tVNS enhanced response selection by preventing a slowing of response speed on certain trials in the serial reaction time (SRT) task. However, Chapter Ten reports on a different technique to enhance neural inhibition and demonstrates that this impairs rather than enhances response selection processes. In this chapter, tDCS is used to stimulate the cerebellum,

which exerts an inhibitory tone over the primary motor cortex. As such, excitatory stimulation of the cerebellum strengthens this inhibition, thereby decreasing excitability of the motor cortex. Chapter Ten reports that this stimulation produces an increase in reaction times on the SRT task, consistent with the idea that inhibition of the motor cortex hinders the initiation of responses. In sum, Chapter Nine reports that increased neural inhibition enhances response selection whereas Chapter Ten reports that this impairs response selection.

These findings highlight that regional specificity might play an important role in the effects of neural inhibition on response selection. Indeed, previous studies have demonstrated that higher GABA concentration (associated with stronger inhibition) in some but not other regions predicts better response selection ability (Boy et al., 2011; Dharmadhikari et al., 2015; Sumner et al., 2010). These regions include, for example, the dorsolateral prefrontal cortex, the thalamus and striatum. In contrast, excitatory tDCS directly applied to the motor cortex (which presumably decreases inhibition) has also been reported to enhance response selection (Nitsche, Schauenburg, et al., 2003). Based on these studies, it is conceivable that tVNS might have enhanced response selection by promoting GABA activity in for example thalamic and striatal regions, whereas the inhibitory effects of cerebellar tDCS mainly targeted the primary motor cortex and led to an impairment in response selection ability. However, the important lesson to take away in this case is that in order to better understand and predict the effects of stimulation techniques such as tVNS and cerebellar tDCS, it is crucial to investigate how and which brain regions are affected by the different techniques.

In conclusion, the biological underpinnings of cognitive-behavioral control are highly complex, involving many different neurotransmitters and their interactions, as well as various different brain regions that contribute differentially to control. This dissertation has shown that it is possible to non-invasively estimate individual differences in neural chemistry and use them to predict performance on various experimental tasks. Furthermore, it demonstrated that some but not all available techniques for manipulation of

neural chemistry and inhibition can enhance performance. Better understanding how and why these techniques work is an endeavor that is sure to stimulate research in many more years to come.

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Summary in Dutch

Nederlandse samenvatting

Zonder het door te hebben leveren mensen dagelijks uitzonderlijke prestaties. We navigeren een steeds complexere en uitdagende wereld door gebruik te maken van onze verfijnde vaardigheid om habituele neigingen te overkomen. Bovendien kunnen we ons handelen zorgvuldig plannen, uitvoeren en aanpassen om zodoende de doelen te behalen die we voor onszelf stellen. Er wordt gerefeerd naar dit vermogen tot doelgericht gedrag—vaak beschouwt als een kenmerk van de superioriteit van de mens boven andere diersoorten—als 'cognitieve controle' of 'executive functie'. Dit zijn bijzonder vage, meestal synonieme concepten die meer dienen als een parapluterm voor veel verschillende processen dan dat ze refereren naar één functie. Tientallen jaren aan neuropsychologisch onderzoek zijn gewijd aan het begrijpen van cognitieve controle en zijn deelprocessen, de manier waarop het geïmplementeerd is in het brein, en hoe we de effectiviteit kunnen beïnvloeden—en mogelijk verbeteren. Deze vraagstukken dragen het onderzoek dat gepresenteerd wordt in deze dissertatie. Het onderzoek in deze dissertatie betreft voornamelijk de overkoepelende vragen van hoe chemische processen in het brein cognitieve controle mogelijk maken en beïnvloeden, en of we deze biologische onderleggingen van doelgericht gedrag non-invasief kunnen meten en manipuleren.

Cognitieve controle

Wat betreft het definiëren en operationaliseren van cognitieve controle is deze dissertatie geïnspireerd door twee invloedrijke en zeker niet wederzijds-exclusieve theoretische kaders. Het eerste kader is gerepresenteerd in het werk van Miyake et al. (2000), dat zich focuste op het identificeren van drie belangrijke executieve functies en het bepalen van hun onderscheidbaarheid. Miyake et al. veronderstellen dat cognitieve controle uit drie hoofdfuncties bestaat, namelijk *inhibition* (i.e., het vermogen om prepotente/dominante

responsen te weerhouden), *updating* (i.e., het vermogen om werkgeheugen representaties vast te houden en bij te werken), en *shifting* (i.e., het vermogen om te wisselen tussen doelen en taken). Een belangrijke bevinding van Miyake et al. is dat deze functies (maar) middelmatig gecorreleerd zijn met elkaar, wat impliceert dat dit scheidbare processen zijn die gevoelig zijn voor verschillende manipulaties. In lijn met dit idee laten cognitieve trainingsstudies zien dat het trainen van een van deze functies zelden gegeneraliseerde effecten heeft op de andere functies. Echter onderstreept de middelmatige correlatie van deze functies ook het feit dat executieve functies een gedeelde onderlegging hebben—waarop straks wordt teruggekomen—en dat hun effectiviteit afhangt van elkaar (zie Diamond, 2013).

Het tweede theoretische kader betreft in mindere mate specifieke cognitieve functies en stelt in plaats daarvan voor dat er verschillende cognitieve controle 'modi' of 'staten' zijn die beïnvloeden hoe de eerdergenoemde functies opereren. Met name wordt gedacht dat controle modus varieert van (i) een meer stabiele setting die het vasthouden van doelen ondersteunt en hen beschermt tegen afleiding, tot (ii) een meer flexibele setting die ontkoppeling van en wisselen tussen doelen en taken bevordert (Cools & D'Esposito, 2011; Goschke, 2003; Hommel, 2015). Elke controle modus is in verschillende situaties voordelig, maar heeft ook noemenswaardige nadelen. Hoewel een stabiele controle modus het navolgen van een specifiek doel toelaat, brengt dit het risico met zich mee dat iemand te rigide is om zich aan te passen aan een verandering in de omgeving. In tegendeel, een flexibele controle modus laat het efficiënt wisselen tussen doelen toe, maar kan iemand ook afleidbaar maken wanneer dit wisselen niet selectief gebeurt. Zodoende vereist adaptieve cognitieve controle een balans tussen de tegenstrijdige vereisten van cognitieve stabiliteit en flexibiliteit, wat ook wel bekend staat als de *cognitieve control paradox* of de *paradox van de flexibele geest*.

Er is grote compatibiliteit tussen deze twee theoretische kaders van cognitieve controle. Bijvoorbeeld, Miyake et al. (2000) rapporteren noemenswaardige individuele verschillen in prestaties op taken die de drie voorgestelde executieve functies meten, en deze verschillen zijn mogelijk het

gevolg van individuele variabiliteit in cognitieve controle modus. Dat wil zeggen, iemand met een meer stabiele controle modus zou plausibel beter zijn in het inhiberen van handelingen uitgelokt door afleidende, taak-irrelevante stimuli, terwijl iemand met een meer flexibele controle modus beter is in het updaten van hun werkgeheugen representaties en het wisselen tussen doelen en taken. Dit idee wordt ondersteund door verschillende studies (bijvoorbeeld, Colzato, Ozturk, & Hommel, 2012; Colzato, Sellaro, Samara, & Hommel, 2015; Colzato, Szapora, Lippelt, & Hommel, 2017; Fischer & Hommel, 2012; Fröber & Dreisbach, 2017). De vraag *waarom* sommige individuen superieure inhibitievermogen of cognitieve flexibiliteit vertonen betreft nog een overeenkomst tussen deze twee theoretische kaders en de gedeelde biologische onderlegging van executieve functies waar eerder naar verwezen werd: dopamine activiteit in het brein.

Dopamine

Er wordt gedacht dat de neurotransmitter dopamine in grote mate individuele verschillen in cognitieve controle modus en de efficiëntie van de drie grote executieve functies bepaalt. Dopamine wordt vaak een neuro*modulator* genoemd vanwege zijn wijdverspreide, complexe effecten op neurale activiteit (Nieoullon, 2002; Seamans & Yang, 2004). In plaats van het volgen van een 'meer is beter' regel, volgt de relatie tussen dopamine activiteit en cognitieve prestatie vaak een karakteristieke omgekeerde-U relatie (Cools & D'Esposito, 2011; Cools, 2006; Goldman-Rakic, Muly, & Williams, 2000). Dat wil zeggen, een middelmatig niveau van dopaminerge activiteit is veelal geassocieerd met optimale prestatie, terwijl zowel lagere als hogere dopamine activiteit gepaard gaat met suboptimale prestatie.

Hoewel dopamine wellicht het best bekend is bij de algemene bevolking voor zijn rol in beloning, de ervaring van plezier, en verslaving, is het moeilijk om zijn belang bij cognitieve controle te overdrijven. Om dit belang te begrijpen is het noodzakelijk om een onderscheid te maken tussen twee dopaminerge paden in het brein die op verschillende wijze bijdragen aan cognitieve controle. Dit zijn (i) het mesocorticale pad dat projecteert naar

cingulate en prefrontale cortex, en (ii) het nigrostriatale pad dat projecteert naar de basale ganglia. Kort gezegd wordt gedacht dat dopaminerge activiteit in het eerste pad cognitieve stabiliteit ondersteunt terwijl activiteit in het tweede pad cognitieve flexibiliteit ondersteunt (Cools & D'Esposito, 2011; Cools, 2006).

In meer detail, binnen de prefrontale cortex (PFC) moduleert dopamine cognitieve controle via twee verschillende families van receptoren: de D1-achtige en D2-achtige receptoren. Zoals uiteengezet in de *dual-state theory* van PFC functie (Durstewitz & Seamans, 2008) leidt dopaminerge stimulatie van prefrontale D1-achtige receptoren tot het inhiberen van vuren van neuronen in een lage, spontane activatiestaat terwijl het vuren van neuronen in een hoge, persistente activatiestaat wordt bevorderd. Dit verhoogt de corticale signaal-tot-ruis ratio en faciliteert de stabiliteit van mentale representaties in PFC. Aan de andere kant, activatie van D2-achtige receptoren leidt tot een algehele vermindering in inhibitie van PFC neuronen, wat hun spontaan vuren faciliteert en daarmee flexibele maar ook storingsgevoelige representaties bevordert (Robbins, 2005; Seamans, Gorelova, Durstewitz, & Yang, 2001; Seamans & Yang, 2004; Trantham-Davidson, Neely, Lavin, & Seamans, 2004). Zodoende wordt aangenomen dat dopamine in de PFC de balans tussen een stabiele en flexibele controle modus moduleert door middel van de ratio tussen D1 en D2-achtige receptor activatie.

Binnen de basale ganglia bevordert dopamine flexibele controle via een *input-gating* mechanisme dat bepaalt of de PFC open is voor nieuwe informatie. Het *prefrontal-cortex basal-ganglia working memory model* (Frank, Loughry, & O'Reilly, 2001; Hazy, Frank, & O'Reilly, 2006; O'Reilly, 2006) stelt voor dat *phasic* dopamine activiteit in de basale ganglia een zogenaamde poort opent naar de PFC, wat corticale representaties vatbaar maakt voor updaten en storing, terwijl een gebrek aan dopamine activiteit in de basale ganglia zorgt dat de poort dicht blijft en daarmee corticale representaties beschermd zijn tegen afleiding (zie ook Braver & Cohen, 2000). Belangrijk is dat dopaminerge stimulatie van D1-achtige receptoren in de basale ganglia het doorlaten van informatie faciliteert terwijl D2 receptoren dit tegengaan, en een verhoogd *tonic* dopamine niveau in de basale ganglia leidt

voornamelijk tot stimulatie van D1 over D2 receptoren (Hazy et al., 2006; O'Reilly & Frank, 2006; van Schouwenburg, Aarts, & Cools, 2010). Het gevolg hiervan is dat hogere niveaus van dopamine in de basale ganglia flexibiliteit bevorderen door toegang van informatie tot de PFC te faciliteren. Tegelijkertijd verhoogt dit echter ook het risico dat taak-irrelevante informatie interfereert met het vasthouden van informatie van in PFC, waardoor niet alleen flexibiliteit maar ook afleidbaarheid wordt verhoogd.

Samengevat is dopamine bijzonder belangrijk bij het begrijpen van cognitieve controle. Via regio-specifieke effecten in corticale en subcorticale netwerken kan het cognitieve processen meer stabiel of flexibel maken, en zodoende de effectiviteit van *inhibition*, *updating* en *shifting* beïnvloeden. Echter zou het nalatig zijn om te impliceren dat dopamine de enige neurotransmitter is dat van belang is bij cognitieve-gedragsmatige controle. Het is bekend dat andere neurotransmitters ook een belangrijke rol spelen, zoals noradrenaline (Robbins, 2005), serotonine (Cools, Roberts, & Robbins, 2008), en glutamaat en GABA (de la Vega et al., 2014; Munakata et al., 2011). Daarom zullen de laatste hoofdstukken van deze dissertatie de focus verschuiven naar de laatste twee neurotransmitters, glutamaat en GABA, en onderzoeken hoe manipulatie van deze neurotransmitter system controle beïnvloeden.

Glutamaat en GABA

Als de primaire exciterende en inhiberende neurotransmitters, respectievelijk, spelen glutamaat en GABA een belangrijke rol in de controle over handelingen. Kort gezegd wordt gedacht dat glutamaat en GABA (en met name de balans tussen de twee) bepalend zijn voor het niveau van intracorticale inhibitie, wat vervolgens het vermogen beïnvloedt om een specifieke representatie of handeling te kiezen uit verschillende alternatieven (de la Vega et al., 2014; Munakata et al., 2011). Dit kan van invloed zijn op alledaagse situaties zoals het kiezen welk woord te gebruiken in een zin of een besluit te nemen wanneer er niet een duidelijk beste optie is.

Kort gezegd, hogere niveaus van glutamaat (en omgekeerd, lagere GABA-niveaus) onderdrukken de competitie tussen representaties in PFC, waardoor de kans groter is dat alternatieve, wellicht zelfs taak-irrelevante concurrenten actief worden. Dit kan resulteren in het kiezen van de verkeerde handeling, of het proces van het kiezen van de juiste handeling vertragen. In tegendeel, meer competitie (als gevolg van lagere glutamaat en/of hogere GABA niveaus) heeft het tegenovergestelde effect door de activatie van concurrerende responsen te onderdrukken (de la Vega et al., 2014; Jocham, Hunt, Near, & Behrens, 2012). Verschillende studies hebben dit model van actie selectie binnen het brein bevestigd, bijvoorbeeld door te laten zien dat hogere GABA concentraties in zekere regionen voorspellend zijn voor snellere (Dharmadhikari et al., 2015) en meer accurate (Haag et al., 2015) responsen in de Simon taak, een klassieke respons-interferentie taak (Hommel, 2011).

In het kader van dit model van actie selectie en inhibitie in het brein zullen de laatste drie hoofdstukken in deze dissertatie onderzoeken hoe een veronderstelde verhoging of verlaging van neurale inhibitie een effect heeft op respons selectie. Dit wordt onderzocht door gebruik te maken van het *serial reaction time* (SRT) paradigma (Abrahamse & Noordzij, 2011), waarin men een sequentie van knoppen snel moet indrukken. Deze sequentie kan willekeurig zijn, of een ingebedde *second-order conditional* (SOC) sequentie bevatten. Terwijl een willekeurige response sequentie sterk berust op een stimulus-georiënteerde, reactieve modus van controle, is het in een SOC-sequentie mogelijk om kennis van de vorige twee responsen te gebruiken om te anticiperen wat de volgende respons zal zijn. Zodoende laten SOC sequenties een meer plan-georiënteerde, proactieve modus van controle toe (Tubau, Hommel, & López-Moliner, 2007) die steeds snellere en accurate responsen toelaat. Zodoende is het mogelijk om met de SRT-taak te onderzoeken hoe response selectie, inhibitie van irrelevante responsen, en de impliciete formering van response sequentie structuren gevoelig zijn voor een verandering in het niveau van neurale inhibitie.

Overzicht

Deze dissertatie kan worden onderverdeeld in drie overkoepelende thema's. Het eerste deel (Hoofdstukken 1-2) presenteert een literatuur review en een empirische studie die focussen op non-invasieve markers van individuele verschillen in dopamine functie en of het mogelijk is cognitieve controle prestatie te voorspellen op basis van deze verschillen. Het tweede deel (Hoofdstukken 3-7) verschuift van deze correlationele aanpak naar milde experimentele manipulaties van het dopaminerge systeem en hun geassocieerde veranderingen in cognitieve controle, zoals besproken in twee literatuur reviews en twee empirische studies. Als laatst betreft het derde deel (Hoofdstukken 8-10) drie empirische studies die verschillende methoden gebruiken om neurale inhibitie te manipuleren om zodoende de effecten op actie selectie te onderzoeken.

Hoofdstuk Een presenteert een uitgebreide review van literatuur die het spontane oog knipper gehalte (*eye blink rate*; EBR) gebruikt als indirecte marker van dopaminerge activiteit. Zoals besproken in dit hoofdstuk is er veel literatuur die een positieve relatie aantoont tussen EBR en dopaminerge activiteit. Kort gezegd laten farmacologische studies zien dat dopamine agonisten en antagonisten respectievelijk EBR verhogen en verlagen, en klinische populaties gekenmerkt door hypo-actieve dopamine activiteit vertonen lage EBR terwijl populaties gekenmerkt door hyper-actieve dopamine activiteit een hoge EBR vertonen. Met name interessant is de bevinding dat EBR in gezonde individuen de cognitieve prestatie op verschillende experimentele paradigma's kan voorspellen. In lijn met het idee dat EBR vooral geassocieerd is met dopaminerge activiteit in de basale ganglia, voorspelt hogere EBR meer cognitieve flexibiliteit zoals gemeten, bijvoorbeeld, op paradigma's van taak-wisselen en divergent denken.

Aangezien er al omvangrijke literatuur is over EBR als marker van dopaminerge activiteit zal **Hoofdstuk Twee** een studie presenteren dat focust op een ander aspect van onze ogen dat mogelijk dopaminerge activiteit voorspelt. Met name blijkt dat kleurenvisie, i.e., het vermogen om kleuren te onderscheiden, voorspellend is van individuele verschillen in dopamine en

gerelateerde cognitieve functies. Dit werd onderzocht door het testen van kleurenvisie en prestatie op een *action cascading* (ook bekend als *multitasking* of taak-wisselen) paradigma. *Action cascading* refereert naar het vermogen om verschillende doelen achter elkaar uit te voeren en tussen doelen te wisselen. Dit kan gedaan worden in een meer seriële, stap-voor-stap wijze waarbij het volgende doel pas geactiveerd wordt wanneer het vorige doel volledig is afgerond, of in een meer parallelle, overlappende wijze waarbij verschillende doelen tegelijkertijd geactiveerd worden. *Action cascading* is gerelateerd aan dopamine functie, aangezien een vorige studie heeft aangetoond dat individuen met een genetische predispositie voor meer dopamine D2 receptor activiteit (wat met name prevalent is in de basale ganglia) de neiging hebben om doelen in een meer parallelle wijze te verwerken. De resultaten in Hoofdstuk Twee laten zien dat, op vergelijkbare wijze, individuen met goede kleurenvisie presteren op een manier die consistent is met een meer parallelle dan seriële modus van doelen verwerken. Dit suggereert onder voorbehoud dat goede kleurenvisie met name voorspellend is van de dopamine D2 receptor en cognitieve flexibiliteit. Een discussie van deze interpretatie, en een alternatief perspectief, wordt uiteengezet in de Discussie sectie van deze dissertatie.

Hoewel markers als EBR en kleurenvisie ons in staat stellen om veronderstelde individuele verschillen in dopamine functie te onderzoeken, is deze aanpak correlationeel van nature en kan daarom niet een causale rol van dopamine in de onderzoeksresultaten bevestigen. Daarom zullen de volgende hoofdstukken focussen op een milde maar effectieve methode om dopaminerge activiteit te manipuleren. In **Hoofdstuk Drie** wordt een uitgebreide review gepresenteerd met betrekking tot de cognitief-gedragsmatige effecten van het toedienen van het voedingssupplement L-tyrosine, wat de biochemische voorloper is van dopamine. Aangezien tyrosine omgezet kan worden in dopamine in het brein, hebben veel studies onderzocht of tyrosine supplementatie gunstige effecten heeft op cognitieve processen die gemoduleerd worden door dopamine. Inderdaad, het is aangetoond dat tyrosine de drie executieve functies uiteengezet door Miyake et al. (2000) kan verbeteren, dat wil zeggen *inhibition* (Colzato, Jongkees, Sellaro, van den

Wildenberg, & Hommel, 2014), *task-switching* (Steenbergen, Sellaro, Hommel, & Colzato, 2015), en met name werkgeheugen (Colzato, Jongkees, Sellaro, & Hommel, 2013; Jongkees, Sellaro, et al., 2017; Thomas, Lockwood, Singh, & Deuster, 1999). Noemenswaardig is het feit dat de effecten van tyrosine enkel betrouwbaar lijken te zijn wanneer men blootgesteld wordt aan een externe stressor zoals hitte, kou, of lawaai, of een interne stressor zoals hoge cognitieve belasting. Daarom wordt voorgesteld dat tyrosine een '*depletion reverser*' is, aangezien het alleen effectief is in omstandigheden waarin prestatie normaliter verslechterd zou zijn door de uitputting van cognitieve middelen, motivatie, of dopamine niveaus.

Hoofdstuk Vier dient als uitbreiding van het vorige hoofdstuk, door te benadrukken dat de effecten van tyrosine waarschijnlijk afhankelijk zijn van individuele verschillen in dopamine functie. Inderdaad wordt vaak geobserveerd dat het effect van een dopaminerge manipulatie staat-afhankelijk is en verschillend is voor hen met een laag of hoog baseline dopamine niveau. Doorgaans worden individuen met een lager dopamine niveau omhooggeschoven op de omgekeerde-U-curve die dopamine aan cognitieve prestatie relateert wanneer hen een verhoging in dopamine activiteit wordt toegediend. In tegendeel, individuen met een hoger dopamine niveau zouden als gevolg hiervan omlaag schuiven naar de rechterzijde van de curve. Voor laag en hoog baseline niveau individuen zou dit respectievelijk leiden tot een geobserveerde toename en afname in cognitieve prestatie in vergelijking met baseline[2]. Dit is waarom de korte review in Hoofdstuk Vier verschillende mogelijke markers van individuele verschillen in dopamine functie voorstelt die wellicht de effectiviteit van tyrosine supplementatie voorspellen. Deze markers zijn onder andere EBR en kleurenvisie zoals onderzocht in eerdere hoofdstukken, en genetische markers van dopamine functie in PFC of basale ganglia. Een recente studie heeft een van deze hypotheses bevestigd door aan te tonen dat tyrosine supplementatie meest effectief was in individuen met een

[2] Echter moet worden opgemerkt dat een dergelijk patroon van resultaten ook verklaard kan worden door het fenomeen 'regressie naar het gemiddelde' (zie Barnett, van der Pols, & Dobson, 2005). Toekomstige studies moeten deze alternatieve verklaring in acht nemen, wat in de huidige literatuur zelden wordt gedaan.

genetische predispositie naar lagere dopamine activiteit in de basale ganglia (Colzato et al., 2016), van wie verondersteld wordt dat ze de meeste ruimte hebben om omhoog te schuiven op de curve die dopamine activiteit relateert aan prestatie.

Hoofdstuk Vijf presenteert een van de empirische studies van tyrosine supplementatie die onderdeel uitmaakt van de review in Hoofdstuk Drie. Dit hoofdstuk onderzoekt met name de effectiviteit van tyrosine supplementatie bij het verbeteren van inhibitievermogen, waarvan bekend is dat het afhangt van dopamine activiteit. Dit is onderzocht door gebruik te maken van het *stop-signal* paradigma, waarbij proefpersonen een simpele geforceerde-keuze reactietijd taak zo snel mogelijk uitvoeren tenzij een stopsignaal aangeeft dat ze hun respons moeten inhouden. Door te variëren wanneer het stopsignaal verschijnt is het mogelijk om te schatten hoe veel tijd iemand nodig heeft om hun respons succesvol te inhiberen. Zoals verwacht laten de resultaten zien dat proefpersonen sneller waren in het inhouden van hun respons na tyrosine supplementatie zoals vergeleken met een placebo. In tegendeel was respons executie niet beïnvloedt, wat onderstreept dat tyrosine supplementatie alleen effectief is in het verbeteren van prestatie op bijzonder uitdagende taken.

In **Hoofdstuk Zes** wordt een andere aanpak genomen tot dopaminerge manipulatie, door gebruik te maken van *transcranial direct current stimulation* (tDCS). Dit is een non-invasieve methode van hersenstimulatie waarvan bekend is dat het corticale excitabiliteit en neurale plasticiteit kan beïnvloeden. Er wordt gedacht dat tDCS niet direct maar indirect dopamine kan beïnvloeden door een effect op GABA, wat vervolgens een modulerende invloed heeft op dopaminerge activiteit. Hoewel er veel studies zijn die laten zien dat tDCS cognitieve prestaties kan beïnvloeden, is er ook veel twijfel over de betrouwbaarheid van deze effecten aangezien resultaten variëren tussen studies. Dit is waarschijnlijk deels te wijden aan methodologische verschillen tussen studies, maar er is ook gesuggereerd dat individuele verschillen in dopamine kunnen bijdragen aan variabiliteit in respons op tDCS (Wiegand, Nieratschker, & Plewnia, 2016). Er zijn enkele studies die dit idee ondersteunen. In acht nemend dat er een omgekeerde-U-curve is in de relatie

tussen dopamine activiteit en cognitieve prestaties, hebben voorgaande studies laten zien dat het toepassen van exciterende (anodale) stimulatie bij individuen die al een hoog niveau van dopamine activiteit hebben leidt tot een afname in prestatie. Ook leidt het toepassen van inhiberende (kathodale) stimulatie bij individuen die al een laag niveau hebben van dopaminerge activiteit tot een afname in prestatie. Dit patroon van resultaten is waargenomen door een onderscheid te maken tussen individuen met een genetische predispositie naar hogere of lagere dopamine activiteit in de PFC. Echter is het belangrijk om te erkennen dat genetische studies enkel correlationeel bewijs kunnen leveren en niet kunnen spreken tot de causale rol van dopamine in de effecten van tDCS. Dat is waarom de studie gepresenteerd in Hoofdstuk Zes een meer experimentele aanpak zocht door tDCS te combineren met tyrosine supplementatie en het effect op werkgeheugen te testen, welke de meest onderzochte cognitieve functie is in tDCS studies. Zoals in het overgrote deel van voorgaande studies werd tDCS toegepast over de dorsolaterale PFC, welke een regio is dat belangrijk is voor cognitieve control en met name werkgeheugen. In lijn met de eerdergenoemde bevindingen met genetica, lieten de resultaten zien dat de combinatie van tyrosine met exciterende stimulatie leidde tot een afname in werkgeheugenprestatie. Deze bevinding ondersteunt het idee dat tDCS beïnvloedt kan worden door dopamine in het brein, en kan leiden tot een afname in prestatie wanneer deze wordt gecombineerd met een manipulatie die ook dopamine activiteit verhoogd.

Gezien het bewijs dat een rol voor dopamine in de effecten van tDCS ondersteund, onderzoekt **Hoofdstuk Zeven** of het patroon van resultaten uit het vorige hoofdstuk nagebootst kunnen worden met al-bestaande individuele verschillen in dopamine activiteit in plaats van een experimentele manipulatie daarvan. Als dit het geval blijkt, dan zouden dit en het vorige hoofdstuk de belangrijke implicaties hebben dat (i) dopamine een rol speelt in de effecten van tDCS en dat (ii) individuele verschillen in dopamine activiteit mogelijk bijdragen aan de variabiliteit in de effecten van tDCS. Om deze tweede hypothese te testen presenteert dit hoofdstuk een studie dat dezelfde experimentele opzet gebruikt als in Hoofdstuk Zes. In plaats van een tyrosine

manipulatie, worden proefpersonen ditmaal gegenotypeerd voor het COMT Val[158]Met polymorfisme, welke het niveau van dopaminerge activiteit in de PFC bepaalt. Vergelijkbaar met het patroon van resultaten dat werd geobserveerd in Hoofdstuk Zes, was hierbij de hypothese dat het toedienen van exciterende stimulatie bij hen met een predispositie voor hogere dopamine activiteit zou leiden tot een verslechtering van werkgeheugen prestatie. Opmerkelijk genoeg leverde de studie enkel nul-bevindingen. Dat wil zeggen, verschillende COMT polymorfismen waren niet geassocieerd met verschillende responsen op de tDCS. In combinatie met de bevindingen van het vorige hoofdstuk, impliceert dit dat resultaten van studies met farmacologische manipulaties (bijvoorbeeld tyrosine) enkel voorzichtig gegeneraliseerd moeten worden naar bevindingen met individuele verschillen (bijvoorbeeld het COMT-polymorfisme). In dit specifieke geval lijken *state* (i.e., een manipulatie van) en *trait* (i.e., baseline) verschillen in dopamine een verschillend effect te hebben op tDCS.

Hoofdstuk Acht maakt de overgang van dopamine naar het onderwerp van neurale inhibitie en respons selectie. De volgende hoofdstukken, elk op hun eigen manier, onderzoeken hoe een veronderstelde toename of afname in neurale inhibitie een effect heeft op het vermogen om de juiste respons te selecteren uit verschillende alternatieven. In Hoofdstuk Acht wordt de eerste studie gerelateerd aan dit onderwerp gepresenteerd, waarbij gefocust wordt op het voedingssupplement glutamine. Zoals tyrosine de voorloper is van dopamine, is glutamine de voorloper van glutamaat en GABA. Dit zijn respectievelijk de voornaamste exciterende en inhiberende neurotransmitters en daarom kan supplementatie van glutamine mogelijk het niveau van neurale inhibitie beïnvloeden. Hoewel glutamine een populair supplement is dat vaak gebruikt wordt door bodybuilders, zijn de cognitief-gedragsmatige effecten ervan weinig onderzocht tot op heden. Om te onderzoeken of en hoe glutamine de response selectie beïnvloedt, werden proefpersonen gesupplementeerd met glutamine of een placebo en voerden zij vervolgens een SRT-taak uit, wat zowel *sensorimotor* (i.e., stimulus-georiënteerde) controle meet als impliciet sequentieel leren. De resultaten lieten geen effect zien van glutamine op

motorische leerprocessen, maar zij die glutamine kregen maakten wel meer respons fouten, voornamelijk wanneer de taak vereiste dat ze wisselden van reageren met de ene naar de andere hand. Deze bevinding impliceert dat glutamine het niveau van glutamaat ten opzichte van GABA verhoogde, met als gevolg meer corticale excitabiliteit en response competitie tussen verschillende alternatieven. Deze vermindering in prestatie bleek alleen betrouwbaar wanneer men moest wisselen van hand gedurende de taak, wat indiceert dat de verhoogde corticale excitabiliteit ervoor zorgde dat de lateraliteit van de vorige respons interfereerde met die van de huidige respons. Dit is de eerste demonstratie dat glutamine de response selectie kan verhinderen via een veronderstelde afname in neurale inhibitie.

In **Hoofdstuk Negen** werd onderzocht of het tegenovergestelde ook bewezen kan worden. Dat wil zeggen, of een toename in neurale inhibitie de respons selectie kan verbeteren. Correlationeel bewijs voor dit idee bestaat al, aangezien studies hebben aangetoond dat individuen met hogere GABA-niveaus in striatale and thalamische gebieden beter zijn in het selecteren van de juiste respons uit verschillende concurrerende alternatieven. Om causaal bewijs te vinden voor dit idee werd in de studie in Hoofdstuk Negen gebruik gemaakt van transcutane (door de huid) vagus zenuwstimulatie (*transcutaneous vagus nerve stimulation*; tVNS), een non-invasieve methode van hersenstimulatie die het niveau van GABA in het brein kan verhogen. Deze manipulatie werd wederom gecombineerd met de SRT-taak, om te bepalen of tVNS respons selectieprocessen kan verbeteren. Vergelijkbaar met het vorige hoofdstuk was er geen verschil in impliciet sequentieel leren tussen hen die actieve (echte) of *sham* (placebo) stimulatie kregen. Echter, zoals verwacht verbeterde tVNS de response selectie. Om precies te zijn, actieve tVNS elimineerde een fenomeen vergelijkbaar met '*inhibition of return*', waarbij proefpersonen langzamer zijn wanneer de huidige respons dezelfde is als de respons op twee trials eerder. In andere woorden, terwijl zij die *sham* tVNS kregen wel deze *inhibition of return* vertoonde, ook wel het *reversal effect* genoemd, lieten zij die actieve tVNS kregen niet dergelijke respons vertraging zien. Deze bevinding valt samen met eerdere studies die suggereren dat tVNS,

via een veronderstelde toename in GABA, een effectieve methode is om cognitief-gedragsmatige controle te verbeteren.

Als laatst werd in **Hoofdstuk Tien** neurale inhibitie gemanipuleerd met tDCS. Echter, terwijl voorgenoemde tDCS studies typisch direct gericht waren op PFC-regionen, werd in dit hoofdstuk tDCS toegepast op het cerebellum. Dit gebied is noemenswaardig voor het feit dat het tot wel 80% van alle neuronen in het gehele brein bevat, en het is bekend dat het een belangrijke rol speelt in het plannen, initiëren, en coördineren van beweging. Maar een klein aantal studies heeft tot op heden onderzocht of cerebellaire tDCS de respons selectie kan beïnvloeden, maar er is bewijs voor deze mogelijkheid afkomstig van een studie die laat zien dat cerebellaire tDCS een fenomeen genaamd cerebellaire-brein inhibitie (CBI) kan beïnvloeden. Dit refereert naar het feit dat het cerebellum een inhiberende werking heeft op de primaire motor cortex, en deze inhibitie kan versterkt worden door exciterende en verzwakt worden door inhiberende stimulatie van het cerebellum. Dit kan vervolgens beïnvloeden hoe moeilijk of makkelijk het is om beweging te initiëren. Om te onderzoeken of deze modulatie van CBI inderdaad zich vertaalt in een verandering in het vermogen om responsen te selecteren, kregen in Hoofdstuk Tien proefpersonen exciterende (anodale), inhiberende (kathodale), of *sham* (placebo) stimulatie over het cerebellum terwijl zij de SRT-taak uitvoerden. Zoals in de vorige hoofdstukken leek deze manipulatie niet direct een effect te hebben op impliciet motor sequentie leren, maar de exciterende stimulatie in vergelijking met de inhiberende en *sham* stimulatie beïnvloedde wel response selectie zoals bleek uit een algehele toename in reactietijd. Deze bevinding is consistent met het idee dat exciterende stimulatie van het cerebellum de CBI kan versterken en daarmee het vermogen beperkt om beweging te initiëren. Opmerkelijk is het feit dat deze studie ook een follow-up sessie 24 uur na de stimulatie bevatte, om te bepalen of de stimulatie gedurende de taak wellicht van invloed was op consolidatieprocessen die plaats vinden nadat de taak is afgerond. Deze follow-up liet een patroon van resultaten zien dat vergelijkbaar was met de vorige dag: zij die eerder exciterende stimulatie kregen lieten nog steeds verhoogde reactietijden zien, maar alleen wanneer zij twee

verschillende response sequenties moesten uitvoeren in hetzelfde SRT-blok. Dit indiceert dat wellicht de exciterende stimulatie van het cerebellum van invloed was op hoe robuust proefpersonen de motor sequentie leerde, wat vervolgens alleen te merken was wanneer een niet-getrainde sequentie op dag twee interfereerde met de getrainde sequentie. Deze resultaten zijn een van de eerste die vaststellen dat cerebellaire tDCS een potentiele methode is om response selectie te moduleren, en zij suggereren dat de effecten gemedieerd worden door een verandering in de inhiberende werking van het cerebellum op de primaire motor cortex.

Om dit overzicht af te sluiten: de hoofdstukken in deze dissertatie bieden inzicht in of en hoe het mogelijk is om individuele verschillen in neurochemie onderliggend aan cognitief-gedragsmatige controle te meten. Daarnaast verkent het verschillende methoden voor het non-invasief manipuleren van deze biologische basis en levert het bewijs dat sommige van deze methoden veelbelovend zijn voor cognitief-gedragsmatige verbetering.

Curriculum Vitae

Bryant J. Jongkees was born on December 12, 1991 in Nieuwegein, the Netherlands. In 2010, he obtained his pre-university level high school diploma from the Oosterlicht College in Nieuwegein. Thereafter he studied Psychology at Leiden University, graduating (cum laude/with honors) from the Bachelor program in Psychology in 2013. Bryant then started the Research Master's program in Psychology, Cognitive Neuroscience track at Leiden University, during which he worked as a research assistant investigating the relationship between dopamine and goal-directed behavior. In 2015, he graduated (summa cum laude/with honors) from the Master program and immediately started a PhD at Leiden University. Under the supervision of Prof.dr. Sander Nieuwenhuis and Prof.dr. Lorenza Colzato, Bryant has investigated the effects of individual differences in and mild manipulation of several neurotransmitter systems on various cognitive functions including working memory, task-switching, and motor sequence learning. The results of his doctoral work are outlined in this dissertation.

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