Cognitive enhancement : toward the integration of theory and practice

Steenbergen, L.

Cognitive Enhancement

Toward the integration of theory and practice

Laura Steenbergen

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Introduction

As a species, humans have always relied on their adaptability and intelligence in order to survive. It seems that all humans, regardless of their country, culture or race, have a natural tendency to always grow, develop, and learn. Because of this, there has always been great interest in how to grow, develop, and learn even more. This has been one of the most important drives for the field of cognitive enhancement. Cognitive enhancement is the use of any means (e.g., brain stimulation, video-gaming, food supplements) aimed at enhancing cognitive performance (e.g. creativity, memory, etc.) in healthy individuals. Cognitive enhancement has gained great attention over the past years, as the economic problems of the welfare system (i.e., increasing costs) have boosted the interest in procedures and activities that make welfare more affordable for society. That is, from an economical point of view, cognitive enhancement may help to decrease the costs of the welfare system. Especially with regard to the aging population, cognitive enhancement techniques may be used to delay cognitive decline in the elderly, which would extend the time people can live autonomously and, as such, reduce welfare costs. Similarly, the risk of behavioral problems and pathology in children can be reduced by training them – which likewise implies considerable decreases in the costs of our welfare systems. Enhancing cognitive functions may also speed up their education, which benefits society's educational systems.

In addition to the economic benefits that cognitive enhancement may bring, there is another viewpoint from which cognitive enhancement is gaining interest. That is, Western societies seem to continuously be driven towards more individualism, which emphasizes the existence and importance of individual differences. This includes the view of the individual as a director of his or her own life and a rather systematic deconstruction of the collective welfare system. This ideological turn towards individualism offers a natural breeding ground for a growing public interest in procedures and activities that help to express individual needs as well as to minimize weaknesses and further support strengths. As a result of this ideological

trend and the economical trend as described above, research on cognitive enhancement has benefited from a great increase in political, public and academic interest.

Need for theory

"*There is nothing so practical as a good theory*" – Kurt Lewin (quoted in Marrow, 1969)

Findings showing that individuals become better in a certain task after being stimulated, trained, or supplemented with one of the means of cognitive enhancement are meaningful from a practical point of view, although often not new. Many cognitive enhancement approaches do not go beyond concluding that the applied method has an enhancing effect. The typical problem that these approaches then run into is the inability to replicate the effect in related processes in subsequent studies, or to report any effect at all. One possible cause of this problem is that existing studies on cognitive enhancement have mainly been driven by practice (i.e., effect-driven), demonstrating enhancing effects of certain interventions. However, they often do not explain why cognitive enhancement should occur, or which mechanisms could have caused or modulated the effects. However, in order to reach interesting levels of enhancement, and in order to be able to apply this in other fields, clear ideas about the mechanisms underlying the cognitive functions one aims to improve are needed. That being said, it may be clear that Kurt Lewin's claim of nothing being as practical as a good theory applies nowhere better than in the field of cognitive enhancement, with practice being the observed effect and theory being the knowledge that explains the underlying mechanism(s). The present dissertation therefore aims to get a better understanding of the underlying mechanisms of how enhancing techniques affect cognition in healthy humans.

Overview

The means of cognitive enhancement involve various devices, drugs, and food supplements used to enhance cognitive functions. For example, brain stimulating devices targeting specific brain areas aimed at improving attention or working memory, or pills (e.g., methylphenidate) taken by students to help focus on their studies. As can be inferred from these two examples, cognitive enhancement is generally aimed at improving executive functioning including attentional control, inhibitory control, working memory, and cognitive flexibility, but can be aimed at improving social cognition as well. That is, social cognition and social behavior stem from numerous cognitive processes (e.g. attention), and can therefore be targeted by cognitive enhancement. In this thesis, enhancing effects on both cognitive and social functioning, and their underlying mechanisms are therefore discussed.

Brain stimulation

The stimulation of certain brain areas and/or the synthesis and release of certain neurotransmitters by applying electrical stimulation has been done for decades already. Techniques such as magnetic stimulation (TMS), transcranial direct current stimulation (tDCS), and vagus nerve stimulation (VNS), which use electrical stimulation have received considerable attention over the past years. In contrast to imaging techniques, which provide only correlational evidence, these techniques allow us to infer causal relations between the stimulated neurotransmitter system or brain area and the related cognitive function measured. In **Chapter one**, we introduce a technique called transcutaneous vagus nerve stimulation (tVNS) that, in contrast to direct vagus nerve stimulation (VNS), provides an easy, non-invasive and relatively safe method to stimulate the tenth cranial nerve (i.e., vagus nerve) in healthy subjects. This technique is applied by placing an electrode medial of the tragus at the entry of the acoustic meatus in the left ear. The electrode stimulates the afferent auricular branch of the vagus

nerve by applying a weak electrical current through the skin. This technique provides a relatively safe and easy way to investigate the role of the gamma-aminobutyric acid (GABA)-ergic and noradrenergic systems in cognitive processes. In this chapter, we investigate the role of these two systems in action cascading processes. That is, the ever-changing environment we are living in requires us to apply different action control strategies in order to fulfill a task goal. Indeed, when confronted with multiple response options it is fundamental to prioritize and cascade different actions. So far, very little is known about the neuromodulation of action cascading, although there is evidence showing that the GABA-ergic system is important because of its inhibitory features. There is also evidence showing that stress modulates action cascading processes, and stress is known to affect the noradrenergic system. So there is tentative support for the idea that norepinephrine (NE), playing an important role in stress responses, may affect functions during action cascading and lead to slowing of responses during multitasking. Given the idea that GABA – the main inhibitory neurotransmitter - and NE impact action selection, it was expected that active tVNS would improve action cascading processes. That is, tVNS would decrease reaction times on trials where responses have to be inhibited and changed to an alternative response, both when a person has to stop and change to an alternative response simultaneously, and when a person has to change a response when the first action is already successfully inhibited. This hypothesis is further supported by the fact that, from an anatomical point of view, action cascading efficiency is related to a neural network that includes the anterior cingulate cortex (ACC). Indeed, functional magnetic resonance imaging (fMRI) studies have shown an increase in activity in the cingulate cortex during active tVNS. Importantly, the vagal nerve is connected to the ACC, and the ACC is a crucial area for the execution of multi-component behavior. In this chapter, we demonstrate that active tVNS indeed modulates action cascading efficiency, providing considerable support for the idea of a crucial role of the GABA-ergic and noradrenergic pathways in action cascading.

Besides affecting the GABA-ergic and noradrenergic systems, two recent functional magnetic resonance imaging studies showed increased activation in the thalamus, prefrontal cortex (PFC) and insula during active

tVNS in healthy humans. Importantly, these areas are key areas related to social cognition such as social pain and mentalization (i.e., the ability to understand the mental state of oneself and others), and are linked to vicarious ostracism (i.e., the observation of other people being socially ignored and/or excluded). Interestingly, observing ostracism increases activity in the insula and ACC, areas that are also activated when directly experiencing ostracism. Moreover, observing ostracism activates the PFC and precuneus—brain regions associated with mentalization. Brain activation of both the mentalization areas and social pain-related regions correlates with individual differences in empathy when observing ostracism and with prosocial behavior toward the victim. This has been taken to suggest that differences in experiencing vicarious ostracism may also reflect individual differences in trait empathy. In **Chapter two** we assessed the causal role of this PFC-insula network in mediating vicarious ostracism and investigated whether active tVNS can modulate vicarious ostracism using an adapted version of the Cyberball game (Williams, 2009), a virtual ball-tossing game designed to measure prosocial compensation for ostracism. Given the available correlational evidence that vicarious ostracism involves the PFC-insula network, we hypothesized that tVNS would enhance prosocial helping behavior (i.e., increase the amount of ball-tosses to the ostracized person) in the Cyberball game. However, in this study we found that active tVNS did not increase prosocial helping behavior toward an ostracized person, as compared to sham (placebo) stimulation. Corroborated by Bayesian inference, which allows us to make inferences about non-significant effects by estimating the probability of their occurrence, we therefore conclude that tVNS does not modulate reactions to vicarious ostracism, as indexed by performance in a Cyberball game.

As described in the Introduction, cognitive enhancement and its methods have received considerable attention from the greater public. That is, the increased individualistic society stresses individual differences and encourages minimizing our weaknesses. Techniques such as tDCS, which has been shown to be effective in enhancing cognitive processes such as working memory, have therefore been brought on the market by what is called the ‘brain-training industry’. Commercial tDCS devices are supposed to have the same effects as medical tDCS devices, which are used to deliver

a weak electrical current to the brain by placing two electrodes on the head. Depending on the placement of the electrode, neuronal activity under the 'anodal' electrode is supposedly increased, whereas that under the 'cathodal' electrode decreased. The electrical current delivered by the tDCS device depolarizes (anodal) or hyperpolarizes (cathodal) membrane potentials, as such causing a relative increase or decrease in spontaneous neuronal firing. Although the actual effectivity of tDCS in modulating cognitive functions remains topic of debate because of the various factors (e.g. stimulation parameters, individual differences like genetic predispositions and hair thickness, anatomical differences, experimental design, etc.) influencing the effectivity of tDCS, consumers are told that using the commercial tDCS devices or playing so-called 'brain games' will make them smarter, better able to focus, and quicker learners. In the long run, this is said to perhaps even reduce cognitive decline associated with aging, and improve everyday functioning and memory. However, a recent consensus signed by several prominent researchers calls for a more critical and active role of the scientific community in evaluating the sometimes far-reaching, sweeping claims from the brain training industry with regard to the impact of their products on cognitive performance. In **Chapter three** we investigated whether the commercial tDCS headset *foc.us* (V.1), can indeed improve working memory, as advertised in the media. We applied the commercial tDCS headset to healthy participants, who then received a low intensity current to the frontal part of the brain administered by electrodes. Either during or after the stimulation, we asked participants to perform a working memory task in which they had to update remembered information. Findings showed that, compared to when the participants received sham stimulation, active stimulation actually impaired working memory performance. Even if preliminary, we believe that these results show the importance of a critical and active role of the scientific community in evaluating the claims made by the brain-training industry. More specifically, given the potential risks of misusing tDCS, and the fact that its long-term effects on the brain have not yet been fully explored, we believe that there is a need for regulations or official guidelines for the commercial use of tDCS.

Cognitive training

Chapter four focuses on the idea that certain lifestyles can enhance cognitive abilities because they train certain cognitive functions in itself. In this chapter, we test the idea that action videogames (AVGs), especially first person shooter games, require gamers to develop different action control strategies to rapidly react to fast moving visual and auditory stimuli, and to flexibly adapt their behavior to the constantly changing context of the game, and that this generalizes to cognitive control abilities. It is expected that playing first person shooter videogames is associated with enhanced action cascading performance. Replicating previous findings, it was demonstrated that, compared to non-videogame players, videogame players showed higher efficiency in response execution, but similar performance with regard to response inhibition (i.e. inhibitory control). Videogame players showed enhanced action cascading processes both when an interruption (stop) and a change towards an alternative response were required simultaneously, as well as when such a change had to occur after the completion of the stop process. The findings in this chapter suggest that playing AVGs is associated with enhanced action cascading and multi-component behavior without affecting inhibitory control. The latter finding is particularly intriguing as it challenges the anecdotal idea that AVG players are more impulsive than non-videogame players. If this would indeed have been the case, AVG players would have shown lower inhibitory efficiency than non-videogame players – but this was not the case. These findings may therefore represent an important first step in stimulating further research to assess whether videogames can be used to optimize cognitive control. Importantly, given the importance of action control in daily activities and the known difficulties shown by older adults in response selection and action cascading processes, the findings can have important practical implications for designing intervention/training studies aimed at overcoming or slowing down action control deficits associated with aging. However, one of the drawbacks of this chapter with regard to the implications it has for the general public, at least for now, is that the AVG players that were shown to have enhanced action cascading efficiently

played first person shooter videogames for at least five hours a week in the past year. Future studies are needed to investigate how much experience with AVGs is needed to obtain enhancing effects, and to investigate for how long these effects last. Whereas playing videogames is a rather time-intensive manner to enhance cognitive performance, the next chapters study cognitive enhancement means that result in rather acute effects.

Food supplements

From the first three chapters, it may become clear that brain stimulation techniques in itself are promising tools if used correctly. However, further investigation is needed before they will ever be ready to be used commercially (if ever). In the next chapters, we therefore focus on an even safer and healthier method to enhance cognition: food supplements. Food supplements denote a nutrient or group of nutrients such as vitamins, minerals, proteins, fats or oils, that are meant to supplement, but not substitute, a healthy diet. They provide a safe, healthy, and easy way to modulate cognitive processes. This idea is not new though, as several decades ago the German philosopher Ludwig Feuerbach already claimed that “Der Mensch ist, was er iβt” (i.e., you are what you eat, 1862, as cited in Feuerbach, 1960). Feuerbach was probably the first to promote the idea that the food one eats affects a person’s state of mind. With the recent economical, societal and ideological developments of supporting health and remaining vital in aging, the idea that the food we eat influences the way we think and perceive the world has received increasing attention (e.g., think about all the “superfoods” that are on the market nowadays). In the remaining chapters, based on knowledge about the physical effects (i.e., metabolic, chemical, etc.) several food supplements are put forward as “cognitive enhancers”.

As discussed earlier, active tVNS enhanced action cascading performance, most likely through affecting GABA and NE neurotransmitter levels in the brain. However, the exact role of each separate neurotransmitter cannot be investigated using tVNS. That is, tVNS targets different neurotransmitters simultaneously, which makes it impossible to

ascertain whether the observed effects resulting from tVNS are due to NE, GABA, or both. However, based on previous studies and theories as discussed in Chapter one, there is reason to believe that GABA plays a possible causal role in action cascading performance. In **Chapter five** therefore, it is investigated whether the intake of the food supplement GABA, which mimics the chemical structure of GABA and leads to increases of GABA in the brain, enhances action selection processes. That is, the general consensus is that action cascading processes rely on fronto-striatal networks, and GABA is likely to play an important role in the neuromodulation of action control processes. GABA plays a pivotal role in information encoding and behavioral control, in the regulation of motor functions, and in motor learning. More importantly, GABA also seems involved in action selection and response inhibition processes occurring in the frontal-striatal networks. Previous studies have also shown that superior performance in action cascading tasks is associated with increased concentrations of GABA in the brain. Taken together, these findings indicate an important role of GABA in the neuromodulation of action cascading processes, where slight increases of GABA are associated with better action cascading performance. In this chapter, it is indeed demonstrated that the intake of GABA directly influences the efficiency of action cascading. It is shown that the administration of a low dose of synthetic GABA reduced the time needed to change to an alternative response, regardless of whether this shift was required to occur simultaneously to a stopping process or when the stopping process had already finished. In addition, the intake of GABA reduced the time that people needed to inhibit the unwanted response. These findings offer substantial support for the idea of a crucial role of the GABA-ergic system in action cascading. Given that in daily life we are often confronted with multiple response options and need to efficiently prioritize and cascade our actions in order to successfully fulfill a task, this has important implications. That is, the intake of the food supplement GABA, and possibly foods containing GABA, could help to efficiently handle the ever-changing environment we are living in.

Building upon the previous chapter, action cascading involves a component called task-switching, in which dopamine (DA) seems to play an important role. **Chapter six** evaluates the intake of the amino acid tyrosine

(TYR), the chemical precursor of DA, as a method to enhance task-switching (i.e. cognitive flexibility). We suggest that TYR administration selectively counteracts DA depletion, a process in which performance levels decline corresponding to the decrease DA function in the brain: When exposed to a cognitively challenging task, the rate of DA synthesis rises and resources become depleted. Under these circumstances, TYR may provide the resources necessary to allow DA synthesis to carry on and DA to remain at a level that allows optimal performance. The findings from this study demonstrate that, when participants are given enough time to prepare to switch between the two tasks (i.e. proactive control), TYR improved performance and made participants significantly faster at switching, but not when the switching had to be done very rapidly (i.e. reactive control). Even though we need to be careful in interpreting a null effect, the absence of a reliable impact of TYR on the preparatory task-switching component might thus be taken to suggest that TYR has little effect on processes underlying the retrieval, implementation, and maintenance of task sets. As these functions are commonly attributed to the frontal dopaminergic pathway, we speculate that this pathway does not belong to the main targets of TYR-induced increases of DA. In contrast, the residual component of task-switching costs is likely to reflect the online resolution of conflict induced by inertia or stimulus-triggered reactivation of the old task set. The significant effect of TYR on the residual component can thus be taken to reflect TYR-induced support of processes underlying such conflict-resolving processes. Future studies are however needed to varify these interpretations.

In **Chapter seven,** the amino acid tryptophan (TRP), one of the most investigated amino acids and the chemical precursor of serotonin (5-HT), is introduced. TRP supplementation can increase 5-HT levels in the brain and for this reason, numerous studies have investigated whether administration of TRP can positively influence social behavior that relies on serotonergic function. It is thought that increasing levels of 5-HT leads to improvements in social functioning. In this chapter, it is demonstrated that the oral intake of TRP, supposedly increasing levels of 5-HT, increased the amount of money that subjects donated to charity. Importantly, charitable donating is defined as a prosocial behavior (i.e. behavior intended to help

others such as such as helping, sharing, donating, and volunteering). Although preliminary, these findings may indicate that the intake of TRP promotes prosocial behavior, which could have important implications for society. That is, promoting prosocial behavior by stimulating the intake of TRP may benefit society as a whole.

Based on the previous chapter, **Chapter eight** provides a review of TRP as a modulator of social behavior. In this chapter, the available studies on TRP supplementation are reviewed to clarify if, and under what circumstances, TRP supplementation might modulate social behavior. A rising theory in this field is that TRP re-biases attention away from negative stimuli and towards more positive ones, which fits also with the findings that TRP and 5-HT play important roles in affective processing. Consistent with that, studies demonstrate that TRP supplementation seems to improve control over social behavior in patients and individuals suffering from disorders or behaviors associated with dysfunctions in serotonergic functioning. In contrast, in healthy humans TRP supplementation seems to promote social behavior. Although more research is needed to disentangle and understand the relations between individual differences (e.g. metabolic rate and pathways, genetic predisposition, enzymatic activity, gender, age, etc.), 5-HT functioning, and social interactions, TRP seems a promising tool for modulating social behavior. Even though the food supplements (i.e., GABA, TYR, TRP) put forward in these chapters were administered in pure form, these amino acids are also naturally present in our food. Although more research is needed to understand how these amino acids affect cognition and behavior when administered through food (which always contains other ingredients as well), together, these chapters seem to support the idea that the food we eat may have important implications for our cognition and behavior.

The idea that the food we eat affects the way we think and perceive the world, which can then be used to enhance cognitive and social functioning, is further supported by the existence of the ‘gut-brain axis’, which involves bidirectional communication via neural, endocrine and immune pathways between the brain and the intestines. In recent years it has become increasingly evident that this communication also involves interactions with the intestinal microbiota, which release immune-

activating and other signaling molecules that may play an important role in regulating the brain and behavior. These novel insights have fueled the hypothesis that modification of microbial ecology, for example by supplements containing microbial species (probiotics), may be used therapeutically to modify stress responses and symptoms of anxiety and depression. The increasing incidence of depression is alarming and development of preventive measures has been identified as a priority (World Health Organization, 2012). According to cognitive theories of depression, cognitive reactivity plays a central role in the development, maintenance, and recurrence of depression and therefore is a relevant target for interventions. **Chapter nine** therefore focuses on the question whether probiotics can reduce cognitive reactivity to sad mood (i.e. vulnerability to develop a depression) in healthy participants not currently diagnosed with a mood disorder. Results of this chapter demonstrate that, compared to participants who received a placebo intervention, participants who received a 4-week multispecies probiotics intervention showed a significantly reduced overall cognitive reactivity to sad mood, which was largely accounted for by reduced rumination and aggressive thoughts. These results provide the first evidence that the intake of probiotics may help reduce negative thoughts associated with sad mood. As cognitive reactivity seems to be critical in determining whether sad mood will be a transient state or will become protracted, thus increasing the risk of developing clinical depression, probiotics supplementation warrants further research as a potential preventive strategy for depression.

To conclude this overview, the chapters presented in this dissertation provide further evidence for the idea that brain stimulation, video gaming, and food supplements provide promising tools in enhancing cognitive performance and social behavior in healthy humans. In addition, important insights in the (possibly) underlying mechanisms of the effects of enhancement techniques, which are needed if we ever want to be able to apply these methods in other fields, are provided.

Openness in science

This dissertation supports the mission of the Center for Open Science (COS) to increase openness, integrity, and reproducibility of scientific research. The (raw) data of all studies reported in this dissertation are therefore stored in the Open Science Framework (OSF). This data can be accessed with the following web links:

Chapter 1 : Transcutaneous vagus nerve stimulation (tVNS) enhances response selection during action cascading processes.
https://osf.io/xed5m/?view_only=84789d588e48409cb4d61e638ba6489f

Chapter 2 : Transcutaneous vagus nerve stimulation (tVNS) does not increase prosocial behavior in Cyberball.
https://osf.io/wb2zt/?view_only=032e4ab33218414085c5e90b443e5877

Chapter 3 : "Unfocus" on foc.us: Commercial tDCS headset impairs working memory.
https://osf.io/43kix/?view_only=423f8fe402af43aa86e2155d47d50a8e

Chapter 4 : Action video gaming and cognitive control: playing first person shooter games is associated with improved action cascading but not inhibition.
https://osf.io/sbvni/?view_only=8bec928941724b30acbfa1d9302be434

Chapter 5 : γ-Aminobutyric acid (GABA) administration improves action selection processes: a randomized controlled trial.
https://osf.io/8g3hr/?view_only=7b4ceb65be744cc286c5d07c6d9d48d3

Chapter 6 : Tyrosine promotes cognitive flexibility: Evidence from proactive vs. reactive control during task switching performance.
https://osf.io/aqrzb/?view_only=906a14b7676145278728a6a2cbfb24ef

Chapter 7 : Tryptophan promotes charitable donating.
https://osf.io/6szjq/?view_only=b31635d6bed544a29d545a21f5832479

Chapter 8 : Tryptophan supplementation modulates social behavior: a review.
Not applicable

Chapter 9 : A randomized controlled trial to test the effect of multispecies probiotics on cognitive reactivity to sad mood.
https://osf.io/enxmq/?view_only=ff13ba53293246bc82d09568515ca193

Chapter one

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

Steenbergen, L., Sellaro, R., Stock, A.K., Verkuil, B., Beste, C. & Colzato, L.S. (2015). Transcutaneous vagus nerve stimulation (tVNS) enhances response selection during action cascading processes. *European Neuropsychopharmacology, 25*(6), 773-778*.* doi: 10.1016/j.euroneuro.2015.03.015

Abstract

The ever-changing environment we are living in requires us to apply different action control strategies in order to fulfill a task goal. Indeed, when confronted with multiple response options it is fundamental to prioritize and cascade different actions. So far, very little is known about the neuromodulation of action cascading. In this study we assessed the causal role of the gamma-aminobutyric acid (GABA)-ergic and noradrenergic system in modulating the efficiency of action cascading by applying transcutaneous vagus nerve stimulation (tVNS), a new non-invasive and safe method to stimulate the vagus nerve and to increase GABA and norepinephrine concentrations in the brain. A single-blind, sham-controlled, between-group design was used to assess the effect of on-line (i.e., stimulation overlapping with the critical task) tVNS in healthy young volunteers (n=30)–on a stop-change paradigm. Results showed that active, as compared to sham stimulation, enhanced response selection functions during action cascading and led to faster responses when two actions were executed in succession. These findings provide evidence for the important role of the GABA-ergic and noradrenergic system in modulating performance in action cascading.

1. Introduction

The ever-changing environment we are living in requires us to apply different action control strategies in order to fulfill a task goal. Indeed, when confronted with multiple response options it is fundamental to prioritize and cascade different actions (Mückschel, Stock, & Beste, 2014). So far, very little is known about the neuromodulation of action cascading, although there is evidence showing that dopaminergic and the gamma-aminobutyric acid (GABA)-ergic system are important (Stock, Arning, Epplen, & Beste, 2014; Stock, Blaszkewics, & Beste, 2014; Beste & Saft, 2013). Concerning the GABA-ergic system, recent findings using magnetic resonance spectroscopy (MRS) showed that superior performance in action cascading was associated with increased concentrations of striatal GABA (Yildiz et al., 2014). Given the correlational nature of MRS studies, it is, however, hard to infer the exact role of GABA in mediating action cascading. There is also evidence that stress modulates action cascading processes (Yildiz, Wolf, & Beste, 2014). Stress is known to affect the noradrenergic system (Glavin, 1985). So there is tentative evidence for the idea that norepinephrine (NE), playing an important role in stress responses, may affect functions during action cascading and lead to slowing of responses when two actions are executed in succession.

In this study we assessed the causal role of the GABA-ergic and noradrenergic system in modulating the efficiency of action cascading by applying transcutaneous vagus nerve stimulation (tVNS), a new non-invasive method to stimulate the vagus nerve, introduced for the first time by Ventureyra (2000; for a recent review see Vonck et al., 2014). tVNS stimulates the afferent auricular branch of the vagus nerve located medial of the tragus at the entry of the acoustic meatus (Kreuzer et al., 2012). tVNS is safe and is accompanied only by minor side effects such as a burning or itching sensation under the electrodes. Very recently, it has been suggested that tVNS may be a useful tool to further investigate the neuromodulation of cognitive processes related to NE and GABA, two of the main neurotransmitters targeted by VNS (van Leusden, Sellaro, & Colzato, 2015). In rats, it has been demonstrated that VNS leads to an intensity-dependent

increase in brain NE in response to stimulation of the left vagus nerve (Raedt et al., 2011; Roosevelt, Smith, Clough, Jensen, & Browning, 2006). These increases in NE are transient and return to baseline levels when the stimulation is stopped and the vagus nerve is no longer being stimulated (Roosevelt, Smith, Clough, Jensen, & Browning, 2006). Besides NE, the other main neurotransmitter targeted by VNS is GABA. So far, tVNS has mainly been used to treat patients with epilepsy (Vonck et al., 2014), who suffer from an abnormal reduction of GABA-ergic function (Treiman, 2001). Indeed, VNS seems to increase the levels of free GABA in the cerebrospinal fluid (Ben-Menachem et al., 1995). Moreover, in epileptic patients receiving VNS for a year, GABA-A receptor density was significantly increased as compared to controls (Marrosu, Serra, Maleci, Puligheddu, Biggio, & Piga, 2003).

Given the available, correlational evidence that action cascading is modulated by the GABA-ergic system, we tested whether tVNS, via GABA and NE release, ameliorates the efficiency of action cascading. This hypothesis is supported by the fact that, from an anatomical point of view, action cascading efficiency is related to a neural network that includes the anterior cingulate cortex (ACC; Mückschel, Stock, & Beste, 2014). Importantly, the vagal nerve is connected to the ACC (Mayer, 2011), and the ACC is a crucial area for the execution of multi-component behavior (Duncan, 2010; 2013). We assessed action cascading by means of a well-established stop-change paradigm (Verbruggen et al., 2008), in which we varied the interval between “stopping” and “changing” (stop-change delay; SCD) and hence varied the time available for preparation before executing the change response (Mückschel, Stock, & Beste, 2014). Given the idea that GABA and NE impact action selection (Yildiz et al., 2014; Yildiz, Wolf, & Beste, 2014), we expected the active tVNS to ameliorate the action cascading processes (i.e. decrease reaction times on the change stimuli) when (i) an interruption, i.e. stopping a response, and a change toward an alternative response are required simultaneously (SCD0), and when (ii) the change to another response is required once the stopping process has already finished (SCD300).

2. Method

2.1. Participants

Thirty undergraduate students of the Leiden University (26 females, 4 males, mean age = 19.8 years, range 18-27) participated in the experiment. Participants were recruited via an on-line recruiting system and were offered course credit for participating in a study on the effects of brain stimulation on cognition. Once recruited, participants were randomly assigned to one of the two following experimental groups: sham stimulation (N=15; 2 male; mean age=20.2, SD=3.0), and active stimulation (N=15; 2 male; mean age=19.3, SD=1.4). Groups did not differ in terms of age, $t(28)=1.0$, $p=.32$, or gender, $\chi^2<.01$, $p>.9$. All participants were naïve to tVNS. Participants were screened individually via a phone interview by the same lab-assistant using the Mini International Neuropsychiatric Interview (M.I.N.I.). The M.I.N.I. is a short, structured, interview of about 15 minutes that screens for several psychiatric disorders and drug use, often used in clinical and pharmacological research (Sheehan et al., 1998; Colzato, Kool, & Hommel, 2008; Colzato, Hertsig, van den Wildenberg, & Hommel, 2010). Participants were considered suitable to participate in this study if they fulfilled the following criteria: (i) age between 18 and 30 years; (ii) no history of neurological or psychiatric disorders; (iii) no history of substance abuse or dependence; (iv) no history of brain surgery, tumor or intracranial metal implantation; (v) no chronic or acute medications; (vi) no pregnancy; (vii) no susceptibility to seizures or migraine; (viii) no pacemaker or other implanted devices.

All participants were naïve to tVNS. Prior to the testing session, they received a verbal and written explanation of the procedure and of the typical adverse effects (i.e., itching and tingling skin sensation, skin reddening, and headache). No information was provided about the different types of stimulation (active vs. sham) or about the hypotheses concerning the outcome of the experiment. 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).

2.2. Apparatus and procedure

Single-blinded, sham-controlled, randomized two-arms trials were used to assess the effect of on-line (i.e., stimulation overlapping with the critical task) tVNS in healthy young volunteers in a stop-change paradigm. All participants were tested individually. After having read and signed the informed consent, heart rate (HR) was collected from the non-dominant arm with an OSZ 3 Automatic Digital Electronic Wrist Blood Pressure Monitor (Spiedel & Keller). Immediately after, participants performed the stop-change paradigm, which included a practice phase (about 20 minutes) and a testing phase (about 25 minutes). Thus, tVNS was applied throughout the whole task. Once finished, participants had their HR measured for the second time.

After completion of the session, participants were debriefed and asked to complete a tVNS adverse effects questionnaire requiring them to rate, on a five-point (1–5) scale, how much they experienced: 1) headache, 2) neck pain, 3) nausea, 4) muscles contraction in face and/or neck, 5) stinging sensation under the electrodes, 6) burning sensation under the electrodes, 7) uncomfortable (generic) feelings, 8) other sensations and/or adverse effects. None of the participants reported major complains or discomfort during or after tVNS.

2.2.1. tVNS

We used a tVNS instrument consisting of two titan electrodes mounted on a gel frame and connected to a wired neurostimulating device (CM02, Cerbomed, Erlangen, Germany). Following the suggestions by Dietrich et al. (2008) for optimal stimulation, the tVNS® device was programmed to a stimulus intensity at 0.5mA, delivered with a pulse width of 200-300µs at 25 Hz. Stimulation was active for 30 seconds, followed by a break of 30 seconds. Following Kraus et al. (2007), in the sham condition, the stimulation electrodes were attached to the center of the left ear love instead of the outer auditory canal. Indeed, the ear lobe has been found to be free of cutaneous vagal innervation (Peuker & Filler, 2002; Fallgatter et

al., 2003) and a recent fMRI study showed that this sham condition produced no activation in the cortex and brain stem (Kraus et al., 2013).

None of the participants were able to determine whether or not they received real or sham stimulation. Since efferent fibers of the vagus nerve modulate cardiac function, cardiac safety has always been a concern in the therapeutic use of vagus nerve stimulation (Cristancho, Cristancho, Baltuch, Thase, & O'Reardon, 2011). Efferent vagal fibers to the heart are supposed to be located on the right side (Nemeroff et al., 2006). In order to avoid cardiac side effects, electrodes were always placed on the left ear (Nemeroff et al., 2006). While placing electrodes on the left side, a clinical trial showed no arrhythmic effects of tVNS (Kreuzer et al., 2012).

2.2.2. Stop-Change paradigm

The experiment was controlled by an Asus laptop running on an Intel Core i3-3217U processor, attached to a LG Flatron 776FM 16 inch monitor (refresh rate of 60 Hz). Stimulus presentation and data collection were controlled using the Presentation software system (Neurobehavioral Systems, Inc., Berkeley, CA). The stop-change (SC) paradigm was adapted from Yildiz, Wolf, and Beste (2014), and Dippel and Beste (2015), see Figure 1. Responses were given using the index and middle fingers of the right hand during the GO trials and those of the left hand for the SC trials.

Throughout each trial, a white rectangle of 55 x 16 mm was displayed on a black background in the centre of the screen. Within this rectangle, four vertically aligned circles (diameter 7mm) were separated by three horizontal reference lines (line thickness 1 mm, width 13 mm). 250 ms after the onset of each trial, one of the circles was filled white, as such becoming the GO target stimulus. In the GO condition (67% of all trials), participants were expected to indicate whether the white circle was located above or below the middle reference line. Responses were given by pressing the outer right key with the right middle finger (i.e. above) or by pressing the innermost right key with the right index finger (i.e. below). All stimuli remained visible until either the participant responded or 2500 ms had elapsed. In case of RTs longer than 1000 ms, the word "Quicker" was presented above the box until the participant responded.

The SC condition (consisting of the remaining 33% of the trials) began with the presentation of a white GO stimulus, as described above. However, after a variable stop signal delay (SSD), which was adjusted using a staircase procedure, a STOP signal was presented. The STOP signal consisted of the white frame of the rectangle turning red. This STOP signal requested the participant to try to inhibit the right-handed response to the GO stimulus and stayed on the screen until the end of the SC trial. The SSD was initially set to 250 ms and was adapted to each participant's performance by means of a staircase procedure. This procedure ensures a 50% success rate of inhibiting the GO response, which gives an accurate estimate of the stop-signal reaction time (SSRT), a quantitative estimate of the duration of the covert response-inhibition process (Logan & Cowan, 1984). In the case of a completely correct SC trial (no response to GO stimulus, no response prior to the CHANGE stimulus in the SCD300 condition (explained below) and a correct left-hand response to the CHANGE stimulus), the SSD of the following SC trial was adjusted by adding 50 ms to the SSD of the evaluated trial. In the case of an incorrect SC trial, the SSD for the next SC trial was adjusted by subtracting 50 ms from the SSD of the current trial. To limit the SSD, values were set to not become lower than 50 ms or to exceed 1000 ms. Irrespective of whether participants successfully inhibited the GO trial or not, every stop signal was combined with a CHANGE stimulus. Notably, in 50% of the SC trials, the STOP and CHANGE stimuli were presented simultaneously (SCD0 condition), and in the remaining 50% of the trials there was a stop change delay (SCD) with a stimulus onset asynchrony (SOA) of 300 ms between the STOP and the CHANGE signals (SCD300 condition). The CHANGE stimulus could be a high (1300 Hz), medium (900 Hz) or low (500 Hz) sine tone presented for 100 ms via headphones at 75 dB SPL. This tone indicated that the CHANGE target (i.e. the white circle previously indicating the GO trial) had to be evaluated with regard to a new reference line. That is, if the tone was high in pitch (i.e. high tone), the highest of the three lines had to be used as the new reference, the medium tone indicated re-referencing to the middle line and the low tone indicated the lowest of the three lines had to be used as the new reference line (see Figure 1). All three reference lines were used with equal frequency. The required CHANGE response to this had to be

executed using the index and middle fingers of the left hand. Which key to press had to be decided upon evaluating the white circle with regard to the new reference line (i.e. as indicated by the tone). If the target was located above the reference line corresponding to the tone, an outer left key press (left middle finger) was required; if the target circle was located below the reference line, a left innermost key press (left index finger) was required. For these responses, the reaction time (RT2) was measured. In the case of RT2s longer than 2000 ms, the English word “Quicker” was presented above the rectangle until the participant responded. During the inter-trial interval (ITI; 900 ms), a fixation cross was presented in the center of the screen. Participants first received explanation and practiced the task, whereafter they were presented with 864 test trials. In total, it took the participants approximately 45 minutes to finish.

2.3. Statistical analyses

HR was analyzed by means of repeated-measures analyses of variance (ANOVAs) with group (active vs. sham) as between-subjects factor and effect of time (first vs. second measurement) as within-subjects factor. The effect of tVNS on action cascading was assessed by means of repeated-measures ANOVAs with condition (Go, SCD0, SCD300) as within-subject factor and group (active vs. sham) as between-subject factor. The effect of tVNS on SSRT was assessed by independent samples t-tests. LSD-Fisher post-hoc tests were performed to clarify mean differences in case of significant interactions. Trials with errors were excluded from the reaction times (RTs) analysis. A significance level of $p<0.05$ was adopted for all statistical tests.

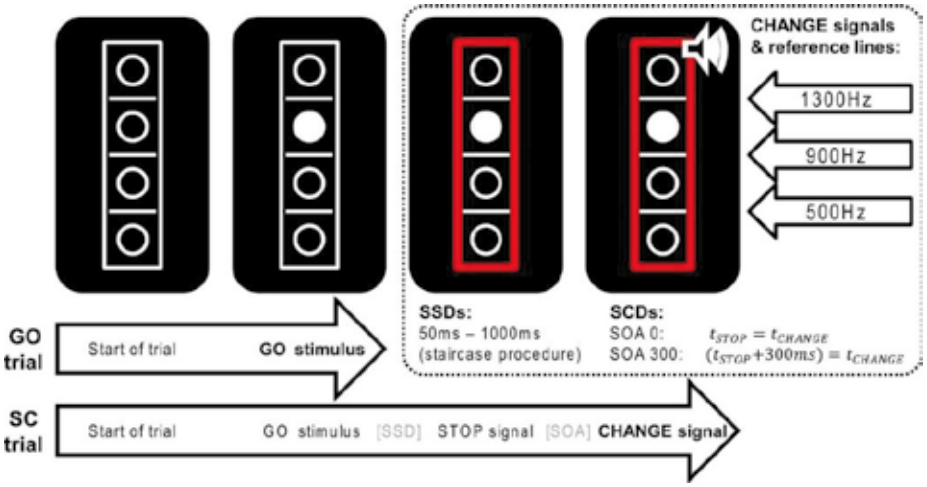

Figure 1. Schematic illustration of the stop-change paradigm. GO1 trials end after the first response to the GO1 stimulus (bold). In contrast, SC trials end after the first response to the CHANGE signal (bold). The stop-signal delay (SSD) between the onset of the GO1 stimulus and the STOP signal was adjusted using a staircase procedure described in Section 2. The stimulus onset asynchrony (SOA) between the onset of the STOP and CHANGE stimuli was set to either 0 or 300 ms. As indicated in the upper right corner, the three CHANGE stimuli were associated with one of the three reference lines.

3. Results

3.1. Stop-Change paradigm

Table 1 shows the behavioral parameters for the Stop-Change paradigm separately for the active and sham group.

Table 1. Behavioral parameters (reaction times; RTs in miliseconds and error rates in percentages) separated for the active (tVNS) and sham group (mean±SEM)

	Active tVNS		**Sham**	
	RTs	**Error rates**	**RTs**	**Error rates**
GO	542±30	4.8±0.7	539±30	4.7±0.7
SCD 0	977±52	40.3±1.8	1139±52	42.9±1.8
SCD 300	802±60	17.3±2.4	1000±60	17.9±2.4
SSRT	255±13		270±13	

There was a main effect of group, F (1,28) = 4.97, p = .034, η^2_p = .151, indicating that RTs where faster in the active group (774ms) as compared to the sham group (893ms). There was also a main effect of condition, F (2,56) = 98.22, p < .001, η^2_p = .778. LSD-Fisher post-hoc tests showed that RTs were longer in the SCD0 condition (1058 ±37), as compared to the SCD300 (901±42) and the Go condition (541±21) (both p < .001). The latter conditions (i.e., SCD300 and Go) differed significantly from each other too, p < .001. Most importantly, the two-way interaction involving condition and group was significant, F(2,56) = 4.00; p = .024; η^2_p = .125. LSD-Fisher post-hoc tests revealed a difference in RTs between groups in the SCD0 condition, p = .02, and in the SCD300 condition, p = .006, but not in the GO condition, p = .96. Specifically, for the SCD0 and the SCD300 conditions, the

sham group had longer RTs (SCD0 1139ms ±52; SCD300 1000ms ±60) than the active group (SCD0 977ms ±52; SCD300 802ms ±60). The error rate analysis revealed a main effect of condition, F (2,56) = 448.558 p < .001, η^2_p = .94: the SCD0 condition (41.6%±1.3) produced more errors as compared to the SCD300 (17.6%±1.7) and the Go conditions (4.8%±0.5) (both p < .001), which differed significantly from each other too (p < .001). The main effect of group and the two-way interaction between group and condition were not significant, F_s < 1, p_s ≥ .55 (see Table 1). Analyzing SSRTs, as calculated after Logan and Cowan (1984), did not reveal differences between the active and sham groups (t_{28}=.75, p > .45).

3.2. HR measurements

ANOVA showed a main effect of time, F(1,27)=11.27, p<.002, η^2_p = .295, indicating that heart rate decreased during the experiment (85 vs. 75 BPM). However, HR did not significantly differ between groups (85 vs. 75 and 85 vs. 75 in the active and sham group, respectively), F(1,27)<.001, p=.98. This suggests that we can rule out an account of our results in terms of physiological changes.

4. Discussion

Our findings show that tVNS, likely via GABA and NE release and because of connections between the vagus nerve and the ACC, modulates the efficiency of action cascading as measured by a stop-change paradigm. The observation that tVNS boosts performance on a well-established diagnostic index of action cascading (Verbruggen, Schneider, & Logan, 2008) provides considerable support for the idea of a crucial role of GABA-ergic and noradrenergic pathways in action cascading (Yildiz et al., 2014; Yildiz, Wolf, & Beste, 2014). tVNS modulates action cascading processes when (i) an interruption, i.e. stopping a response, and a change toward an alternative response are required simultaneously (SCD0 condition) and when (ii) the change to another response is required once the stopping process has already finished (SCD300 condition). As revealed by the lack of tVNS effects on the stop-signal reaction time (SSRT), tVNS did not modulate the efficiency to stop an ongoing response. This is not surprising given that SSRT seems to be affected, instead, by dopaminergic manipulations (Colzato, van den Wildenberg, & Hommel, 2013; Colzato, Jongkees, Sellaro, van den Wildenberg, & Hommel, 2014; but see Stock, Gohil, & Beste, 2014; Stock, Blaszkewicz, & Beste, 2014).

Our results are partially inconsistent with a previous study by Yildiz et al. (2014) in which airplane pilot trainees (associated with increased GABA concentrations) were better than controls only in the SCD0 condition, when participants were confronted with stop and change stimuli at the same time. Given that tVNS, besides GABA, also targets NE it may be possible that the noradrenergic release contributed to ameliorating action cascading in the SCD300 condition, when participants have enough time to prepare for the change response. Indeed, a previous study showed that stress, a factor known to affect the noradrenergic system (Galvin, 1990), impacted the SCD300 but not the SCD0 condition (Yildiz, Wolf, & Beste, 2014). As the data pattern is hence more consistent to what was found for stress responses, the results suggest that in the SCD300 condition the impact of tVNS is stronger on the NE-system than on the GABA-ergic system.

Future studies require a more systematic examination of this issue. Further investigations testing acute neuromodulatory effects of highly selective GABA and NE agonists on the efficiency of action cascading are necessary to determine the precise role of the GABA-ergic and noradrenergic system in modulating response selection processes. Of particular interest would be also to look into the genetic variability associated with GABA (Mulligan et al., 2012) and NE (Stöber et al., 1996), which may predict individual differences in the efficiency of action cascading.

Even though VNS, besides GABA and NE, is also associated with acetylcholine (ACh) release (Borovikova, et al., 2000), previous literature suggest that it is less plausible that ACh is responsible for our results. Indeed, animal literature proposes that ACh is responsible for, more than action selection processes, the proper development of action *coordination* in rats (e.g., Watanabe, Shimizu, & Matsumoto, 1990) and that it plays an essential role in neural communication in brain networks implicated in movements and actions (Bartus, Dean, Pontecorvo, & Flicker, 1985). That is, if ACh would have significantly contributed to our results, we would have found an improvement in action accuracy; however, in the current study, we failed to found such evidence in the Go trials.

The present study has some limitations that deserve discussion. First, we did not explicitly assess participants' blinding by asking them if they could guess the stimulation received. Second, it would have been ideal to have the application of tVNS accompanied by appropriate physiological assays, such as the vagus-evoked potentials (See Bestmann, de Berker, & Bonaiuto, 2015 for a related discussion).

In sum, the available observations provide converging evidence for the idea that GABA and NE-related processes only affect the change to an alternative response, once an ongoing response has stopped. Taken altogether, our results support the idea that tVNS is a promising non-invasive brain stimulation technique to enhance cognitive processes.

Chapter Two

Transcutaneous vagus nerve stimulation (tVNS) does not increase prosocial behavior in Cyberball

Sellaro, R., Steenbergen, L., Verkuil, B., van IJzendoorn, M.H., Colzato, L.S. (2015). Transcutaneous vagus nerve stimulation (tVNS) does not increase prosocial behavior in Cyberball. *Frontiers in Psychology, 6,* 499. doi: 10.3389/fpsyg.2015.00499

Abstract

Emerging research suggests that individuals experience vicarious social pain (i.e., ostracism). It has been proposed that observing ostracism increases activity in the insula and in the prefrontal cortex (PFC), two key brain regions activated by directly experiencing ostracism. Here, we assessed the causal role of the insula and PFC in modulating neural activity in these areas by applying transcutaneous Vagus Nerve Stimulation (tVNS), a new non-invasive and safe method to stimulate the vagus nerve that has been shown to activate the insula and PFC. A single-blind, sham-controlled, within-subjects design was used to assess the effect of on-line (i.e., stimulation overlapping with the critical task) tVNS in healthy young volunteers (n = 24) on the prosocial Cyberball game, a virtual ball-tossing game designed to measure prosocial compensation of ostracism. Active tVNS did not increase prosocial helping behavior toward an ostracized person, as compared to sham (placebo) stimulation. Corroborated by Bayesian inference, we conclude that tVNS does not modulate reactions to vicarious ostracism, as indexed by performance in a Cyberball game.

1. Introduction

People vicariously experience others' (social) pain. Several recent studies have demonstrated vicarious ostracism (i.e., the observation of other people being socially ignored and excluded). These studies show that spectators identify with an ostracized individual's pain and also feel ostracized themselves (Over & Carpenter, 2009; Wesselmann, Bagg, & Williams, 2009; Masten, Eisenberger, Pfeifer, & Drapetto, 2010; Masten, Morelli, & Eisenberger, 2011; Masten, Eisenberger, Pfeifer, Colich, & Drapetto, 2013; Masten, Eisenberger, Pfeifer, & Drapetto, 2013; Beeney, Franklin, Leby, & Adams, 2011; Meyer et al., 2012; Will, Crone, van den Bos, & Güroğlu, 2013). As pointed out by Wesselmann, Williams, and Hales (2013), not only adults (Wesselmann, Bagg, & Williams, 2009; Beeney, Franklin, Levy, & Adams, 2011; Masten, Morelli, & Eisenberger, 2011; Meyer et al., 2012; Will, Crone, van den Bos, & Güroğlu, 2013) but also children and adolescents (Over & Carpenter, 2009; Masten, Eisenberger, Pfeifer, & Drapetto, 2010; Masten, Eisenberger, Pfeifer, & Drapetto, 2013; Masten, Eisenberger, Pfeifer, Colich, & Drapetto, 2013; Will, Crone, van den Bos, & Güroğlu, 2013) exhibit vicarious ostracism.

In the literature, a reliable index of vicarious ostracism is an adapted version of the Cyberball game (Williams, 2009), a virtual ball-tossing game in which participants observe someone else being ostracized. Perceiving someone being ostracized during the Cyberball game presents the participant with a moral conflict: helping the ostracized person by throwing the ball to the victim more often, or following the other computer-controlled confederates by excluding the victim (Williams & Jarvis, 2006). Using this version of the Cyberball game, previous research has shown that people typically tend to compensate for other individuals' ostracism by throwing the ball toward the ostracized person more often (Riem, Bakermans-Kranenburg, Huffmeijer, & van IJzendoorn, 2013; Wesselmann, Wirth, Pryor, Reeder, & Williams, 2013), unless they are induced to think that the ostracized individual deserved this treatment (Wesselmann, Wirth, Pryor, Reeder, & Williams, 2013). Interestingly, observing ostracism increases activity in the insula and anterior cingulate

cortex, the key social pain-related regions that are activated also by directly experiencing ostracism (Eisenberger & Lieberman, 2004). Moreover, observing ostracism activates the prefrontal cortex (PFC) and precuneus—brain regions associated with mentalization (i.e., ability to understand the mental state of oneself and others; Masten, Eisenberger, Pfeifer, & Drapetto, 2010; Masten, Morelli, & Eisenberger, 2011; Masten, Telzer, & Eisenberger, 2011; Masten, Eisenberger, Pfeifer, Colich, & Drapetto, 2013). Brain activation of both the mentalization areas and social pain-related regions correlates with individual differences in empathy when observing ostracism and with prosocial behavior toward the victim, which has been taken to suggest that differences in experiencing vicarious ostracism may also reflect individual differences in trait empathy (Masten, Eisenberger, Pfeifer, & Drapetto, 2010; Masten, Morelli, & Eisenberger, 2011; Masten, Telzer, & Eisenberger, 2011; Masten, Eisenberger, Pfeifer, & Drapetto, 2013).

Here, we assessed the causal role of this PFC-insula network in mediating vicarious ostracism by applying transcutaneous Vagus Nerve Stimulation (tVNS), a new non-invasive and safe method to stimulate the vagus nerve, introduced for the first time by Ventureyra (2000; for a recent review see Vonck et al., 2014). tVNS stimulates the afferent auricular branch of the vagus nerve located medial of the tragus at the entry of the acoustic meatus (Kreuzer et al., 2012). tVNS is safe and is accompanied only with minor side effects such as an itching sensation under the electrodes. Very recently, it has been suggested that tVNS may be a valuable tool for modulating cognitive processes in healthy humans (van Leusden, Sellaro, & Colzato, 2015). Two functional magnetic resonance imaging (MRI) studies in healthy humans have shown increased activation during active tVNS in the locus coeruleus and the solitary tract, as an indication of effective stimulation of the vagal afferences and both the insula and PFC (Dietrich et al., 2008; Kraus et al., 2013), which are key areas related to social pain and mentalization, and linked to vicarious ostracism.

Given the available correlational evidence that vicarious ostracism involves the PFC-insula network, we tested whether tVNS enhances prosocial helping behavior toward an ostracized person who was unknown to the participant. This hypothesis is supported by the findings that tVNS

produces a reliable activation in both the insula and the PFC (Dietrich et al., 2008; Kraus et al., 2013). Accordingly, we assessed the effect of on-line (i.e., stimulation overlapping with the critical task) tVNS on an adapted version of the Cyberball game to measure compensation for other players' ostracism. A positive effect of tVNS during Cyberball would be indicated by an increased number of tosses toward the ostracized person.

2. Method

2.1. Participants

Twenty-four Leiden University undergraduate students (21 females, three males, mean age = 19.13 years, range 18–22) participated in the experiment. Participants were recruited via an on-line recruiting system and were offered course credit for participating in a study on the effects of brain stimulation on social decision-making. Participants were screened individually via a phone interview by the same lab-assistant using the Mini International Neuropsychiatric Interview (M.I.N.I.). The M.I.N.I. is a short, structured interview of about 15 min that screens for several psychiatric disorders and drug use, often used in clinical and pharmacological research (Sheehan et al., 1998; Colzato, Kool, & Hommel, 2008). Participants were considered suitable to participate in this study if they fulfilled the following criteria: (i) age between 18 and 30 years; (ii) no history of neurological or psychiatric disorders; (iii) no history of substance abuse or dependence; (iv) no history of brain surgery, tumors, or intracranial metal implantation; (v) no chronic or acute medications; (vi) no pregnancy; (vii) no susceptibility to seizures or migraine; (viii) no pacemaker or other implanted devices.

All participants were naïve to tVNS. Prior to the testing session, they received a verbal and written explanation of the procedure and of the typical adverse effects (i.e., itching and tingling skin sensation, skin reddening, and headache). No information was provided about the different types of stimulation (active vs. sham) or about the hypotheses concerning the experiment. The study conformed to the ethical standards

of the Declaration of Helsinki and the protocol was approved by the medical ethics committee (Leiden University Medical Center).

2.2. Apparatus and Procedure

A single-blinded, sham/placebo-controlled, randomized cross-over within-subjects study with counterbalanced order of conditions was used to assess the effect of on-line (i.e., stimulation overlapping with the critical task) tVNS on a prosocial Cyberball game in healthy young volunteers.

All participants took part in two sessions (active vs. sham) and were tested individually. In both sessions, upon arrival, participants were asked to rate their mood on a 9 × 9 Pleasure × Arousal grid (Russell, Weiss, & Mendelsohn, 1989) with values ranging from -4 to 4. Heart rate (HR) and systolic and diastolic blood pressure (SBP and DBP) were collected from the non-dominant arm with an OSZ 3 Automatic Digital Electronic Wrist Blood Pressure Monitor (Speidel & Keller) for the first time (T1). Immediately after, participants performed either the Empathy Quotient (EQ; in session 1) or the interpersonal reactivity index (IRI; in session 2). The EQ is a self-report questionnaire designed to assess empathy in normal adult populations (Cronbach's alpha is 0.92; Baron-Cohen & Wheelwright, 2004). It comprises 60 questions (20 items are filler questions) that, taken together, provide an overall measure of cognitive perspective taking, affective empathy, and social skills (range 0–80, higher scores = more empathy). The IRI is a self-report questionnaire that assesses perceived individual differences in the tendency to be empathetic. It consists of 28 Likert-type items on a response scale with five alternatives ranging from 0 (Does not describe me well) to 4 (Describes me very well). It comprises four subscales assessing affective (empathic concern and personal distress) and cognitive (fantasy and perspective taking) components of empathy (Davis, 1980, 1983). Cronbach' s alphas are 0.73, 0.77, 0.83, and 0.73 for the emphatic concern, personal distress, fantasy, and perspective taking subscales, respectively (De Corte et al., 2007). Afterwards, participants rated again their mood and HR, SBP, and DBP were collected for the second time (T2). Then, they performed for 8 min each two unrelated computer tasks tapping into emotional working memory and implicit biased attitudes

(data not reported here) before rating their mood and having HR, SBP, and DBP measured for the third time (T3). After that, participants performed the prosocial Cyberball game, which lasted for 10 min. Once completed the Cyberball, mood, HR, SBP, and DBP were measured for the fourth time (T4). tVNS was applied throughout all three computer tasks.

2.2.1. Transcutaneous Vagus Nerve Stimulation (tVNS)

We used a tVNS wired neurostimulating device connected with two titan electrodes fastened on a gel frame (CM02, Cerbomed, Erlangen, Germany). Following the suggestions by Dietrich et al. (2008) and Steenbergen et al. (2015) for optimal stimulation, the tVNS®device was programmed to a stimulus intensity at 0.5 mA, delivered with a pulse width of 200–300 µs at 25 Hz. Stimulation alternated between On/Off periods every 30 s. In the sham (placebo) condition, the stimulation electrodes were placed on the center of the left ear lobe instead of the outer auditory canal. Indeed, the ear lobe has been found to be free of cutaneous vagal innervation (Peuker & Filler, 2002; Fallgatter et al., 2003) and a recent fMRI study showed that this sham condition produced no activation in the cortex and brain stem (Kraus et al., 2013).

Importantly, following safety criteria to avoid cardiac side effects, the stimulation was always applied to the left ear (Nemeroff et al., 2006; Cristancho et al., 2011). Indeed, although efferent fibers of the vagus nerve modulate cardiac function, such a modulation seems to relate only to the efferent vagal fibers connected to the right ear (Nemeroff et al., 2006). Consistently, a clinical trial showed no arrhythmic effects of tVNS when applied to the left ear (Kreuzer et al., 2012).

2.2.1. Prosocial Cyberball

The Cyberball game was an adapted version of the task used in the study by Riem, Bakermans-Kranenburg, Huffmeijer, and van IJzendoorn (2013). The game was a virtual online group interaction involving four players throwing a ball to each other. Participants were led to believe that they would play this game via Internet with three other unknown peers. In reality, the other

players were virtual computer-controlled confederates. The participants' glove was at the bottom of the screen. The gloves, names, and pictures of the unknown victim and of the other two unknown players were displayed in the upper part of the screen, and to the left and to right of the screen, respectively (see Figure 1). A computer keyboard was used by the participants to throw the ball to the other players.

The game consisted of two parts with a short break in between, each comprising three 48-trial blocks. The first block was programmed to create a fair situation where all players received the ball equally often (i.e., fair play block). The second (i.e., unfair play block 1) and the third (i.e., unfair play block 2) blocks were programmed to establish an unfair situation in which one of the players (i.e., the victim) never received any throw from the two unknown players. The third block included an additional manipulation: the facial expression of the ostracized player changed from neutral to sad (i.e., unfair play block 2 with sad victim), or remained neutral (i.e., unfair play block 2 with neutral victim). Half of the participants were confronted with the ostracized player showing a sad expression in the third block of the first part, and with the ostracized player showing a neutral expression in the third block of the second part. The remaining participants experienced the two conditions in the reversed order. The sad facial expression did not change when the participant threw the ball to the ostracized victim.

The dependent variable was the number of ball tossing to the victim, calculated as the ratio between the number of throws of the participant to the victim and the total number of throws by the participant to any of the players. Ratios were calculated for each play block. A ratio larger than 0.33 in the unfair play block indicates that participants compensate for the other player' ostracism by throwing the ball to the victim more often.

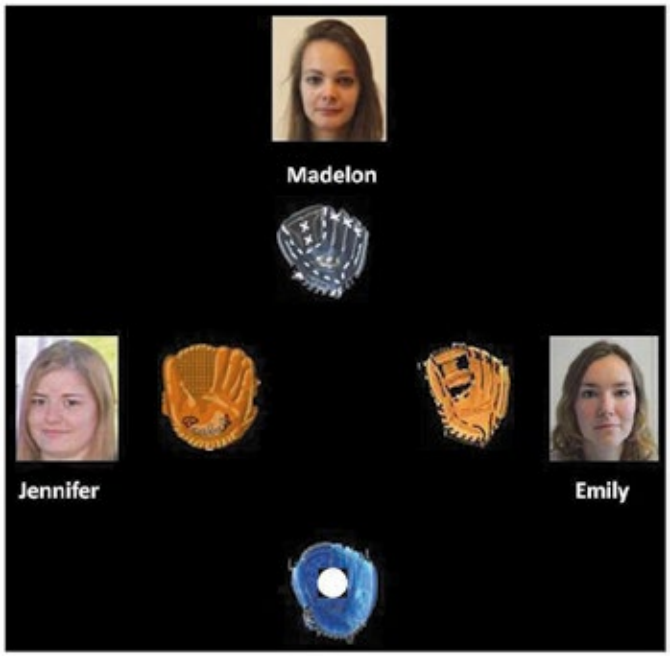

Figure 1. Set-up Cyberball task in the neutral condition. The participants' glove was at the bottom of the screen. The glove, name, and picture of the unknown victim with a neutral or sad expression were at the upper part of the screen. The gloves, names, and pictures of the other unknown players were to the left and right of the screen center. Figure taken from Riem, Bakermans-Kranenburg, Huffmeijer, and van IJzendoorn (2013).

2.3. Statistical Analyses

To examine whether active tVNS, as compared to sham (placebo) stimulation, influenced prosocial behavior, as indexed by the number of tossing to the ostracized player, repeated-measures analysis of variance (ANOVA) was carried out with the ratio of ball throws to the victim as dependent variable and play block (fair play blocks, unfair play block 1, unfair play block 2 with neutral victim, unfair play block 2 with sad victim) and session (active vs. sham) as within-participants factors. Mood (i.e., pleasure and arousal scores), HR, SBP, and DBP were analyzed separately by means of repeated-measures ANOVAs with effect of time (first vs. second

vs. third vs. fourth measurement) and session (active vs. sham) as within-participants factors.

A significance level of $p < 0.05$ was adopted for all statistical tests. Tukey HSD post hoc tests were performed to clarify mean differences. Furthermore, we calculated Bayesian (posterior) probabilities associated with the occurrence of the null [$p(H_0|\ D)$] and alternative [$p(H_1|\ D)$] hypotheses, given the observed data. Bayesian analyses allow making inferences about both significant and non-significant effects by estimating the probability of their occurrence, with values ranging from 0 (i.e., no evidence) to 1 (i.e., very strong evidence; see Raftery, 1995). To calculate Bayesian probabilities we used the method proposed by Wagenmakers (2007) and Masson (2011). This method uses Bayesian information criteria (BIC), calculated using a simple transformation of sum-of-squares values generated by the standard ANOVA, to estimate Bayes factors and generate $p(H_0|\ D)$ and $p(H_1|\ D)$, assuming a "unit information prior" (for further details, see Kass & Wasserman, 1995; see also Jarosz & Wiley, 2014).

3. Results

3.1. Cyberball task

ANOVA revealed a significant effect of play block [$F(3,69) = 29.58$, $p < 0.001$, $\eta 2p\eta p2 = 0.56$, $p(H_1|\ D) = 0.83$]. *Post hoc* tests showed that participants threw the ball more often to the victim in the unfair blocks compared to the fair block ($p_s < 0.001$, Cohen's $d_s \geq 1.45$). There were no significant differences between the three types of unfair blocks ($p_s \geq 0.36$, Cohen's $d_s \leq 0.27$). Importantly, neither the main effect of session [$F(1,23) < 1$, $p = 0.99$, $\eta 2p\eta p2 < 0.001$, $p(H_0|\ D) > 0.99$] nor the session by play block interaction [$F(3,69) < 1$, $p = 0.76$, $\eta 2p\eta p2 = 0.02$, $p(H_0|\ D) > 0.99$] reached statistical significance, see Figure 2.

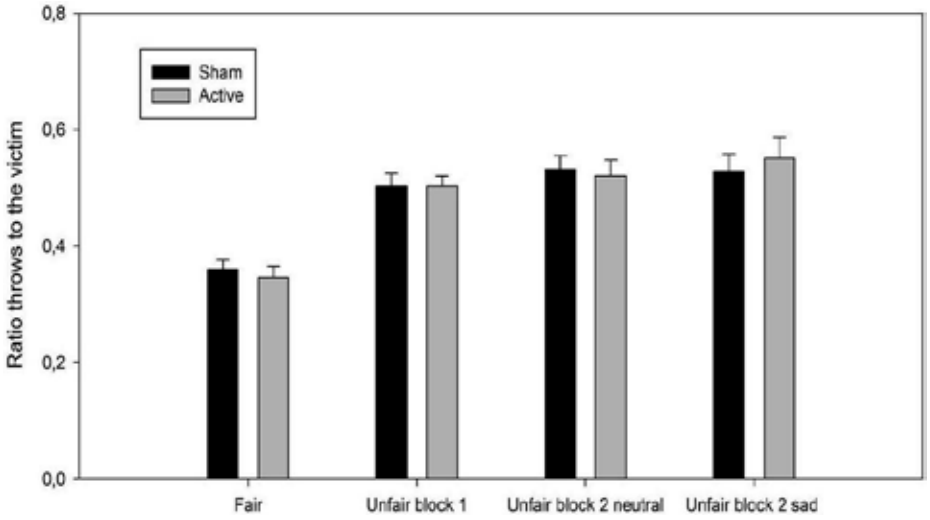

Figure 2. Ratio of throws (M, SEM) to the excluded player as a function of play block (fair play block, unfair play block 1, and unfair block 2 with the neutral and sad victim) and session (active and sham).

3.2. Empathy Quotient (EQ) and Interpersonal Reactivity Index (IRI)

For both the EQ and IRI, participants' scores were comparable to the values typically observed in healthy participants: EQ (47.96, SD = 9.8); $IRI_{totalscore}$ (66.75, SD = 12.11); $IRI_{perspectivetaking}$ (18.42, SD = 4.8); $IRI_{fantasyscale}$ (16.79, SD = 5.8); $IRI_{emphaticconcern}$ (18.79, SD = 4.0); $IRI_{personal\ distress}$ (12.75, SD = 3.3). In order to examine the possible role of individual differences in empathy, Pearson correlations coefficients were computed between the ratio of ball throws to the victim and participants' EQ and IRI scores, separately for the four blocks (fair play blocks, unfair play block 1, unfair play block 2 with neutral victim, unfair play block 2 with sad victim) and the two sessions (active and sham). No significant correlations were observed ($p_s \geq 0.07$).

3.3. Physiological and mood measurements

Table 1 provides an overview of the outcomes for physiological and mood measurements. ANOVAs showed a main effect of timing for pleasure [$F(3,69) = 4.15$, $p = 0.009$, η2pηp2 = 0.15, but $p(H_1 | D) = 0.39$], but not for the other variables ($F_s \leq 1.0$, $p_s \geq 0.39$, $\eta_{ps}^2 \leq 0.04$, $p_s(H_0 | D) \geq 0.99$). Pleasure levels dropped at the third measurement and rose again at the fourth one (1.5 vs. 1.5 vs. 1.2 vs. 1.5). Indeed, *post hoc* tests revealed that pleasure levels at the third measurement were significantly different from levels at the first, second, and forth measurements ($p_s \leq 0.05$, Cohen's $d_s \geq 0.42$). No significant differences were observed when comparing scores at the first, second, and forth measurements to each other ($p_s \geq 0.99$, Cohen's $d_s \leq 0.11$). Importantly, HR, DBP, SBP, pleasure, and arousal did not significantly differ between the two sessions. Indeed, neither the main effects of session nor the two-way interactions involving session and time were significant [$F_s \leq 1.76$, $p_s \geq 0.16$, $\eta_{ps}^2 \leq 0.07$, $p_s(H_0 | D) \geq 0.71$]. Significant differences between the two sessions were not observed either when considering only participants' scores measured immediately before (T3) and at the end of the Cyberball game (T4) [$F_s \leq 2.7$, $p_s \geq 0.12$, $\eta_{ps}^2 \leq 0.11$, $p_s(H_0 | D) \geq 0.60$].

Table 1. Mean heart rate (HR) values (in beats per minute), systolic and diastolic blood pressure (SBP and DBP; in mmHg), and arousal and pleasure scores as function of effect of time [first (T1) vs. second (T2) vs. third (T3) vs. fourth (T4) measurement; see text for more details] for active and sham (placebo) sessions. Standard errors of the mean are shown in parentheses.

	T1		T2		T3		T4	
	Active	Sham	Active	Sham	Active	Sham	Active	Sham
HR	79.9	81.5	82.4	76.1	78.6	79.4	79.	74.0
	(2.8)	(2.7)	(3.0)	(1.8)	(2.6)	(4.2)	(2.8)	(2.3)
SBP	118.0	118.5	116.7	114.0	118.8	117.2	116.3	118.8
	(3.1)	(3.3)	(3.0)	(2.8)	(2.6)	(3.0)	(3.1)	(2.8)
DBP	70.4	72.1	72.9	72.6	72.8	70.0	71.4	72.5
	(2.1)	(2.1)	(2.1)	(2.8)	(1.8)	(1.6)	(1.8)	(2.1)
Arousal	0.8	0.7	0.5	0.8	0.4	0.7	0.4	0.5
	(0.3)	(0.2)	(0.3)	(0.2)	(0.3)	(0.3)	(0.3)	(0.3)
Pleasure	1.5	1.5	1.6	1.5	1.3	1.0	1.5	1.5
	(0.2)	(0.2)	(0.2)	(0.2)	(0.2)	(0.3)	(0.2)	(0.2)

4. Discussion

Our results, corroborated by Bayesian inference, suggest that tVNS does not directly modulate reactions to vicarious ostracism in a Cyberball game: participants did not throw more balls toward the unknown ostracized person in the active stimulation compared to sham (placebo). Given that the insula and the PFC seem to be involved in vicarious ostracism (Masten, Morelli, & Eisenberger, 2011; Masten, Eisenberger, Pfeifer, & Drapetto, 2013) and that tVNS produces a reliable activation in both the insula and the PFC (Dietrich et al., 2008; Kraus et al., 2013), we expected active tVNS to enhance prosocial helping behavior, leading participants to increase their tendency to compensate the victim for the other players' ostracism. We can only speculate what the reasons for this outcome pattern are. First, we considered just one index of vicarious ostracism. Even though this index is frequently used and well-established, it remains to be seen whether other measurements of vicarious ostracism can be affected by tVNS. In our current study the victim was unknown to the participant, and an interesting example to consider would be to use a Cyberball game in which the ostracized player is known to the participant and/or to manipulate the group membership (in-group vs. out-group) of the victim. That being said, it is possible that the version of the task we used was not sensitive enough to allow possible tVNS-induced modulations to be detected. Second, and related to the previous point, the lack of a tVNS modulation on vicarious ostracism may be related to the sample of participants tested in the current study, who showed high empathy. As mentioned in the introduction, compensatory behavior following vicarious ostracism is reckoned to reflect trait empathy (Masten, Eisenberger, Pfeifer, & Drapetto, 2010). Indeed, people high in trait empathy tend to experience augmented vicarious ostracism and show higher activation in empathy-related brain regions, that is, in the same regions that are activated when observing ostracism (Masten, Morelli, & Eisenberger, 2011; Masten, Telzer, & Eisenberger, 2011; Masten, Eisenberger, Pfeifer, & Drapetto, 2013; Masten, Eisenberger, Pfeifer, & Drapetto, 2010) and that were targeted by tVNS stimulation. Thus, it is plausible that tVNS was not effective at modulating reactions to vicarious ostracism because participants already displayed a lot of empathy

(i.e., hitting a ceiling effect), which prevented a possible tVNS-induced effect from emerging. This may also explain why we failed to observe any significant correlation between trait empathy and compensatory behavior. Furthermore, individual differences in family background may at least partially account for the lack of effectiveness of our manipulation. For instance, in a previous study applying intranasal oxytocin, behavioral effects were only found in participants with rather warm relationships with their parents (Riem, Bakermans-Kranenburg, Huffmeijer, & van IJzendoorn, 2013), and similar neural effects moderated by childhood experiences have also been suggested (Bakermans-Kranenburg & van IJzendoorn, 2013). Thus, it would be crucial for follow-up studies to assess the role of past experiences and/or the quality of early relationships in moderating the possible effectiveness of tVNS in promoting prosocial behavior. Third, in our study we used a current of 0.5 mA. While this intensity was sufficient to enhance response selection (Steenbergen et al., 2015), changing vicarious ostracism may require greater intensities.

Finally, there are some limitations of the current study that warrant discussion. First, it would have been optimal to have linked the implementation of tVNS with appropriate physiological assays, such as the vagus-evoked potentials (see Bestmann, de Berker, & Bonaiuto, 2015, for a related discussion). Follow-up studies might consider a more thorough exploration of vicarious ostracism through scalp-EEG measures, such as P3b component and frontal EEG asymmetry, two cortical correlates of ostracism (Kawamoto, Nittono, & Ura, 2013). Second, we did not explicitly assess participants' blinding by asking them if they could guess the stimulation received.

In sum, we failed to obtain any evidence that tVNS, by increasing insula and PFC neural activity, is effective at modulating reactions to vicarious ostracism in a Cyberball game. Notwithstanding, our results may be useful. First, they can inform future studies on how to better design tVNS experiments to possibly affect vicarious ostracism and prosocial compensation and, second, to suggest potential future directions in this field.

Chapter Three

"Unfocus" on foc.us: Commercial tDCS headset impairs working memory

Steenbergen, L., Sellaro, R., Hommel, B., Lindenberger, U., Kuhn, S., & Colzato, L.S. (2016). "Unfocus" on foc.us: Commercial tDCS headset impairs working memory. *Experimental Brain Research, 234*(3), 637-643. doi: 10.1007/s00221-015-4391-9

Abstract

In this study we tested whether the commercial transcranial direct current stimulation (tDCS) headset foc.us improves cognitive performance, as advertised in the media. A single-blind, sham-controlled, within-subject design was used to assess the effect of on-line and off-line foc.us tDCS–applied over the prefrontal cortex in healthy young volunteers (n=24) on working memory (WM) updating and monitoring. WM updating and monitoring, as assessed by means of the N-back task, is a cognitive-control process that has been shown to benefit from interventions with CE-certified tDCS devices. For both on- and off-line stimulation protocols, results showed that active stimulation with foc.us, compared to sham stimulation, significantly decreased accuracy performance in a well-established task tapping WM updating and monitoring. These results provide evidence for the important role of the scientific community in validating and testing far-reaching claims made by the brain training industry.

1. Introduction

A recent initiative supported by several eminent research institutes and scientists calls for a more critical and active role of the scientific community in evaluating the sometimes far-reaching, sweeping claims from the brain training industry with regard to the impact of their products on cognitive performance (Max Planck Institute on Human Development, Stanford Center on Longevity, 2014). Following this prominent suggestion, we tested whether and to what degree the commercial transcranial direct current stimulation (tDCS) headset *foc.us* improves cognitive performance, as advertised in the media.

tDCS is a non-invasive brain stimulation technique that involves passing a constant direct electrical current through the cerebral cortex (via electrodes placed upon the scalp) flowing from the positively charged anode to the negatively charged cathode (Paulus, 2011; Nitsche & Paulus, 2011). By doing so, spontaneous cortical excitability is either enhanced or reduced depending on the current polarity: Anodal stimulation leads to a resting-membrane depolarization in the cortical region under the electrode, thus increasing the probability of neural firing, whereas cathodal stimulation leads to a resting-membrane hyperpolarization, thus reducing the probability of neural firing (Nitsche & Paulus, 2000; Nitsche et al., 2003a). This technique has developed into a promising tool to boost human cognition (Fregni et al., 2005; Fox, 2011; Kuo & Nitsche, 2012; Kuo & Nitsche, 2015). Previous studies using tDCS CE-certified devices have shown that excitability-enhancing anodal tDCS applied over the left dorsolateral prefrontal cortex promotes working memory (WM) updating in healthy individuals and patients (for recent reviews, see Brunoni & Vanderhasselt, 2014; Kuo & Nitsche, 2015), both when combined with excitability-diminishing cathodal tDCS over the right prefrontal cortex, either the right supraorbital region (e.g., Fregni et al., 2005; Boggio et al., 2006; Ohn et al., 2008; Jo et al., 2009; Keeser et al., 2011; Teo, Hoy, Daskalakis, & Fitzgerald, 2011) or the right dorsolateral prefrontal cortex (e.g., Oliveira et al., 2013), and when combined with a contralateral extracephalic return electrode (Seo, Park, Seo, Kim, & Ko, 2011; Zaehle, Sandmann, Thorne, Jäncke, & Herrmann, 2011). Such improvements were observed under both on-line

(i.e., stimulation overlapping with the critical task; e.g., Fregni et al., 2005; Ohn et al., 2008; Teo, Hoy, Daskalakis, & Fitzgerald, 2011) and off-line (e.g., Ohn et al., 2008; Zaehle et al., 2011; Keeser et al., 2011; Oliveira et al., 2013) stimulation. The ability to monitor and update information in the WM is considered a key cognitive-control function (Miyake et al., 2000) that strongly relies on prefrontal cortex functioning (Curtis & D'Esposito, 2003). Interestingly, WM performance can also be enhanced by video game playing (Colzato, van den Wildenberg, Zmigrod, & Hommel, 2013), an activity for which the use of the tDCS headset *foc.us* is recommended to boost performance via (left anodal-right cathodal) prefrontal cortex stimulation.

The aim of the current study was to investigate whether the commercial tDCS headset *foc.us* does in fact improve cognitive performance, as advertised in the media. Given the link between prefrontal cortex activity and WM and the aforementioned studies proving evidence that enhancing left prefrontal cortex activation by means of CE-certified tDCS devices can boost WM performance, we tested whether comparable enhancing effects can be obtained with the commercial tDCS headset *foc.us*. Consistent with previous studies assessing tDCS-induced effects on WM performance (Fregni et al., 2005; Ohn et al., 2008; Jo et al., 2009; Seo et al., 2011; Zaehle et al., 2011; Teo, Hoy, Daskalakis, & Fitzgerald, 2011, Keeser et al., 2011; Oliveira et al., 2013), WM updating was assessed by means of the well-established N-back task, (for a review, see Kane, Conway, Miura, & Colflesh, 2007).

In this task, participants are to decide whether each stimulus in a sequence matches the one that appeared n items ago—a task that requires on-line monitoring, updating, and manipulation of remembered information (Kane, Conway, Miura, & Colflesh, 2007). The task gets more difficult as n increases, since this requires more online monitoring, updating, and manipulation of remembered information. We used two conditions: In the 2-back condition, each stimulus was to be compared with the one presented two trials before. In the 4-back condition, each stimulus was to be compared with the one presented four trials before, which implies a higher memory load and greater demands on control resources. In contrast with previous studies, we preferred to include a more challenging

4-back condition instead of the 3-back condition (Teo, Hoy, Daskalakis, & Fitzgerald, 2011; Fregni et al., 2005; Ohn et al., 2008), in order to increase the chance to detect possible WM improvements following active *foc.us* tDCS, thereby minimizing potential ceiling effects (cf. Teo, Hoy, Daskalakis, & Fitzgerald, 2011; Kuo & Nitsche, 2015).

To the degree that the *foc.us* device is comparable to traditional tDCS, we expected participants to be more accurate in monitoring and updating WM when receiving active *foc.us* tDCS than when receiving sham stimulation.

2. Method

2.1. Participants

The sample size was calculated on the basis of previous studies investigating the effect of tDCS on WM (Fregni et al., 2005; Ohn et al., 2008). Twenty-four undergraduate students of Leiden University (20 females, 4 males, mean age = 19.6 years, range 18-26) participated in the experiment. Participants were recruited via an on-line recruiting system and offered course credits for participating in a study on the effects of brain stimulation on memory. Once recruited, participants were randomly assigned to one of the two following experimental groups: off-line stimulation (N=12; 2 male; mean age=20.1, SD=2.5), and on-line stimulation (N=12; 2 male; mean age=19.7, SD=2.3). Groups did not differ in terms of age, $F < 1$, or gender, $\chi^2=.00$, p=1.00. All participants were naïve to *foc.us* tDCS. Participants were screened individually via a phone interview by the same lab-assistant using the Mini International Neuropsychiatric Interview (MINI). The MINI is a short, structured, interview of about 15 minutes that screens for several psychiatric disorders and drug use, often used in clinical and pharmacological research (Sheehan et al., 1998; Colzato, Kool, & Hommel, 2008; Colzato, Hertsig, van den Wildenberg, & Hommel, 2010). Participants were considered suitable to participate in this study if they fulfilled the following criteria: (i) age between 18 and 32 years; (ii) no history of neurological or psychiatric disorders; (iii) no history of substance abuse or dependence; (iv) no history of brain surgery, tumor or intracranial metal implantation; (v) no chronic or acute medications; (vi) no pregnancy;

(vii) no susceptibility to seizures or migraine; (viii) no pacemaker or other implanted devices.

Prior to the first testing session, all participants received a verbal and written explanation of the *foc.us* tDCS procedure and gave their written informed consent to participate in the study. No information was provided about the different types of stimulation (active vs. sham). 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).

2.2. Apparatus and procedure

A single-blinded, sham-controlled, randomized cross-over within-subject design with counterbalancing of the order of conditions was used to assess the effect of off-line and on-line *foc.us* tDCS on WM updating in healthy young volunteers. The *foc.us* headset (v.1) was applied over the prefrontal cortex (PFC) according to the manufacturer's guidelines (see Figure 1) . All participants took part in two sessions (active vs. sham) and were tested individually.

Upon arrival, participants read and signed the informed consent. In the off-line stimulation group, active or sham stimulation was applied for 20 minutes while at rest. Immediately thereafter, participants were asked to perform the N-back task (see Kane et al., 2007, for a review), which lasted for 15 minutes. In the on-line stimulation group, participants performed the N-back task five minutes after the onset of the stimulation, which was applied throughout the whole task.

At the end of each session, participants were asked to complete a foc.us (tDCS) adverse effects questionnaire requiring them to rate, on a five-point (1–5) scale, how much they experienced: (1) headache, (2) neck pain, (3) nausea, (4) muscles contraction in face and/or neck, (5) stinging sensation under the electrodes, (6) burning sensation under the electrodes, (7) uncomfortable (generic) feelings, and (8) other sensations and/or adverse effects. After completion of the second session, participants were debriefed and compensated for their participation.

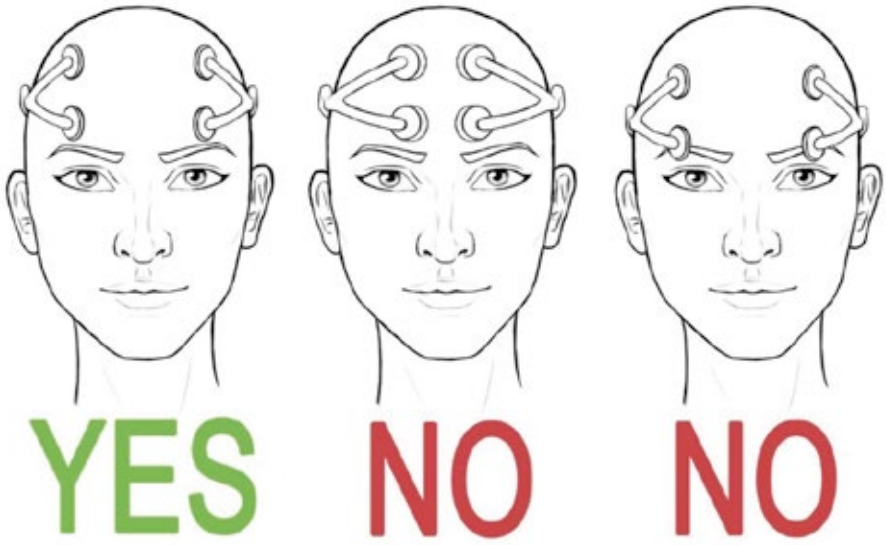

Figure 1. Positioning of the *foc.us* headset on the head as provided by the manufacturer. The correct positioning of *foc.us* is the one displayed in the leftmost panel. Note that this is the only possible allowable montage with this device. Figure designed by the authors.

2.*2.1. Foc.us tDCS commercial device*

Direct current was induced by four circular saline-soaked surface sponge electrodes (2.0 cm diameter) and delivered by a *foc.us* tDCS commercial device v1 (http://www.foc.us/; © FOC.US LABS / EUROPEAN ENGINEERS), a device complying with Part 15 of the Federal Communications Commission (FCC) Rules, but without being CE (European Conformity)-certified. The Federal Code Of Regulation (CFR) FCC Part 15 is a common testing standard for most electronic equipment. FCC Part 15 covers the regulations under which an intentional, unintentional, or incidental radiator may be operated without an individual license. FCC Part 15 also covers technical specifications, administrative requirements and other conditions relating to the marketing of FCC Part 15 devices. Depending on the type of the equipment, verification, declaration of conformity, or certification is the process for FCC Part 15 compliance.

Foc.us tDCS was applied on participants' head according to the instructions provided by the manufacturer, which allow for a single type of electrodes montage, that is, a bipolar-balanced montage (see Nasseri, Nitsche, & Ekhtiari, 2015, for a tDCS electrodes montage classification), with anodal stimulation applied over the left prefrontal cortex and cathodal stimulation applied over the right prefrontal cortex (see Figure 1, leftmost panel). For the active stimulation, a constant current of 1.5 mA was delivered for 20 minutes with a linear fade-in/fade-out of 15 seconds. These parameters are within safety limits established from prior work in humans (Nitsche & Paulus, 2000; Nitsche et al., 2003b; Nitsche et al., 2004; Poreisz, Boros, Antal, & Paulus, 2007). For sham stimulation, the position of the electrodes, current intensity and fad-in/fade-out were the same as in the active tDCS, but stimulation was automatically turned off after 30 seconds, without the participants' awareness. Hence, participants felt the initial short-lasting skin sensation (i.e., itching and/or tingling) associated with tDCS without receiving any active current for the rest of the stimulation period. Stimulation for 30 seconds does not induce after-effects (Nitsche & Paulus, 2000). This procedure has been shown to be effective in blinding participants to the received stimulation condition (see Poreisz, Boros, Antal, & Paulus, 2007; Gandiga, Hummel & Cohen, 2006; Palm et al., 2013). Consistently, none of the participants was able to determine whether or not he/she received real or sham stimulation. The condition (active vs. sham) and duration of stimulation was controlled by the *foc.us* app iOS (version 2.0) using iPad 4.

2.2.2. N-back task

The experiment was controlled by an 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 using a QWERTY computer keyboard. Stimulus presentation and data collection were controlled using E-Prime 2.0. software system (Psychology Software Tools, Inc., Pittsburgh, PA).

The two conditions of the N-back task were adapted from Colzato et al. (2013a; 2013b). A stream of single visual letters (taken from B, C, D, G, P, T, F, N, L) was presented (stimulus–onset asynchrony 2000 ms;

duration of presentation 1000 ms). Participants responded to targets and to nontargets.

Half of the participants pressed the 'z' key in response to a target and the 'm' key in response to a nontarget; the other half of the participants received the opposite mapping. Target definition differed with respect to the experimental condition. In the 2-back condition, targets were defined as stimuli within the sequence that were identical to the one that was presented two trials before. In the 4-back condition, participants had to respond if the presented letter matched the one that was presented four trials before. Each condition consisted of a practice block followed by two experimental blocks. The 2-back condition comprised of 106 trials in total (42 target stimuli and 64 nontarget stimuli), whereas the 4-back condition consisted of 110 trials (42 target stimuli and 68 nontarget stimuli). All participants performed the 2-back condition first and then the 4-back condition.

2.3. Statistical Analyses

Repeated-measures analyses of variance (ANOVAs) including stimulation protocol (off-line vs. on-line) as between-subjects factor and condition (Active vs. Sham) as within-subjects factors were performed to compare participants' self-reports of discomfort about headache, neck pain, nausea, muscles contraction in face and/or neck, stinging sensation under the electrodes, burning sensation under the electrodes, and other uncomfortable (generic) feelings.

For the N-back task, practice blocks and either the first two trials (in the 2-back condition) or the first four trials (in the 4-back condition) of each block were excluded from the analyses. Repeated-measures ANOVAs with load (2-back vs. 4-back) and condition (Active vs. Sham) as within-subjects factors and stimulation protocol (off-line vs. on-line) as between-subjects factor were carried out on reaction times (RTs) on correct trials, as well as for hits, correct rejections, false alarms and misses in percent. Furthermore, the sensitivity index d' was calculated for both active and sham stimulation and the two WM loads separately (see. Haatveit et al., 2010; Buckert, Kudielka, Reuter, & Fiebach, 2012). This index, which derives from signal

detection theory (Swets, Tanner, & Birdsall, 1961), provides a combined measure of correct hits and false alarms and thus reflects participants' ability to discriminate target from nontargets, with higher d' indicating better signal detection. d' was computed from hit rate and false alarm (FA) rate using the following formula: $Z_{HIT} - Z_{FA}$, where Z represents the z-scores of the two rates (Macmillan & Creelman, 1991). The Z transformation was done using the inverse cumulative distribution function in Microsoft Excel 2010 (NORMSINV). Perfect scores were adjusted using these formulas: 1 − 1/(2n) for perfect (i.e., 100%) hits, and 1/(2n) for zero false alarms, where n was number of total hits or false alarms (Macmillan & Creelman, 1991). A significance level of $p<0.05$ was adopted for all statistical tests.

In addition to standard statistical methods, we calculated Bayesian probabilities associated with the occurrence of the null ($p(H_0|D)$) and alternative ($p(H_1|D)$) hypotheses, given the observed data (see Masson, 2011; Wagenmakers, 2007). This method allows making inferences about both significant and nonsignificant effects by providing the exact probability of their occurrence. The probabilities range from with 0 (i.e., no evidence) to 1 (i.e., very strong evidence; see Raftery, 1995).

3. Results

3.1. Foc.us (tDCS) adverse effects

ANOVAs performed on participants' self-reports of discomfort revealed significant main effects of condition on self-reports of stinging sensation under the electrode, $F(1,22)=10.56$, $p=.004$, MSE=1.044, $\eta^2_p=0.32$, burning sensation under the electrode, $F(1,22)=5.11$, $p=.034$, MSE=.587, $\eta^2_p=0.19$, and other uncomfortable (generic) feelings, $F(1,22)=4.64$, $p=.04$, MSE=.544, $\eta^2_p=0.17$, with participants reporting higher discomfort in the active (3.4, 3.0 and 1.9) than in the sham (2.5, 2.5 and 1.4) condition. Finally, a significant interaction involving the factors condition and stimulation protocol was observed on self-reports of headache, $F(1,22)=4.24$, $p=.05$, MSE=.314, $\eta^2_p=0.16$. Newman-Keuls post-hoc analyses showed that for the off-line stimulation participants reported higher discomfort in the active than in the sham condition (2.0 vs. 1.4, $p=.02$), whereas no difference between active and sham conditions was observed for participants who

received the stimulation during the task (on-line stimulation; 1.4 vs. 1.3, p=.72). No other significant source of variance was observed, $F_s \leq 3.12$, $p_s \geq .09$.

3.2. N-back task

Table 1 shows mean RTs (in milliseconds; ms), hits, correct rejections, false alarms and misses (in percent) for the N-back task separately for off-line and on-line stimulations and for active and sham conditions.

Load (i.e. 2-back vs. 4-back) affected all dependent measures, showing that higher load increased RTs (568 vs. 492 ms), $F(1,22)=63.80$, p=.0001, MSE=2148.196, η^2_p=0.74, $p(H_1|D) > .99$, and reduced hit rates (89 % vs. 64 %), $F(1,22)=125.60$, p=.0001, MSE=.012, η^2_p=0.85, $p(H_1|D) > .99$. Higher load also produced fewer correct rejections (92 % vs. 80 %), but more false alarms (8 % vs 20 %), $F(1,22)=38.34$, p=.0001, MSE=.010, η^2_p=0.64, $p(H_1|D) > .99$, and misses (11 % vs. 36 %), $F(1,22)=125.60$, p=.0001, MSE=.012, η^2_p=0.85, $p(H_1|D) > .99$, than the lower load did. Most importantly, with regard to the effect of condition, active stimulation, as compared to sham, significantly reduced hits (75 % vs. 78 %) and increased misses (26 % vs. 22 %), $F(1,22)=5.62$, p=.027, MSE=.006, η^2_p=0.20, $p(H_1|D) = .76$, but it did not affect RTs, false alarms, correct rejections, $F < 1$, $p \geq .71$, $p(H_0|D) \geq .81$, [$d'_{(sham)}$= 2.2, $d'_{(active)}$= 2.0] (see Figure 2). No further significant source of variance was observed, $F_s \leq 2.5$, $p_s \geq .13$, $p_s(H_0|D) \geq .60$.

Table 1. Mean RTs (in ms), hits, correct rejections, false alarms and misses (in percent) for the N-back task as a function of condition (Sham vs. Active) and stimulation protocol (Off-line vs. On-line stimulation). Standard errors are shown within parentheses.

N-back (WM monitoring/ updating)	**Off-line stimulation**		**On-line stimulation**	
	Sham	**Active**	**Sham**	**Active**
2-back				
Reaction times (ms)	480 (19.1)	487 (16.5)	505 (19.1)	496 (16.5)
Hits (%)	90.9 (2.0)	88.5 (2.2)	90.7 (2.0)	85.5 (2.2)
Correct rejections (%)	93.1 (2.8)	92.9 (1.7)	92.1 (2.8)	91.1 (1.7)
False alarms (%)	6.9 (2.8)	7.1 (1.7)	7.9 (2.8)	8.9 (1.7)
Misses (%)	9.1 (2.0)	11.5 (2.2)	9.3 (2.0)	14.5 (2.2)
4-back				
Reaction times (ms)	561 (11.6)	575 (15.7)	575 (11.6)	559 (15.7)
Hits (%)	63.3 (3.7)	59.9 (2.9)	68.7 (3.7)	64.1 (2.9)
Correct rejections (%)	78.5 (3.2)	82.1 (2.3)	78.8 (3.2)	79.0 (2.3)
False alarms (%)	21.5 (3.2)	17.9 (2.3)	21.2 (3.2)	21.0 (2.3)
Misses (%)	36.7 (3.7)	40.1 (2.9)	31.3 (3.7)	35.9 (2.9)

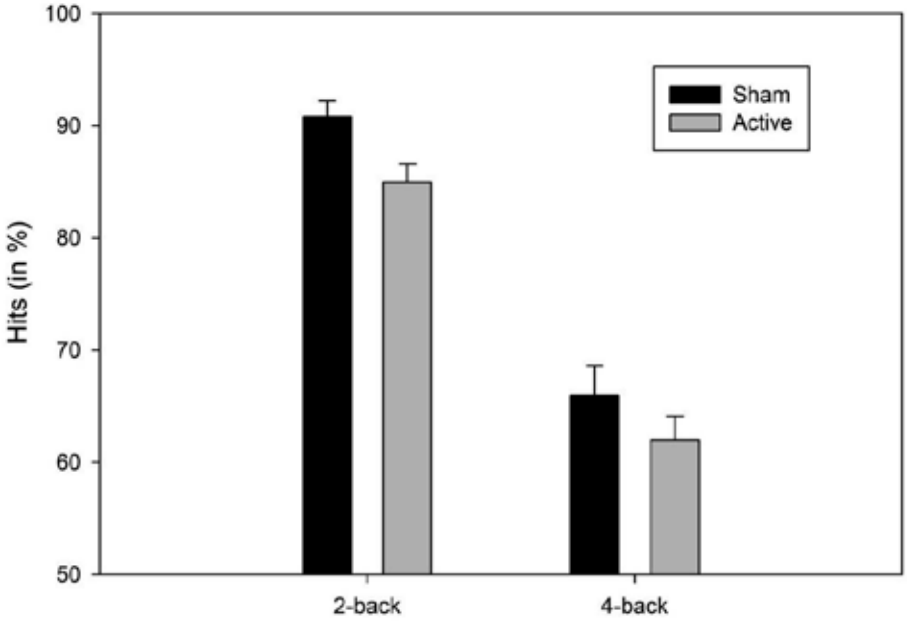

Figure 2. Mean hits (in %) as a function of load (2-back vs.4-back) and condition: Active and Sham. Vertical capped lines atop bars indicate the standard error of the mean.

4. Discussion

The present study is the first to demonstrate that prefrontal cortex stimulation delivered using the commercial *foc.us* tDCS headset (v.1) impairs the ability to monitor and update information in the WM . Results showed that, regardless of the adopted protocol (on-line or off-line stimulation), active stimulation with *foc.us* significantly decreased hits and increased misses in a WM monitoring task compared to sham stimulation. Given that WM updating is a key cognitive control function (Miyake et al., 2000), the present findings do not support the claims that the use of *foc.us* tDCS (v1) headset can improve cognitive performance. Instead, our results suggest that the use of this device can actually be detrimental and, as such, cannot be regarded as an alternative to CE-certified tDCS devices, the use of which has been demonstrated to be successful in promoting WM (Fregni et al., 2005; Kuo & Nitsche, 2012; Boggio et al., 2006; Ohn et al., 2008; Jo et al., 2009; Teo, Hoy, Daskalakis, & Fitzgerald, 2011; Seo et al., 2011; Zaehle et al., 2011). In contrast to such devices, the *foc.us* device is not CE-certified but complies only with Part 15 of the FCC Rules.

Given that, as advertised in the media, the use of *foc.us* is quite popular among young people to improve their gaming performance, future research will need to explore the effects of prolonged use of *foc.us* on the brain. Moreover, given that tDCS has the potential to induce significant alterations of functional connectivity (e.g., Polanía, Nitsche, & Paulus, 2011; Keeser et al., 2011), follow-up studies should assess whether the use of *foc.us* produces prefrontal functional connectivity changes, and how these possible changes relate to behavioral performance decrements.

From a more general point of view, *foc.us* is just one example of a device that can easily be purchased and, without any control or expert knowledge, used by anyone. The results of the study are straightforward in showing that the claims made by companies manufacturing such devices need to be validated, To conclude, even if the consequences of long-term or frequent use of the *foc.us* device are yet to be demonstrated, our findings provide strong support for the claim that the scientific community should play a more critical and active role in validating and testing far-reaching claims made by the brain training industry.

Chapter four

Action video gaming and cognitive control: playing first person shooter games is associated with improved action cascading but not inhibition

Steenbergen, L., Sellaro, R., Stock, A-K., Beste, C., & Colzato, L.S. (2015). Action video gaming and cognitive control: Playing first person shooter games is associated with improved action cascading but not inhibition. *PLoS ONE, 10*(12): e0144364. doi:10.1371/journal.pone.0144364

Abstract

There is a constantly growing interest in developing efficient methods to enhance cognitive functioning and/or to ameliorate cognitive deficits. One particular line of research focuses on the possibly cognitive enhancing effects that action video game (AVG) playing may have on game players. Interestingly, AVGs, especially first person shooter games, require gamers to develop different action control strategies to rapidly react to fast moving visual and auditory stimuli, and to flexibly adapt their behavior to the ever-changing context. This study investigated whether and to what extent experience with such videogames is associated with enhanced performance on cognitive control tasks that require similar abilities. Experienced action videogame-players (AVGPs) and individuals with little to no videogame experience (NVGPs) performed a stop-change paradigm that provides a relatively well-established diagnostic measure of action cascading and response inhibition. Replicating previous findings, AVGPs showed higher efficiency in response execution, but not improved response inhibition (i.e. inhibitory control), as compared to NVGPs. More importantly, compared to NVGPs, AVGPs showed enhanced action cascading processes when an interruption (stop) and a change towards an alternative response were required simultaneously, as well as when such a change had to occur after the completion of the stop process. Our findings suggest that playing AVGs is associated with enhanced action cascading and multi-component behavior without affecting inhibitory control.

1. Introduction

Cognitive control is defined as a set of processes that sustain our ability to interact with the environment in a goal-directed manner, by flexibly and continuously adapting our behavior to the ever-changing environment (Botvinick, Braver, Barch, Carter, & Cohen, 2001). As humans, we are regularly confronted with situations in which cognitive control is needed, for instance, when driving a car, cooking, doing sports, working, and in several other similar and more complex situations.

The importance of cognitive control processes becomes apparent when looking at the consequences its impairments can have on personal life and interpersonal relationships, as is the case for individuals with mental and neurological disorders (e.g., attention deficit/hyperactivity disorder (ADHD), obsessive–compulsive disorder, or dysexecutive syndrome (Konrad, Gauggel, Manz, & Schöll, 2000a,b; Gauggel, Rieger, & Feghoff, 2004; Chamberlain et al., 2006a; Martinussen, Hayden, Hogg-Johnson, & Tannock, 2005; Nakao et al., 2009) in aging (Gazzaley, 2013; Levy, 1994), and for otherwise healthy individuals suffering from maladaptive habits (e.g., alcohol and substance abuse; Curtin & Fairchild, 2003; Ambrose, Bowden, & Whelan, 2001; McCann, Mertl, Eligulashvili, & Ricaurte, 1999; Li, Luo, Yan, Bergquist, & Sinha, 2009; Fillmore & Rush, 2002; Gouzoulis-Mayfrank et al., 2000).

Given the important role of cognitive control processes in daily life, there is great interest in developing efficient methods to improve cognitive control functions and/or to counteract their decline. In this regard, action video game training seems to represent a promising tool (Green & Bavelier, 2015). Indeed, since the seminal work by Green and Bavelier (2003), converging evidence has suggested that in contrast to other types of games, such as life-simulations, playing action video games (AVG)–in particular first-person shooter games such as the Halo, Call of Duty, and Battlefield series, and third-person shooter games such as the Gears of War and Grand Theft Auto series (Kin & Krzywinska, 2002) –is associated with improvements in a wide range of perceptual (Boot, Blakely, & Simons, 2011; Donohue, Woldorff, & Mitroff, 2010, Green, Pouget, & Bavelier, 2010; Green, Li, & Bavelier, 2010; Buckley, Codina, Bhardwaj, & Pascalis,

2010; Appelbaum, Cain, Darling, & Mitroff, 2013; Li, Polat, Makous, & Bavelier, 2009), (visuo-)spatial (Green & Bavelier, 2003, 2006a, 2007; Spence, Yu, Feng, & Marshman, 2009; Spence & Feng, 2010; Feng, Spence, & Pratt, 2007), perceptuo-motor (Hubert-Wallander, Green, & Bavelier, 2011; Chen, Chen, & Li, 2015) and attentional skills (Green & Bavelier, 2003; West et al., 2008; Hubert-Wallander, Green, Sugarman, & Bavelier, 2011; Chisholm & Kingstone, 2012; Chisholm, Hickey, Theeuwes, & Kingstone, 2010). For instance, AVG experience has been found to be associated with a more efficient distribution of visuo-spatial attention (Green & Bavelier, 2003, 2006a), a general increase in central and peripheral visuospatial attention (Green & Bavelier, 2007), an increment in the number of objects that can be apprehended (Green & Bavelier, 2006b), enhanced temporal processing of multisensory stimuli (Donohue, Woldorff, & Mitroff, 2010), enhanced sensorimotor learning (Gozli, Bavelier, & Pratt, 2014), and a general speeding of perceptual reactions (Dye, Green, & Bavelier, 2009). Remarkably, recent studies have complemented the aforementioned findings by showing that the beneficial effects of playing AVGs can generalize to cognitive control, that is, to people's capacity to control their thoughts and action in a goal-directed manner. For instance, research has shown that AVG-players (AVGPs), compared to individuals with little to no videogame experience (NVGPs), have an enhanced ability to flexibly switch between tasks, as indexed by performance on a wide range of task-switching paradigms (Boot, Kramer, Simons, Fabiani, & Gratton, 2008; Cain, Landau, & Shimamura, 2012; Colzato, van Leeuwen, van den Wildenberg, & Hommel, 2010; Colzato, van den Wildenberg, & Hommel, 2014; Green, Sugarman, Medford, Klobusicky, & Bavelier, 2012; Karle, Watters, & Shedden, 2010; Strobach, Frensch, & Schubert, 2012; Andrews & Murphy, 2006), which supports the idea that playing AVGs is associated with increased cognitive flexibility (Colzato, van Leeuwen, van den Wildenberg, & Hommel, 2010). Moreover, AVGPs have been found to outperform NVGPs in the monitoring and updating of working memory (WM) representations (Colzato, van den Wildenberg, Zmigrod, & Hommel, 2013) – another key cognitive-control function that is related to cognitive flexibility (Miyake et al., 2000). Conversely, inhibitory control (also considered an index of behavioral impulsivity) does not seem to be associated with AVGs

experience. Indeed, a previous study (Colzato, van den Wildenberg, Zmigrod, & Hommel, 2013) showed that playing AVGs results in more efficient response execution, but does not affect the ability to stop an ongoing response, as indexed by stop-signal reaction times (SSRTs; Logan, Schachar, & Tannock, 1997) (for similar findings, see Dye, Green, & Bavelier, 2009 This latter finding is particularly intriguing. First, it questions the possibility that the beneficial effects of playing AVGs can transfer to all cognitive-control functions, as that would suggest AVGPs should also show superior inhibitory control (i.e., lower SSRTs) as compared to NVGPs. Second, it challenges the anecdotal idea that AVGPs are more impulsive than NVGPs, based on which AVGPs are expected to show lower inhibitory efficiency (i.e., higher SSRTs) than NVGPs.

In the present study we sought to complement previous findings by gaining a better understanding of the extent to which playing AVGs is associated with improved cognitive control. We focused on first person shooter (FPS) AVGs because it has been suggested that it is in particular the first person perspective that allows for cognitive-control improvements (Colzato, van Leeuwen, van den Wildenberg, & Hommel, 2010). Indeed, compared to strategic and life-simulation games, the new generations of FPS AVGs are not just about pressing a button at the right moment, but they require the players to develop different action control strategies to rapidly react to fast moving visual and auditory stimuli, and to flexibly adapt their behavior to the ever-changing context. This resembles complex daily life situations, such as multitasking conditions, in which we are required to inhibit a planned, ongoing response and to rapidly adapt our behavior (e.g., to execute a different response). Successful performance under multitasking conditions relies on the ability to activate different task goals, and to cascade and prioritize different actions (Boecker, Gauggel, & Drueke, 2013). This leads to the possibility that extensive experience with playing FPS AVGs could be linked with better action cascading/multitasking performance. Yet, empirical evidence supporting this possibility is still missing.

Action cascading is defined as the ability to generate, process, and execute separate task goals and responses in an expedient temporal order and, as such, to be able to display efficient goal-directed multi-component

behavior (Dippel & Beste, 2015; Duncan, 2010; Mückschel, Stock, & Beste, 2014; Stock, Arning, Epplen, & Beste, 2014; Stock, Blaszkewicz, & Beste, 2014). The cascading of and selecting the right action can be done in a serial manner (i.e. step-by-step: a new task goal is activated only after the previous one has been carried out or stopped) or in a parallel manner (i.e. overlapping: a new task goal is activated while the previous one is still being is carried out), depending on the task demands (Mückschel, Stock, & Beste, 2014; Stock, Arning, Epplen, & Beste, 2014; Stock, Blaszkewicz, & Beste, 2014; Beste & Saft, 2015; Steenbergen et al., 2015).

In order to assess whether extensive experience with AVGs can in fact result in an enhanced ability to prioritize and cascade different actions, we employed a stop-change task introduced by Verbruggen, Schneider, and Logan (2008). In this task, the primary goal is to quickly react to a GO stimulus. Occasionally, a STOP stimulus is presented, which requires participants to stop the ongoing response. The STOP stimulus is followed by a CHANGE stimulus signaling the participants to shift to an alternative response. The interval between the STOP and the CHANGE stimulus (stop-change delay; SCD) hence, the duration of the preparation process before the execution of the change response, is manipulated in such a way that the two stimuli occur either simultaneously (0 ms; i.e., SCD 0) or with a short delay (300 ms; i.e., SCD 300; for more details, see Method section and Figure 1). Responses on SC trials depend on the ability to activate different task goals, and to cascade and prioritize different actions so as to succeed in inhibiting an ongoing response and rapidly switching to a different one (Boecker, Gauggel, & Drueke, 2013). As such, reaction times (RTs) on stop-change trials can be taken to reflect the efficiency of action cascading, with shorter RTs reflecting more efficient action selection.

Based on the available findings (Boot, Kramer, Simons, Fabiani, & Gratton, 2008; Cain, Landau, & Shimamura, 2012; Colzato, van Leeuwen, van den Wildenberg, & Hommel, 2010; Colzato, van den Wildenberg, & Hommel, 2014; Green, Sugarman, Medford, Klobusicky, & Bavelier, 2012; Karle, Watters, & Shedden, 2010; Strobach, Frensch, & Schubert, 2012) we expected AVGPs to outperform NVGPs in action cascading processes (i.e., to show faster RTs on the stop-change trials) both when an interruption (stopping) and a change toward an alternative response are required

simultaneously (SCD0) and when the change to another response is required when the stopping process has already finished (SCD300). Aside from providing a measure of action cascading efficiency, the stop-change paradigm also allows an assessment of the efficiency of response execution, as reflected by RTs to the GO stimuli, and a quantitative estimation of the duration of the covert response-inhibition process (i.e., the efficiency of inhibitory control), as indexed by the SSRTs (i.e., the time required to stop the ongoing response; Logan & Cowan, 1984; Logan 1994). Assuming we would replicate previous findings (Colzato, van den Wildenberg, Zmigrod, & Hommel, 2013, see also Miyake et al., 2000), we expected AVGPs, compared to NVGPs, to show higher efficiency in response execution (i.e., faster RTs to the GO stimuli), but comparable performance on response inhibition (i.e., comparable mean SSRTs).

Finally, to rule out between-groups differences in terms of fluid intelligence, which could partially account for possible differences in cognitive control (Kane & Engle, 2002; Engle, Tuolski, Laughlin, & Conway, 1999), we also assessed participant's fluid intelligence by means of the Raven's standard progressive matrices (Raven & Court, 1998). Building on previous studies (Pohl et al., 2014; Cain, Landau, & Shimamura, 2012; Colzato, van Leeuwen, van den Wildenberg, & Hommel, 2010; Colzato, van den Wildenberg, Zmigrod, & Hommel, 2013), AVGPs are expected to show comparable performance to that of NVGPs.

2. Method

2.1. Participants

Thirty-six young healthy adults (28 men and 8 women) participated in the experiment. They constituted the two groups of 18 FPS AVGPs and 18 NVGPs. Participants were selected from a sample of 90 young adults who had previously participated in other studies in our lab and agreed to be contacted to participate in other behavioral studies. Using a covert recruitment strategy, the 90 potential volunteers were required (via e-mail) to fill in a questionnaire that assessed their experience with videogame along with other preferences (i.e., religious belief and preferred temperature). Specifically, participants were asked the following questions:

(1) Are you baptized? (2) How often do you pray? (3) How often are you going to the church? (4) Do you prefer the heater high or low? (5) Do you work/study better when the heater is high or low? (6) Do you play video games? (7) Which kind of video games do you play and how much time do you spend playing them per week? (8) When did you start playing video games?. Following previous studies (Green & Bavelier, 2003, 2006a,b, 2007), participants who reported playing a minimum of 5h/week of FPS AVGs, over the last year were defined as AVGPs. Twenty-two participants felt in this category and were invited to the lab, but only 18 of them showed up for the testing session. Participants assigned to the AVGP group reported to play FPS games such as Call of Duty, Unreal Tournament, Half-Life 2, and Battlefield 2 and later versions. All of these games are situated in a 3D environment and require frequent updating between multiple tasks and stimuli. Eighteen matched participants who reported little to no AVG experience (i.e., one or fewer hours per week on average of action videogame play) were selected to form the NVGP group.

All participants who were invited to the lab were also screened individually by a phone interview by the same lab-assistant using the Mini International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998)–a well-established brief diagnostic tool used in clinical and stress research that screens for several psychiatric disorders and drug use (Sheehan et al., 2008; Colzato, Kool, & Hommel, 2008; Colzato, Ruiz, van den Wildenberg & Hommel, 2011).

Prior to the testing session, all participants were informed that they were participating in a study on the effects of playing videogames on cognitive performance. Written informed consent was obtained from all participants. The protocol and remuneration arrangements of 6.50 Euros were approved by the institutional review board (Leiden University, Institute for Psychological Research). The methods were carried out in accordance with the approved guidelines.

2.2. Apparatus and procedure

All participants were tested individually. Participants started with the practice procedure of the stop-change paradigm, which took about 20 minutes. After completion of the practice, participants performed the task

(25 minutes) and filled out the short version (i.e., 30 items) of the Raven's SPM (Standard Progressive Matrices; Raven & Court, 1998; Keizer, Verschoor, Vermen, & Hommel, 2010), a standard and widely-used test to measure fluid intelligence (Raven & Court, 1998). Each participant was given 10 minutes to perform the SPM test. Participants were allowed to take a short break (maximum of 5 minutes) between tasks.

2.2.1. Stop-Change paradigm

The task was adapted from Steenbergen, Sellaro, Stock, Beste, and Colzato (2015) and Yildiz, Wolf, and Beste (2014), see Figure 1. The experiment was controlled by an Asus laptop running on an Intel Core i3-3217U processor, attached to a LG Flatron 776FM 16 inch monitor (refresh rate of 60 Hz). Stimulus presentation and data collection were controlled using Presentation software system (Neurobehavioral Systems, Inc., Berkeley, CA). Responses were executed via button-presses using the number row of a QWERTY computer keyboard. Throughout the task, the response buttons were marked with yellow stickers. All visual stimuli were presented in white on a black background.

Each trial started with the presentation of four vertically aligned unfilled circles (diameter 7 mm) and three horizontal reference lines (line thickness 1 mm, width 13 mm), embedded in a 55 x 16 mm rectangle presented in the center of the screen. After 250 ms, one of the circle was filled white (GO stimulus). In the GO condition (67% of the trials), participants were to indicate the position (above vs. below) of the white circle relative to the middle reference line. Specifically, participants were instructed to press the "7" key (for below) and the "8" key (for above) with the index and middle finger of their right hand, respectively. Stimuli were shown until response, but not longer than 2500 ms. Instructions emphasized both accuracy and speed. When RTs were longer than 1000 ms, the word "Quicker" was presented above the rectangle until the participant responded.

In the SC conditions, which corresponded to the remaining 33% of the trials, the presentation of the white GO stimulus was followed by a STOP signal (a red rectangle replacing the previous white frame), signaling the participants to try to inhibit their right-handed response to the GO

stimulus. The delay between the onset of the GO stimulus and the onset of the STOP signal (i.e., the stop signal delay, SSD) was initially set to 250 ms and then dynamically adjusted using a staircase procedure to yield a 50% probability of successfully inhibiting the GO response (see Steenbergen, Sellaro, Stock, Beste, & Colzato, 2015; Steenbergen et al., 2015; Beste, Stock, Epplen, & Arning, 2014).

Specifically, after a completely correct SC trial (i.e. no response to GO stimulus, no response prior to the CHANGE stimulus in the SCD300 condition (explained below) and a correct left hand response to the CHANGE stimulus), the SSD of the next SC trial increased by 50 ms. Conversely, after an incorrect SC trial (if any of the above criteria were not met), the SSD of the next SC trial decreased by 50 ms. Additionally, the following restriction was applied to this procedure: the SSD values could not fall below a value of 50 ms and could not exceed a value of 1000 ms. Participants were not informed about the staircase procedure, and were instructed not to wait for the stop signal. Regardless of the stopping (inhibitory) performance, every stop signal was associated with one of three possible CHANGE stimuli. The CHANGE stimuli consisted of 100 ms sine tones presented through headphones at 75 dB sound pressure level and could be high (1300 Hz), medium (900 Hz) or low (500 Hz) in pitch. The presentation of the CHANGE stimulus signaled the participants to execute a left-handed response requiring them to judge the position (above vs. below) of the white circle relative to a new reference line, as indicated by the pitch of the tone. The presentation of the high tone indicated the highest of the three lines as the new reference, the medium tone indicated the middle line and the low tone indicated the lowest line (see Figure 1). The three tones occurred with equal frequency. Participants were instructed to press the “1” key for stimuli located above the newly assigned reference line, and the “2” key for stimuli located below the newly assigned reference line, using the middle and index fingers of the left hand, respectively. The delay between the presentation of the STOP signal and the presentation of the CHANGE stimulus (i.e., the stop change delay, SCD) was manipulated to vary as follows. In half of the SC trials, there was a SCD with a stimulus onset asynchrony (SOA) of 300 ms between the STOP and the CHANGE signals (SCD300 condition); in the other half of SC trials, the

STOP and CHANGE stimuli were presented simultaneously (SOA of 0 ms, SCD0 condition). RTs for the stop-change trials were measured from the onset of the CHANGE stimulus. When RTs for the stop-change trials were longer than 2000 the English word “Quicker” was presented above the rectangle until the participant responded. During the inter-trial interval (ITI) a fixation cross was presented in the center of the screen for 900 ms. Overall, the task comprised 864 experimental trials and lasted about 25 minutes.

2.3. Statistical analysis

A Chi-square test was used to compare gender distribution over the two groups. Independent samples t-tests or non-parametric Mann-Whitney U tests (in case of a violation of the normality assumption) were used to compare the two groups with regard to fluid intelligence, age, and the number of hours spent per week playing different game genres including shooter, strategy, and other games (i.e., role-playing, puzzle and sports games). To assess the effect of AVG practice on action cascading, mean RTs were submitted to a repeated-measures ANOVA with condition (GO, SCD0, SCD300) as within-subjects factor and group (AVGPs vs. NVGPs) as between-subjects factor. Greenhouse—Geisser correction was applied when the sphericity assumption was violated. Tukey HSD post-hoc tests were performed to clarify mean differences in case of significant interactions. Given that for the stop-change trials, the percentage of errors is mainly determined by a staircase procedure and, thus, is artificially fixed at approximately 50% (Verbruggen et al., 2008), we only analyzed the percentages of errors for the GO trials. The non-parametric Mann-Whitney U test was preferred over the independent samples t-test because of a small violation to the normality assumption. To index response inhibition, individual SSRTs for stop-signal trials were calculated, as indicated by Verbruggen, Schneider, and Logan (2008). SSRTs were analyzed by means of the Mann-Whitney U test, as this variable was shown not to be normally distributed. A significance level of $p<0.05$ was adopted for all statistical tests.

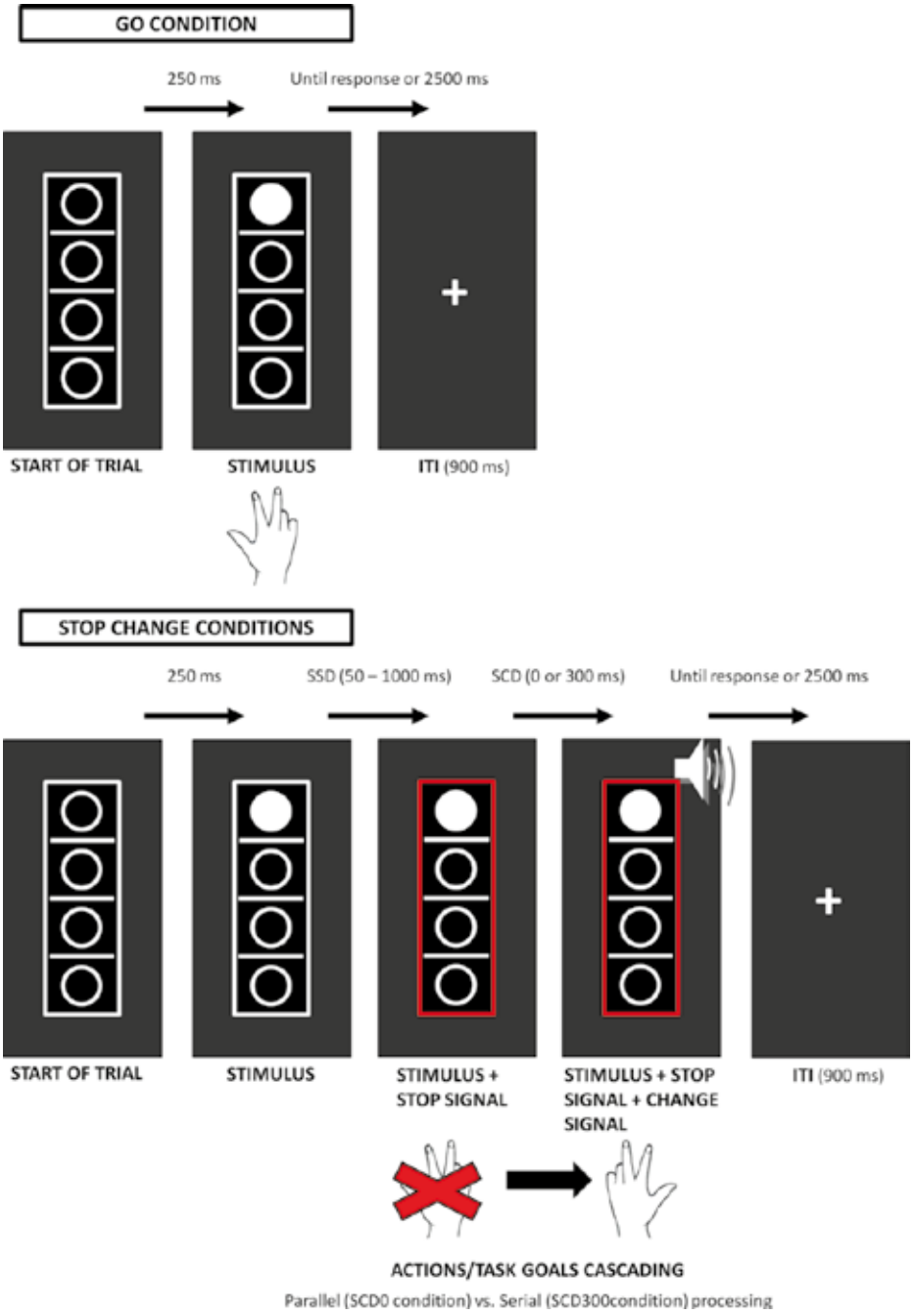

Figure 1. Schematic illustration of the stop-change paradigm. Circles indicate the four possible target locations, while the lines indicate the three possible reference lines. The red rectangle represents the STOP signal, the

presentation of which (SSD) varied according to a staircase procedure (see text, for further details). The speaker icon represents the auditory CHANGE signal, which could be high (1300 Hz), medium (900 Hz) or low (500 Hz) in pitch. The pitch of the CHANGE signal indicates the new reference line to be used to judge the location (above vs. below) of the target stimulus (i.e., the white circle). The figure illustrates the sequence of the events (from left to right) for the GO condition (above panel) and for the STOP-CHANGE conditions (below panel). Each trial starts with the presentation of the four empty circles separated by three lines, with one of the circles becoming white after 250 ms. When no STOP signal is presented (i.e., GO condition–above panel), the presentation of the white circle (i.e., GO stimulus) requires participants to execute a right-handed response to judge its position with respect to the middle reference line. GO trials end after the response to the GO stimulus. Reaction times (RTs) on GO trials reflect the efficiency of response execution. When the STOP signal is presented (i.e., SC condition–below panel), participants are instructed to withdraw their right-handed response to the GO stimulus and to execute a left-handed response instead, judging the position of the white circle with respect to the new reference line (higher, middle, lower), as indicated by the pitch of the CHANGE signal (high, medium, low). The interval between the onset of the STOP and CHANGE stimuli (i.e., stop-change delay; SCD) was set to either 0 or 300 ms to create the SCD0 and SCD300 conditions. SC trials end after the response to the CHANGE stimulus. The time required to stop a planned/ongoing response (i.e., stop-signal reaction times, SSRTs) reflects inhibitory control efficiency. Responses on SC trials require to inhibit a planned, ongoing response and to rapidly execute a different response. Successful performance on these trials relies on the ability to activate different task goals, and to cascade and prioritize different actions (Boecker et al., 2013). Therefore, RTs on these trials are indicative of the efficiency of action cascading, with shorter RTs indicating more efficient action cascading. ITI: intertrial interval; SSD: stop-signal delay; SCD: stop-change delay.

3. Results

Table 1 shows demographic information and the behavioral parameters for the stop-change paradigm separately for the AVGPs and NVGPs group. No significant between group differences were found for age, Z = -.511, p = .628, gender, $\chi 2(1, N = 36) = .643$, p = .423, or fluid intelligence (IQ), $t(34) = -.470$, p = .641. Significant group differences were observed when comparing the two groups with respect to the hours spent at playing shooter, Z = -5.429, $p < .001$, strategic, Z = -2.272, $p < .05$, and other videogames, Z = -3.001, $p < .01$. In all cases, AVGPs reported to have more experience than NVGPs (see Table 1).

Table 1. Demographic characteristics and behavioral parameters for the stop-change paradigm for AVGPs and NVGPs (Mean± SEM).

Variables	*AVGPs*	*NVGPs*
N [M:F]	18 [15:3]	18 [13:5]
Age	21.2±0.6	22.4±1.1
Fluid Intelligence	114±3.0	116±2.7
Hours per week spent playing		
First person shooter games*	9.8±1.6	0.1±0.1
Strategic games*	4.3±1.5	0.4±0.2
Other games*	7.7±1.6	2.6±1.2
STOP-CHANGE PARADIGM		
Mean stop-signal RT (SSRT)	274±12	280±18
Mean RTs on GO trials*	551±30	623±30
Mean RTs SCD 0*	1012± 63	1185±63
Mean RTs SCD 300*	823±63	1018±63

*Significant group difference; * p < 0.05.*
AVGPs: action videogame players, NVGPs: non videogame players, RT: reaction time, SSRT: stop signal reaction time, SCD: stop-change delay

RT analysis showed a main effect of trial type (GO vs. SCD0 vs. SCD300), $F(1.515,39.126) = 135.234$, $p < .001$, $\eta^2_p = .799$, MSE = 31200.879. Tukey HSD post-hoc tests showed that RTs were longer in the SCD0 condition (1098±44), as compared to the SCD300 (920±45) and the GO condition (587±21) (both $p < .001$). The latter conditions (i.e., SCD300 and GO) also differed significantly from each other, $p < .001$. Crucially, as expected, the main effect of group was significant as well, $F(1,34) = 4.746$, $p = .036$, $\eta^2_p = .122$, MSE = 122863.44, indicating that RTs in general where faster in the AVGP group (795ms) as compared to the NVGP group (942ms). Remarkably, the two-way interaction involving group and trial type was not significant, $F(1.515,39.126) = 2.124$, $p = .151$. Therefore, consistent with our expectations, AVGPs outperform NVGPs on both response execution and action cascading. Interestingly, such an improvement in action cascading was observed both when the shift to the alternate response was required to occur simultaneously to a stopping process (i.e., SCD0 condition) and when the stopping process was already finished (SCD300 condition). No differences between groups were observed with regard to the percentage of errors on the GO trials, $p = .226$.

Finally, replicating a previous finding (Colzato, van den Wildenberg, Zmigrod, & Hommel, 2013), the analysis of the SSRTs, did not reveal differences between the two groups: the distribution of SSRTs was the same across groups, $Z = -.063$, $p = .96$.

3.1. Additional analyses

Recent evidence suggests that many of the cognitive enhancements associated with AVG experience can be seen as reflecting the fact that AVG experience allows gamers to learn more quickly and effectively how to perform new tasks, rather than reflecting immediate transfer effects on new tasks (Green & Bavelier, 2015; Gozli, Bavelier, & Pratt, 2014; Bejjanki et al., 2014). Therefore, one may argue that the better performance shown by AVGPs may be due to faster learning rather than to better response execution and action cascading performance. To rule out this possibility, trials were divided into three blocks of 288 trials each. We then re-ran the RTs analysis with the inclusion of the additional within-subjects factor “block”. ANOVA confirmed the main findings, revealing significant main

effects of trial type, $F(1.148, 39.017) = 134.805$, $p < .001$, $\eta^2_p = .799$, MSE = 94109.532, and, crucially, group, $F(1,34) = 4.695$, $p = .037$, $\eta^2_p = .121$, MSE = 370540.019, but no significant interaction between the two factors, $F(1,34) = 2.116$, $p = .128$. Additionally, a significant main effect of block was found, $F(1.573,53.470) = 7.803$, $p = .002$, $\eta^2_p = .187$, MSE = 17794.567. Tukey HSD post-hoc tests showed that RTs decreased with increasing task experience (i.e., RTs were 889ms, 884ms and 831ms in block 1, block 2 and block 3, respectively). Post-hoc analyses revealed no significant difference between block 1 and block 2 ($p = .96$), whereas significant differences were observed between bock 2 and block 3 ($p < .005$), and between block 1 and block 3 ($p < .005$). More importantly, the factor block interacted neither with trial type, nor with group, Fs ≤ 2.323, *ps* ≥0.72, with the latter finding ruling out an interpretation of the observed group differences in terms of learning-related differences (see Mückshel et al., 2015 for similar findings suggesting that performance on the stop-change paradigm is not sensitive to learning effects).

4. Discussion

Research has suggested that playing AVGs can lead to improvements in perceptual (Boot, Blakely, & Simmons, 2011; Donohue, Woldorff, & Mitroff, 2010; Green, Li, & Bavelier, 2010; Green, Pouget, & Bavelier, 2010;, Buckley, Codina, Bhardwaj, & Pascalis, 2010; Appelbaum, Cain, Darling, & Mitroff, 2013; Li, Polat, Makous, & Bavelier, 2009), (visuo-)spatial (Green & Bavelier, 2006a, 2007, 2015; Spence, Yu, Feng, & Marshman, 2009; Spence & Feng, 2010; Feng, Spence, & Pratt, 2007), perceptuo-motor (Hubert-Wallander et al., 2011b; Chen et al., 2015) and attentional abilities (Green & Bavelier, 2003; West, Stevens, Pun, & Pratt, 2008; Hubert-Wallander, Green, Sugarman, & Bavelier, 2011; Chisholm & Kingstone, 2012; Chisholm, Hickey, Theeywes, & Kingstone, 2010), and that such improvements can also extend to cognitive control functions such as cognitive flexibility (Boot, Blakely, & Simmons, 2011; Cain, Landau, & Shimamura, 2012; Colzato, van Leeuwen, van den Wildenberg, & Hommel, 2010; Colzato, van den Wildenberg, & Hommel, 2014; Green, Sugarman, Medford, Klobusicky, & Bavelier, 2012; Karle, Watter, & Shedden, 2010; Strobach, Frensch, & Schubert, 2012; Andrews & Murphy, 2006) and WM updating (Colzato, van

den Wildenberg, Zmigrod, & Hommel, 2013), but not inhibitory control (Colzato, van den Wildenberg, Zmigrod, & Hommel, 2013; Miyake et al., 2000). The present study aimed to extend previous findings by determining the potential effect of FPS AVG playing experience on action cascading, which encompasses cognitive control processes such as task goal manipulation and action selection in multitasking contexts (Boecker, Gauggel, & Drueke, 2013; Verbruggen, Schneider, & Logan, 2008). To this end, AVGPs and NVGPs were confronted with a stop-change paradigm–a well-established diagnostic index of action cascading efficiency (Verbruggen, Schneider, & Logan, 2008). Interestingly, besides providing an index of action cascading efficiency, performance on this task gives additional information on the efficiency of response execution and inhibitory control, thereby providing us with the opportunity to confirm (or disconfirm) previous observations (Colzato, van den Wildenberg, Zmigrod, & Hommel, 2013; Miyake et al., 2000) while simultaneously extending them. Results showed that AVGPs outperformed NVGPs in action cascading efficiency. Indeed, as compared to NVGPs, AVGPs were found to be faster in switching to an alternative response, regardless of whether this shift was required to occur simultaneously to a stopping process (i.e., SCD0 condition–i.e., parallel processing) or when the stopping process had already finished (SCD300 condition–i.e., serial processing). Therefore, the present findings provide support for the idea that FPS AVG playing experience is likely to be associated with a more efficient ability in selecting and applying different action control strategies depending on the task demands. To some extent, this finding is not surprising if one considers that playing FPS AVGs explicitly requires the players to be able to rapidly and flexibly adapt their behavior to the ever-changing context such that, very often, planned actions need to be withheld and rapidly replaced by others–an ability that the current findings suggest can transfer to cognitive tasks tapping similar skills. Our observations fit with previous reports that have associated AVG practice with enhanced cognitive flexibility, as indexed by performance on a wide range of task-switching paradigms (Boot, Blakely, & Simmons, 2011; Cain, Landau, & Shimamura, 2012; Colzato, van Leeuwen, van den Wildenberg, & Hommel, 2010; Colzato, van den Wildenberg, & Hommel, 2014; Green, Sugarman, Medford, Klobusicky, & Bavelier, 2012;

Karle, Watter, & Shedden, 2010; Strobach, Frensch, & Schubert, 2012; Andrews & Murphy, 2006) and WM updating, as indexed by performance on the 2-back task (Colzato, van den Wildenberg, Zmigrod, & Hommel, 2013).

Importantly, in the present study, we also replicated previous observations suggesting that AVG experience is associated with higher efficiency in response execution, but does not affect inhibitory control. Indeed, consistent with a previous study using the stop-signal task (Colzato, van den Wildenberg, Zmigrod, & Hommel, 2013), AVGPs showed faster RTs to GO signals, but were comparable to NVGPs in terms of SSRTs. As mentioned in the Introduction, the converging observations that AVGPs show comparable performance to NVGPs with respect to inhibitory performance in different paradigms (Colzato, van den Wildenberg, Zmigrod, & Hommel, 2013; Miyake et al., 2000) has a twofold importance. On the one hand, the fact that AVGPs are not better than NVGPs in inhibitory control means that the potential beneficial effects associated with gaming experience do not transfer to all cognitive functions. It would be valuable for future studies to shed light on why this is the case. On the other hand, the fact that AVGPs are not worse than NVGPs in inhibitory control do not provide any empirical support for the claim, often seen in the media, that AVGPs are more impulsive, antisocial, or aggressive than non-gamers (but see Ferguson, 2011). Indeed, our findings, along with previous ones (Colzato, van den Wildenberg, Zmigrod, & Hommel, 2013; Miyake et al., 2000), show that AVGPs do not show any dysfunctional impulsivity or impairment in response inhibition, as compared to NVGPs.

The current study has some limitations that warrant discussion. First and foremost, we acknowledge that no causal relation can be drawn between the observed between-groups differences and FPS AVG playing experience. Indeed, our investigation was restricted to how a history of video game experience is associated with action cascading processes, rendering our study correlational in nature–a methodological shortcoming common to most studies reporting gaming effects (for an extensive discussion of this issue, see (Boot, Blakely, & Simmons, 2011; Kristjánsson, 2013). Therefore, one cannot rule out that the differences we found in the stop-change task are actually due to innate differences between the

groups, such as pre-existing neuro-developmental factors and/or a particular pre-gaming learning experience, rather than due to gaming exposure. For instance, individuals with a genetic predisposition associated with better executive control functions might be drawn to video games more strongly, meaning that an effect of experience might actually represent a form of self-selection (Colzato, van Leeuwen, van den Wildenberg, & Hommel, 2010). Interestingly enough, this possibility is in line with other findings showing that cognitive skills are significant predictors of gaming performance in FPS AVGs (Sherry et al., 2006; Bowman et al., 2013; Sherry & Bowman, 2015). Likewise, it seems that gender differences in cognitive skills may be causally linked with the decision to play specific genres of games rather than others, which may explain why males mostly prefer action, shooter, sports, and fighter games, whereas females typically prefer puzzle, card and educational games (Sherry, Rosaen, Bowman, & Huh, 2006; Bowman, Weber, Tamborini, & Sherry, 2013; Sherry & Bowman, 2015). The fact that the two groups did not differ in terms of age, gender, and fluid intelligence allows us to at least exclude the potential confounding influence of these variables. Among these factors, age is probably of particular importance, as a previous study has shown that action cascading performance declines with increasing age (Stock, Gohil, & Beste, 2015). Furthermore, as expected, we found no group differences in terms of fluid intelligence and inhibitory control, replicating previous studies that found no association between these factors and gaming experience (Cain, Landau, & Shimamura, 2012; Colzato, van Leeuwen, van den Wildenberg, & Hommel, 2010; Colzato, van den Wildenberg, Zmigrod, & Hommel, 2013; Pohl et al., 2014). Nevertheless, our study remains correlational in nature and therefore it is crucial for future studies to examine the possible causal nature of our observed group differences. To this end, it is highly advisable to carry out training studies wherein action cascading performance of NVGPs is trained via AVG and thereafter compared with that of NVGPs trained in with control intervention. Perhaps better, longitudinal studies could assess whether and to what extent pre-existing differences between AVGPs and NVGPs may account for the reported effects on cognitive performance, thereby providing this field of research with higher external validity. Lastly, it would

also be interesting to assess whether individual differences in action cascading performance can predict gaming performance, as shown in Sherry, Rosaen, Bowman, and Huh, (2006), Bowman, Weber, Tamborini, and Sherry, (2013), and Sherry and Bowman (2015).

Second, the fact that participants were aware of participating in a study on the effects of playing videogames on cognitive performance– another main methodological shortcoming in this field of research (Boot, Blakely, & Simmons, 2011; Kristjánsson, 2013) – leads to the possibility that the between-groups differences in action cascading performance might have been driven by specific expectations and motivational factors. In other words, one may argue that AVGPs outperformed NVGPs to conform to the expectations wrought by their group membership and/or because they were more motivated to perform well. However, as argued elsewhere (Green, Strobach, & Schubert, 2014; Schubert & Strobach, 2012), for such expectancies-driven effects to occur, participants have to be aware of the specific hypotheses under investigation and of how such hypotheses would translate to the data. Furthermore, this criticism neglects that fact NVGPs may be likewise motivated to perform better than AVGPs. In any case, the fact that the two groups differed only in specific skills such as response execution and action cascading, but not in inhibitory control and fluid intelligence, undermines an interpretation of our results in these terms. Notably, the lack of any AVGP/NVGP difference in tasks tapping inhibitory control and fluid intelligence is consistent with results from a previous study in which a completely covert recruitment strategy was used (Colzato, van den Wildenberg, Zmigrod, & Hommel, 2013). Nevertheless, it would be informative for follow-up studies to replicate our findings in a context in which participants are totally blind to the nature of the study.

A third limitation of the present study is the small sample size, although comparable to that of other studies e.g., (Chisholm, Hickey, Theeywes, & Kingstone, 2010; Donohue, Woldorff, & Mitroff, 2010; Colzato, van Leeuwen, van den Wildenberg, & Hommel, 2010; Green, Sugarman, Medford, Klobusikicy, & Bavelier, 2012, Bejjanki et al., 2014; Mishra, Zinni, Bavelier, & Hillyard, 2011), including mostly male participants. Therefore, more studies are needed in order to verify the reliability and repeatability of our findings in larger samples, possibly balanced for gender. However, it

is important to note that the possibility to test samples balanced for gender is limited by the fact that males are much more likely than females to report playing AVGs (see Sherry, Rosaen, Bowman, & Huh, 2006; Bowman, Weber, Tamborini, & Sherry, 2013; Sherry & Bowman, 2015; Rogers, Bowman, & Oliver, 2015 and above for a possible explanation of why this is the case) and, consequently, there do not seem to be enough females with AVG expertise to allow for gender-balanced groups. This is why in previous cross-sectional groups studies recruitment was restricted to male participants (Donohue, Woldorff, & Mitroff, 2010; Chisholm, Hickey, Theeywes, & Kingstone, 2010; Green, Sugarman, Medford, Klobusikicy, & Bavelier, 2012; Bejjanki et al., 2014). In the present study, we decided to include female participants as well, given that a previous study using the same task has revealed no gender-related differences in action cascading performance (Stock, Gohil, & Beste, 2015). Importantly, even though it is difficult to say whether and to which degree our findings might generalize to female players, the imbalance with respect to gender cannot account for the observed group differences, as the two groups were matched for gender. Again, training studies would be preferable, as they would overcome this limitation as well.

Fourth, in the present study we restricted our hypotheses to AVGPs who played FPS games. This is because it has been suggested that it is in particular the first person perspective (as in the FPS games) that allows for cognitive-control improvements (Colzato, van Leeuwen, van den Wildenberg, & Hommel, 2010). Indeed, success in FPS games requires high levels of action control and a flexible mindset to rapidly react to moving visual and sudden acoustic events, and to switch back and forth between different subtasks, both in a serial and in a parallel manner. However, a more systematic investigation is needed to verify this hypothesis. Specifically, for such a hypothesis to be supported follow-up studies may consider to compare action cascading performance between FPS AVGPs and AVGPs who mainly play third-person shooter games and/or other types of AVGs. Related to the this point, it is important to mention that our sample of AVGPs also had more experience with several other types of game genres including strategic, role-playing, puzzle and sports games. Although this is consistent with previous studies (e.g. Donohue, Woldorff, &

Mitroff, 2010; Cain, Landau, & Shimamura, 2012), it is difficult to ascertain whether the better action cascading performance shown by our AVGPs is specifically due to playing specifically FPS AVGs, other games, or a combination of them. Due to such potential within-group variance, it is possible that our study was underpowered and, therefore, led to an underestimated, relatively small effect size that might seem of little clinical significance. Future studies should aim to decrease within-group variance as much as possible.

A final limitation pertains to the fact that our conclusion that playing AVG is associated with improved action cascading performance relies on participants' performance on a single task. To obtain more reliable results, it would be ideal for future studies to confront AVGPs and NVGPs with different tasks that are reckoned to assess action cascading/multitasking performance, as validated, for instance, by confirmatory statistical analyses (e.g., confirmatory factor analysis and/or structural equation modelling analyses; see Miyake et al., 2000 for an example of application of these methods).

In sum, our findings are promising in suggesting that playing AVG can be associated with enhanced action cascading performance, i.e. more efficient goal-directed multi-component behavior. As such, our findings may represent an important first step in stimulating further research to assess whether videogames can be used to optimize cognitive control. Importantly, given the importance of action control in daily activities and the known difficulties shown by older adults in response selection and action cascading processes (Stock, Gohil, & Beste, 2015; Chmielewski, Yildiz, & Beste, 2014; Verhaegen & Cerella, 2002; Verhaegen, Borchelt, & Smith, 2003), our findings can have important practical implications for designing intervention/training studies aimed at overcoming or slowing down action control deficits associated with aging.

Chapter five

γ-Aminobutyric acid (GABA) administration improves action selection processes: a randomized controlled trial

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Abstract

In order to accomplish a task goal, real-life environments require us to develop different action control strategies in order to rapidly react to fast-moving visual and auditory stimuli. When engaging in complex scenarios, it is essential to priorities and cascade different actions. Recent studies have pointed to an important role of the gamma-aminobutyric acid (GABA)-ergic system in the neuromodulation of action cascading. In this study we assessed the specific causal role of the GABA-ergic system in modulating the efficiency of action cascading by administering 800 mg of synthetic GABA or 800 mg oral of microcrystalline cellulose (placebo). In a double-blind, randomized, between-group design, 30 healthy adults performed a stop-change paradigm. Results showed that the administration of GABA, compared to placebo, increased action selection when an interruption (stop) and a change towards an alternative response were required simultaneously, and when such a change had to occur after the completion of the stop process. These findings, involving the systemic administration of synthetic GABA, provide the first evidence for a possible causal role of the GABA-ergic system in modulating performance in action cascading.

1. Introduction

In order to accomplish a task goal, real-life environments require us to develop different action control strategies in order to rapidly react to fast-moving visual and auditory stimuli. When engaging in complex scenarios, it is essential to priorities and cascade different actions (Mückschel, Stock, & Beste, 2014). Cascading these actions and therefore selecting the appropriate one can be done in either a more serial, step-by-step manner (i.e. a task goal is activated after the previous one has been accomplished or stopped) or in a more parallel, overlapping manner (i.e. a task goal is activated while the previous one is still active), depending on the actions to be carried out (Verbruggen, Schneider, & Logan, 2008; Stock, Arning, Epplen, & Beste, 2014). The general consensus is that action cascading processes rely on fronto-striatal networks (Humphries, Stewart, Gurney, 2006; Bar-Gad, Morris, & Bergman, 2003; Redgrave, Prescott, & Gurney, 1999; Beste, Dziobek, Hielscher, Willemssen, & Falkenstein, 2009; Beste et al., 2012; Ravizza, Goudreau, Delgado, & Ruiz, 2012; Cameron, Watanabe, Pari, & Munoz, 2010; Willemssen, Falkenstein, Schwarz, Müller, & Beste, 2011). Within these networks, gamma aminobutyric acid (GABA) – one of the main inhibitory neurotransmitters – is likely to play an important role in the neuromodulation of action control processes (Humphries, Stewart, Gurney, 2006; Bar-Gad, Morris, & Bergman, 2003; Plenz, 2003). GABA plays a pivotal role in information encoding and behavioral control (Adler, Finkes, Katabi, Prut, & Bergman, 2013), in the regulation of motor functions (Chase & Taminga, 1979; Will, Toniolo, & Brailowsky, 1988; Boy et al., 2010), and in motor learning (Stagg, Bachtiar, & Johansen-Berg, 2011; Floyer-Lea, Wylezinska, Kincses, & Matthews, 2006). More importantly, GABA also seems involved in action selection (Bar-Gad, Morris, & Bergman, 2003) and response inhibition processes occurring in the frontal-striatal networks (Bari & Robbins, 2013; Quetscher et al., 2015).

Given the aforementioned link between GABA and action selection and inhibition, it is reasonable to expect GABA levels to determine the efficacy of action cascading processes. Consistent with this

hypothesis, Yildiz and colleagues (2014) have shown, using magnetic resonance spectroscopy (MRS), that superior performance in action cascading was associated with increased concentrations of striatal GABA. Second, active transcutaneous vagus nerve stimulation (tVNS), which increases GABA and norepinephrine (NE) concentrations in the brain, improved response selection functions during action cascading, compared to sham stimulation (Steenbergen et al., 2015). In contrast, Stock, Blaszkewicz, and Beste (2014) showed that high-dosage alcohol, an unselective GABA-ergic agent (Ticku, 1990), impaired action selection. Taken together, these findings indicate a critical role of GABA in the neuromodulation of action cascading processes and suggest that increased (Yildiz et al., 2014; Steenbergen et al., 2015), but not too high (Stock, Blaszkewics, & Beste, 2014), levels of GABA are associated with better action cascading performance. Yet, because of the correlational nature of MRS studies and the unselective action of tVNS and alcohol on the GABA-ergic system, evidence supporting the possible role of GABA in mediating action cascading is still rather elusive and requires further validation.

The present study aims to provide converging and direct evidence to verify the possible pivotal role of the GABA-ergic system in modulating the efficiency of action cascading. To this end subjects were administered 800 mg of synthetic GABA (Haig et al., 2001; Rizzo et al., 2001) or 800 mg oral of microcrystalline cellulose (placebo). In the literature, there are controversial findings about GABA entering the brain through the blood brain barrier (BBB). The BBB is a tightly sealed layer of cerebral endothelial cells that form continuous tight junctions and prevent most solutes from entering the brain on the basis of size, charge, and lipid solubility. However, as pointed out by Shyamaladevi and colleagues (2002), recent studies have demonstrated that the BBB is much more dynamic than assumed in the past, and some passage of solutes can occur by transcytosis, carrier-mediated transport, or simple diffusion of hydrophobic substances. While there is some evidence in favor of only a limited penetration of GABA into the brain (Knudsen, Poulsen, & Paulson, 1988; Bassett, Mullen, Scholz, Fenstermacher, & Jones, 1990), a more recent study with rats has shown that the administration of GABA alone

increased brain GABA concentration, when compared to untreated rats (Shyamaladevi, Jayakumar, Sujatha, Paul, & Subramanian, 2002). In addition, the synthetic GABA-like agent gabapentin, which mimics the chemical structure of GABA, leads to an overall increase in central GABA levels (Errante, Williamson, Spencer, & Petroff, 2002) and a recent study using 7-T MRS reported an increase in GABA concentration in the visual cortex of healthy participants after gabapentin administration (Cai et al., 2012).

In the present study, action cascading was assessed by means of a well-established stop-change paradigm (Verbruggen, Schneider, & Logan., 2008), in which participants are required to stop an ongoing response to a GO stimulus whenever an occasional STOP stimulus is presented. The STOP stimulus is followed by a CHANGE stimulus, signaling participants to shift to an alternative response. Crucially, the interval between the STOP and the CHANGE stimulus (stop-change delay; SCD) hence, the time of the preparation process before the execution of the change response, is manipulated in such a way that the two stimuli occur either simultaneously (0 ms; i.e., SCD 0) or with a short delay (300 ms; i.e., SCD 300; for more details, see Method section and Figure 1; Mückschel et al., 2014). While reaction times (RTs) to the GO stimuli are assumed to reflect the efficiency of response execution, RTs on stop-change trials can be taken to reflect the efficiency of action cascading, with shorter RTs reflecting a more efficient action selection. Based on previous findings (Bar-Gad, Morris, & Bergman, 2003; Redgrave, Prescott, & Gurney, 1999; Bari & Robbins, 2013; Quetscher et al., 2014; Yildiz et al., 2014; Steenbergen et al., 2015), we expected the administration of synthetic GABA to enhance action cascading processes (i.e. to decrease RTs on the change trials) when (a) an interruption (stop) of the current response and a change towards an alternative response are required simultaneously (SCD0), and when (b) the change to the alternative response is required when the stopping process has already finished (SCD300). In contrast, GABA is not expected to affect the efficiency of response execution, as reflected by RTs to the GO stimuli. Aside from providing a measure of action cascading efficiency, the stop-change paradigm also allows an assessment of the efficiency of inhibitory

control, as indexed by the stop signal reaction time (SSRT), i.e., the time required to stop an ongoing response (Lowan, 1984; Logan, 1994). Typically, longer SSRTs reflect slower inhibitory processes and indicate a lower level of inhibitory efficiency. As previous studies have suggested that higher GABA levels are associated with more efficient response inhibition processes (Boy et al., 2010; Quetscher et al., 2014; Groenewegen, 2003; Draper et al., 2014), we also expected the administration of synthetic GABA to reduce the latency of the stop process.

Given that increases in GABA levels have been found to improve mood (Steeter et al., 2010; Brambilla, Perez, Barale, Schettini, & Soares, 2003) and current mood-state is reckoned to affect cognitive-control processes (Schuch & Koch, 2014; van Steenbergen, Band, & Hommel, 2010), we also assessed participants' subjective affective states, before and 30 minutes after the intake of GABA, as well as at the end of the task. To this end, we used the affect grid (Russel, Weiss, & Mendelsohn, 1989), a single-item scale requiring participants to rate their mood on a 9 × 9 grid, where the horizontal axis stands for affective valence (from –4 to 4; unpleasantness to pleasantness), and the vertical axis for perceived activation (from –4 to 4; sleepiness to high arousal). Moreover, animal studies have suggested that GABA-ergic modulations can have an impact on the cardiovascular system (Zhang & Mifflin, 2010). Although it is unlikely that small doses of GABA, as provided in the present study, can significantly alter cardiovascular functions, alongside the mood assessments we also monitored participants' heart rate (HR), systolic (SBP) and diastolic blood pressure (DBP).

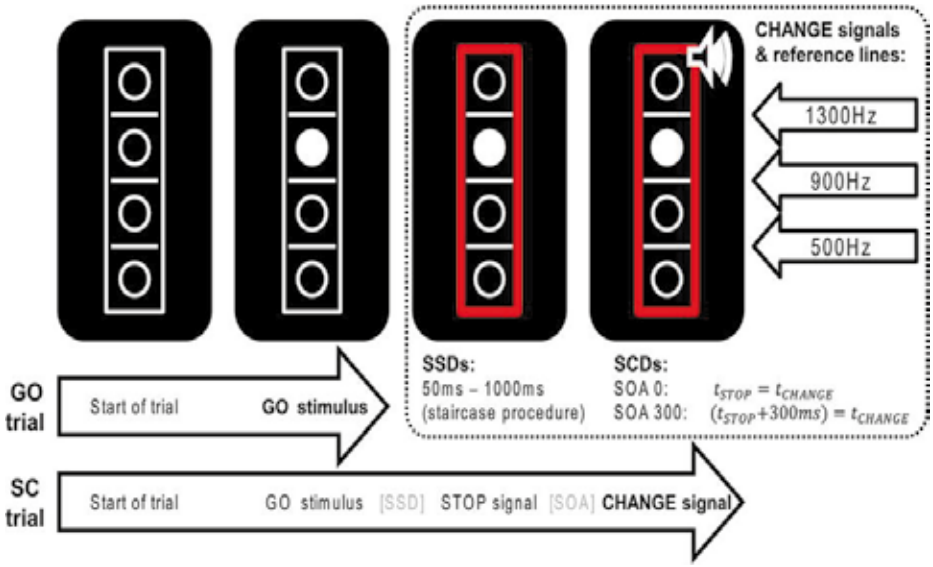

Figure 1. Schematic illustration of the stop-change paradigm. GO trials end after the first response to the GO stimulus (bold). In contrast, Stop-Change trials end after the first response to the CHANGE signal (bold). The stop-signal delay (SSD) between the onset of the GO stimulus and the STOP signal was adjusted using a staircase procedure described in the Method section. The stimulus onset asynchrony (SOA) between the onset of the STOP and CHANGE stimuli was set to either 0 or 300 ms. As indicated in the upper right corner, the three CHANGE stimuli were associated with one of the three reference lines.

2. Method

2.1. Participants

Thirty undergraduate students of the Leiden University (29 females, 1 male, mean age = 19.5 years, range 18–22) participated in the experiment. Participants were recruited via an on-line recruiting system and offered course credits for participating in a behavioral pharmacological study. Participants were screened individually via a phone interview by the same lab-assistant using the Mini International Neuropsychiatric Interview (M.I.N.I.). The M.I.N.I. is a short, structured interview of about 15 minutes that screens for several psychiatric disorders and drug use. The M.I.N.I. is often used in clinical and pharmacological research (Sheehan et al., 1998; Colzato & Hommel., 2008; Colzato, Ruiz, van den Wildenberg, & Hommel, 2011). Participants without cardiac, hepatic, renal, neurological or psychiatric disorders, personal or family history of depression, migraine and medication or drug use were considered suitable to participate in this study. Written informed consent was obtained from all participants, all experimental protocols and remuneration arrangements of course credits were approved by the local ethical committee (Leiden University, Institute for Psychological Research). The methods were carried out in accordance with the approved guidelines.

A double-blind, randomized, between-group design was used. After signing the informed consent, participants were administered an oral dose (powder) of 800 mg of synthetic GABA in the GABA group or 800 mg of microcrystalline cellulose in the placebo group. An independent person not further involved in this study prepared a list that coded for participants to receive either placebo or GABA, and the matching treatment tubes containing either placebo or GABA. Hence, participants were randomly assigned to one of the two experimental groups: placebo (N = 15; mean age = 19.3, SD = 1.1; mean Body Mass Index = 21.6, SD = 1.9), or GABA (N = 15; 1 male; mean age = 19.8, SD = 1.2; mean Body Mass Index = 20.9, SD = 1.3). Both synthetic GABA and placebo were dissolved in 200 ml of orange juice. Following Markus and colleagues (2008) and Colzato, Jongkees, Sellaro, and Hommel

(2013), only women currently using contraception were tested. Participants arrived at the laboratory at 9:30 a.m. and 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. Thirty minutes after the administration of either synthetic GABA or the neutral placebo participants were allowed to eat an apple.

2.2. Apparatus and procedure

All participants were tested individually. Upon arrival, participants were asked to rate their mood on a 9 × 9 Pleasure × Arousal grid (Russel, Weiss, & Mendelsohn, 1989) with values ranging from –4 to 4. Heart rate (HR) and systolic and diastolic blood pressure (SBP and DBP) were collected from the non-dominant arm with an OSZ 3 Automatic Digital Electronic Wrist Blood Pressure Monitor (Spiedel & Keller). Thirty minutes following the administration of synthetic GABA (corresponding to the peak of the plasma concentration, which remains stable until 60 minutes after administration; Abdou et al., 2006) or placebo, participants again rated their mood before having HR, SBP and DBP measured for the second time. Immediately after, participants started with the practice procedure of the stop-change paradigm, which took about 20 minutes. After completing the practice, participants performed the task, which took about 25 minutes. Upon completion, participants again rated their mood before having their HR, SBP and DBP measured for the third time.

2.2.1. Stop-Change paradigm

The experiment was presented on an LG Flatron 776FM 16 inch monitor (refresh rate of 60 Hz), controlled by an Asus laptop running on an Intel Core i3-3217U processor. Presentation software (Neurobehavioral Systems, Inc., Berkeley, CA) was used for stimulus presentation and data collection. The stop-change (SC) paradigm was adapted from Yildiz, Wolf, and Beste (2014), and Dippel and Beste (2015), see Figure 1.

Each trial consisted of the presentation of a white rectangle (on a black background) of 55 × 16 mm in the center of the screen. Within this rectangle, three horizontal reference lines (line thickness 1 mm, width 13 mm) separated four vertically aligned circles (diameter 7 mm). 250 ms after the onset of each trial, one of the circles was filled white, as such becoming the GO target stimulus. Sixty-seven percent of all trials were GO trials, which constituted the GO condition. In this condition, participants were expected to indicate, with their right index and middle finger, whether the target was located above or below the middle horizontal reference line. If the target was located above the middle reference line, participants were supposed to press the outer right key using the right middle finger ("above" judgment). If the target was located below the middle horizontal reference line, participants were supposed to press the inner right key with the right index finger ("below" judgment). All stimuli remained visible until the participant responded. In case of RTs longer than 1000ms, a "Quicker!" sign would appear above the rectangle until the participant responded.

Besides GO trials the task also included stop-change (SC) trials, which constituted the remaining 33% of the trials. Like a GO trial, a SC trial began with the presentation of a white rectangle with 4 vertically aligned circles separated by 3 horizontal reference lines. Again, 250 ms after the onset of the trial, one of the circles would turn white. For this condition however, a STOP signal (a red rectangle replacing the previous white frame) was presented after a variable stop signal delay (SSD). This STOP signal requested participants to try to inhibit the right-handed response to the GO stimulus and remained on the screen until the end of the complete trial. The SSD was initially set to 250 ms and was adapted to each participant's performance by means of a staircase procedure. This procedure yields a 50% probability of successfully inhibiting the GO response. In case of a completely correct SC trial (no response to GO stimulus, no response prior to the CHANGE stimulus in the SCD300 condition (explained below) and a correct left hand response to the CHANGE stimulus), the SSD of the following SC trial was adjusted by adding 50 ms to the SSD of the current trial. In case of an incorrect response to a SC trial (if any of the above criteria were not met), the SSD

was adjusted by subtracting 50 ms from the SSD of the current trial. SSD values were set not to exceed a value of 1000 ms or to fall below a value of 50 ms. Stop-signal reaction times (SSRTs), which index the duration of the stop process, were calculated by subtracting the mean SSD from the mean RT on GO trials (Verbruggen et al., 2008; Cai et al., 2012).

Irrespective of successfully or unsuccessfully inhibiting the GO response, every stop signal was combined with one of three possible CHANGE stimuli. The CHANGE stimuli consisted of a 100 ms sine tone presented through headphones at 75 dB SPL. This tone could be high (1300 Hz), medium (900 Hz) or low (500 Hz) in pitch, and indicated which of the horizontal lines need to be used as a reference line for this trial. That is, the high tone represented the highest of the three lines as the new reference, the medium tone represented the middle line and the low tone represented the lowest line (see Figure 1). All three reference lines were used with equal frequency. Participants were required to make the appropriate CHANGE response with index or middle finger of the left hand. The left middle finger had to be used to press the outermost left key, and the left index finger for the innermost left key. Which button the participant had to press depended on the location of the white circle and the tone presented. In case the target was located above the newly assigned reference line, an outer left key press (left middle finger; above judgement) was required; in case the target circle was located below the newly assigned reference line, a left inner key press (left index finger; below judgement) was required. RTs for the stop-change trials were measured from the onset of the CHANGE stimulus. In the case of a RT-SCD longer than 2000 ms, a "Quicker" sign was presented above the rectangle until the participant responded. Notably, half of the trials in the SC condition, consisted of a STOP signal and a CHANGE stimulus being presented simultaneously (stimulus onset asynchrony (SOA) of 0 ms, SCD0), whereas in the other half of the trials, there was a stop change delay (SCD) with a SOA of 300 ms (SCD300 condition) between the STOP and CHANGE stimuli. In total, 864 trials were administered in the task (576 GO, 144 SCD0 and 144 SCD300), which took the participants approximately 25 minutes to finish.

2.3. Statistical Analyses

Mood (pleasure and arousal), HR, DBP and SBP were analyzed separately by means of repeated-measures analyses of variance (ANOVAs) with treatment group (GABA vs. placebo) as between-subjects factor and effect of time (first vs. second vs. third measurement) as within-subjects factor. To assess the effect of GABA on action cascading, correct reaction times (RTs) were submitted to separate repeated-measures ANOVAs with condition (GO, SCD0, SCD300) as within-subject factor and treatment group (GABA vs. placebo) as between-subject factor. Greenhouse—Geisser correction was applied when the sphericity assumption was violated. The corrected degrees of freedom are reported along with the corrected test values. All post-hoc tests were Bonferroni-corrected. Kolmogorov–Smirnov tests indicated that all variables subsequently tested with t-tests were normally distributed (i.e. BMI, SSRTs and the error percentage for the GO trials), all $z < 0.22$; $p > 0.06$. A significance level of $p < 0.05$ was adopted for all statistical tests.

3. Results

Groups did not differ in terms of age, $p = .187$, as indicated by the non-parametric independent samples Mann-Whitney U test, nor BMI, $t(28) = 1.19$, $p = .245$. Table 1 shows the behavioral parameters for the stop-change paradigm separately for the GABA and placebo group.

Table 1. Behavioral parameters for GABA and Placebo groups (mean ±SEM).

	GABA	Placebo
SSRT**	236±17	316±17
RT GO	611±38	613±38
RT SCD 0**	991±68	1283±68
RT SCD 300**	816±71	1104±71

Significant difference between the two conditions; **p<0.05

For the RTs analysis, a repeated-measures ANOVA using the within-subjects factor "condition" (GO, SCD0, SCD300) and the between-subjects factor "treatment group" (GABA vs. placebo) yielded a main effect of treatment group, $F(1,28) = 7.36$, $p = .011$, $\eta^2_p = .21$, indicating that RTs where faster in the GABA group (806 ms) as compared to the placebo group (1000 ms). There was also a main effect of condition, $F(1.075,30.108) = 82.25$, $p < .001$, $\eta^2_p = .75$. Post-hoc tests showed that RTs were longer in the SCD0 condition (1137 ms ± 48), compared to the SCD300 (960 ms ± 50) and the GO condition (612 ms ± 27) (both $p < .001$). The latter conditions (i.e., SCD300 and GO) differed from each other too, $p < .001$. Most importantly, the interaction involving condition and treatment group was significant, $F(1.075, 30.108) = 7.96$; $p = .007$, $\eta^2_p = .22$. Post-hoc tests revealed a difference in RTs between treatment groups in the SCD0 condition, $p = .02$, and in the SCD300 condition, $p = .02$, but not in the GO condition, $p = .99$. Specifically, for the SCD0 and the SCD300 conditions, the GABA group revealed faster RTs (SCD0 991 ms ± 68; SCD300 816 ms ± 71) than the placebo group (SCD0 1283 ms ± 68; SCD300 1104 ms ± 71).

In the SCD0 and SCD300 conditions errors rates are mainly determined by a staircase procedure and, thus, are artificially fixed at approximately 50% (Verbruggen et al., 2008). For this reason, only error rates in the GO condition were analyzed. The analysis revealed no group effect, $t(28) = 1.49$, $p = .148$. The analysis of the SSRT (Verbruggen et al., 2008) revealed a significant difference between the placebo and GABA groups, $t(28) = 3.32$, $p = .003$. The mean SSRT was longer in the placebo (316 ms ± 16.9) compared to the GABA group (236 ms ± 16.9).

Table 2 provides an overview of the outcomes for physiological and mood measurements. ANOVAs showed a main effect of time only for arousal, $F(1.430,40.044) = 13.42$, $p < .001$, $\eta^2_p = .32$, and HR, $F(1.499,41.902) = 23.91$, $p < .001$, $\eta^2_p = .46$, indicating that arousal levels increased (-0.4 vs. 0.9 vs. 0.9), whereas heart rate decreased during the experiment (78 vs. 71 vs. 67). However, HR, SBP, DBP, pleasure and arousal, did not differ significantly between conditions, and did not show any interaction between condition and time, $Fs \leq 2.8$, $ps \geq .09$. This

suggests we can rule out an account of our results in terms of physiological and mood changes.

Table 2. Mean heart rate values (in beats per minute), systolic (SBP) and diastolic (DBP) blood pressure (in mmHg), and mood and arousal scores as function of effect of time (first (T1) vs. second (T2) vs. third (T3) measurement) for GABA and Placebo groups. Mean±standard error of the mean.

	T1		T2		T3	
	GABA	Placebo	GABA	Placebo	GABA	Placebo
Heart rate	74±4	82±4	68±2	74±2	66±2	67±2
SBP	116±4	118±4	115±4	117±4	109±3	119±3
DBP	72±3	71±3	71±3	74±3	69±2	72±2
Arousal	-0.3±0.3	-0.5±0.3	0.9±0.3	0.9±0.3	0.9±0.4	0.9±0.4
Pleasure	1.3±0.2	1.5±0.2	1.5±0.3	1.6±0.3	1.3±0.3	0.9±0.3

4. Discussion

Our results suggest that systemic administration of synthetic GABA directly influences the efficiency of action cascading as measured by a stop-change paradigm - a well-established diagnostic index of action cascading efficiency (Verbruggen, Schneider, & Logan, 2008). Indeed, we observed that the administration of a low dose of synthetic GABA reduced the time needed to change to an alternative response, regardless of whether this shift was required to occur simultaneously to a stopping process (i.e., SCD0 condition) or when the stopping process had already finished (SCD300 condition). Therefore, the present finding offers substantial support for the idea of a crucial role of the GABA-ergic system in action cascading (Humphries, Stewart, Gurney, 2006; Plenz, 2003; Bar-Gad, Morris, & Bergman, 2003; Redgrave, Prescott, & Gurney, 1999; Yildiz et al., 2014).

In the present study, we also found that synthetic GABA administration affects the efficiency to stop an ongoing response, as

indexed by the SSRTs, but not the efficiency of response execution, as reflected by the null effect on the GO-trials. Therefore, our outcome is consistent with, and further supports, previous findings suggesting that response inhibition processes are modulated by the GABA-ergic system (Boy et al., 2010; Quetscher et al., 2014; Groenewegen, 2003; Draper et al., 2014). In addition, the lack of any group difference in responding to the GO trials demonstrates the specific importance of synthetic GABA for stop-change processes, as opposed to (easy) automatic responding processes. This is in line with the idea that the GABA-ergic system plays a crucial and specific role in the selection of and the coordination between different actions by suppressing competing response options (Bar-Gad, Morris, & Bergman, 2003; Redgrave, Prescott, & Gurney, 1999).

It is worth mentioning that our findings that increases in GABA levels lead to improved action cascading and to shorter SSRTs seem at odds with the results of a recent study showing that high dosage of the GABA-ergic agent alcohol impairs action cascading and significantly increases SSRTs (Stock, Blaszkewicz, & Beste, 2014). This inconsistency might be explained by speculating that GABA may relate to cognitive performance through an inverted U-shaped function: while moderate increases in GABA levels lead to an enhancement of action cascading and to more efficient inhibitory control, large increases in GABA level cause impairments, just like very low levels (possibly) do. Follow-up studies comparing the effects of different GABA dosages are needed to verify this hypothesis. Moreover, to further support the causal role of the GABA-ergic system in mediating action cascading processes, future studies may consider to test patient populations suffering from disorders of the GABA-ergic system. For instance, we predict epilepsy patients, who suffer from an abnormal reduction of GABA-ergic function (Shyamaladevi, Jayakumar, Sujatha, Paul, & Subramanian, 2002), to show inferior performance in action cascading compared to matched controls.

An important limitation of the present study is the small sample size, including predominantly female participants. Therefore, further studies are needed in order to verify the reliability and repeatability of our findings in larger samples that are balanced for gender.

In sum, our findings on the systemic administration of synthetic GABA provide straightforward evidence for a possible causal role of the GABA-ergic system in modulating performance in action cascading. GABA seems to modulate performance both when a more parallel, overlapping strategy was needed (i.e., when interruption (stopping) of a current task goal and a change toward an alternative response were required simultaneously), and when a more serial, step-by-step strategy was required (i.e., when the change toward the alternative response was required after the stopping process had already finished).

Chapter six

Tyrosine promotes cognitive flexibility: Evidence from proactive vs. reactive control during task switching performance

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Abstract

Tyrosine (TYR), an amino acid found in various foods, has been shown to increase dopamine (DA) levels in the brain. Recent studies have provided evidence that TYR supplementation can improve facets of cognitive control in situations with high cognitive demands. Here we investigated whether TYR promotes cognitive flexibility, a cognitive-control function that is assumed to be modulated by DA. We tested the effect of TYR on proactive vs. reactive control during task switching performance, which provides a relatively well-established diagnostic of cognitive flexibility. In a double-blind, randomized, placebo-controlled design, 22 healthy adults performed in a task-switching paradigm. Compared to a neutral placebo, TYR promoted cognitive flexibility (i.e. reduced switching costs). This finding supports the idea that TYR can facilitate cognitive flexibility by repleting cognitive resources.

1. Introduction

One of the most investigated amino acids is tyrosine (TYR). TYR is the biochemical precursor of norepinephrine (NE) and dopamine (DA), which are neurotransmitters of the catecholinergic system. Early research has shown that TYR supplementation, or a TYR-rich diet, increases plasma TYR levels in the blood (Glaeser, Melamed, Growdon, & Wurtman, 1979) and enhances DA and NE release in the brain of rats (Sved & Fernstrom, 1981; Gibson, Watkins, & Wurtman, 1983; Acworth, During, & Wurtman, 1988) and humans (Growdon, Melamed, Logue, Hefti, & Wurtman, 1982; Wurtman, 1992; Deijen, 2005, for a review). Once the optimal level of DA is reached, TYR is no longer transformed to DA because tyrosine hydroxylase, the enzyme that converts TYR into DA, is inhibited (Udenfriend, 1966; Weiner, Lee, Barnes, & Dreyer, 1977). Previous studies on the effect of TYR on cognition focused mainly on deficits in TYR to DA conversion (e.g. phenylketonuria; Pietz et al., 1995; van Spronsen et al., 1996), on the depletion of TYR (Fernstrom & Fernstrom, 1995; Harmer, McTavish, Clark, Goodwin, & Cowen, 2001), or on DA-related diseases (e.g. Parkinson's disease; Growdon, Melamed, Logue, Hefti, & Wurtman, 1982). 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 physical 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; Shurtleff, Thomas, Schrot, Kowalski, & Harford, 1994; Mahoney, Castellani, Kramer, Young, & Lieberman, 2007).

Only recently, the focus has shifted to the possible beneficial effects of TYR on challenging cognitive performance in the absence of physical stress. Indeed, even without exposure to stress, the supplementation of TYR has been shown to have an acute beneficial effect on challenging task performance thought to be related to DA, such as multitasking (Thomas, Lockwood, Sing, & Deuster, 1999), the updating and monitoring of working memory (Colzato, Jongkees, Sellaro, & Hommel, 2013), stopping on time (Colzato, Jongkees, Sellaro, van den Wildenberg, & Hommel, 2014), and convergent thinking (Colzato, de Haan, & Hommel, 2015).

The primary goal of the present study was to examine the effect of TYR on cognitive flexibility, a key cognitive-control function (Miyake et al., 2000). A well-established, reliable indicator of cognitive flexibility is task-switching performance (Monsell, 2003; Miyake et al., 2000). The amount of the time needed to switch between two different tasks can be taken to indicate the efficiency in adapting and restructuring cognitive representations, so that smaller switching costs would reflect a higher level of cognitive flexibility. In this kind of paradigm, the sequence of tasks is often regular and predictable (e.g., AABBAABB…). Accordingly, participants know when to prepare for a task switch, so that the interval between the previous response and the upcoming stimulus (the response-stimulus interval or RSI) represents a preparation interval.

Switching costs in tasks as used in the present study are thought to consist of two major components: a preparatory component and a residual component (e.g., Meiran, Chorev, & Sapir, 2000). In switch trials participants can use the preparation interval (if sufficiently long and sufficiently predictable: Rogers & Monsell, 1995) to reconfigure their cognitive task set to meet the demands of the upcoming task. The shorter the interval the less likely this reconfiguration will be completed before the stimulus is presented, which fits with the observation that switching costs (i.e., the increase of reaction time in task-switching trials relative to task-repetition trials) are more pronounced with short than with long RSIs (Rogers & Monsell, 1995). However, when the RSI is long, the preparatory component is nearly eliminated (Meiran, 1996). What remains is the residual component, the component that is resistant to preparation, e.g., because the stimulus triggers the involuntary activation of the previous task set and/or because completely inhibiting the previous set requires the actual activation of the new task set (see Kiesel et al., 2010). In any case, the residual component reflects processes that occur after target onset on switch trials, regardless of the amount of preparation time (e.g., Monsell, 2003).

According to Cools and D'Esposito (2010), DA modulates cognitive flexibility by facilitating the update of information in working memory such as the current task set. Indeed, the DA-D2 receptor agonist bromocriptine was found to reduce switching costs and was accompanied by a drug-

induced potentiation of striatal activity in participants with a low-span baseline in working memory capacity (Cools, Sheridan, Jacobs, & D'Esposito, 2007). The hypothesis that dopaminergic pathways are crucial in driving cognitive flexibility clearly predicts a beneficial effect of TYR, which in our design translates into the prediction of reduced switching costs. However, the existence of multiple DA pathways with to some degree opposite and counter-acting impact on performance (e.g., a frontal pathway associated with goal maintenance and focusing, and a nigrostriatal pathway associated with inhibition and flexibility; Cools, 2008; Cools & D' Esposito, 2010; van Schouwenburg, Aarts, & Cools, 2010) makes it difficult to predict whether the preparatory component or the residual component or both would be affected. Accordingly, we manipulated the RSI, so that we were able to dissociate possible effects of TYR on these two components. An effect of TYR on the preparatory component would be visible in a particularly strong TYR effect on switching costs when RSI is short, while an effect of TYR on the residual component would be visible in a particularly strong TYR effect on switching costs when RSI is long.

2. Method

2.1. Participants

Twenty-two undergraduate students of the Leiden University (all females, mean age=19.3 years, SD=1.5, range 17–23; mean Body Mass Index=20.9, SD=1.5, range 19–23; all right-handed) 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. All participants were selected individually via a phone interview by the same lab-assistant 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 and pharmacological research that screens for several psychiatric disorders and drug use (Colzato & Hommel, 2008; 2009; Sheehan et al., 1998). Written informed consent was obtained from all subjects; the protocol and the remuneration arrangements of 20 euro in cash payment were approved by the local ethical committee (Leiden University, Institute for Psychological Research).

A double blind, placebo-controlled, randomized cross-over design with counterbalancing of the order of conditions was used to avoid expectancy effects. Participants were exposed to an oral dose (powder) of 2.0 g of L-Tyrosine (TYR) (supplied by Bulkpowders Ltd.) in the TYR condition and to 2.0 g of microcrystalline cellulose (Sigma-Aldrich Co. LLC), a neutral placebo, in the placebo condition. TYR and placebo doses were dissolved in 400 ml of orange juice and were administered in two different experimental sessions, separated by 3-7 days.

Following Markus et al. (2008) and Colzato, Jongkees, Sellaro, and Hommel (2013) 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 the experiment or to drink alcohol the day before their participation and arrival at the laboratory. Thirty minutes after the administration of either TYR or the neutral placebo participants were allowed to eat an apple.

2.2. Apparatus, stimuli, and task

The experiment was controlled by a PC attached to a 17-inch monitor with a refresh rate of 100 Hz. The task was modeled after Colzato, van Leeuwen, van den Wildenberg and Hommel (2010). Throughout each block, a 10-cm square divided into four quadrants was displayed on the computer screen. On each trial, a character pair was presented in a white uppercase Triplex font in the center of one quadrant. Each pair subtended a visual angle of 1.4° both horizontally and vertically. The next stimulus was displayed clockwise in the next quadrant. One pair of adjacent display positions was assigned to the letter task and the other pair to the digit task, so that the display location served as a task cue, and the task changed predictably every second trial. Depending on the task, the relevant character was either a letter or a digit. The second and irrelevant character was either a member of the other category, so that the response afforded by this character was either congruent or incongruent with the task-relevant response, or was drawn from a set of neutral characters (see Figure 1).

Consonants were sampled randomly from the set [G, K, M, R] vowels from the set [A, E, I, U], even digits from the set [2, 4, 6, 8], odd digits from the set [3, 5, 7, 9] and neutral characters from the set [#,?,*,%], with the restriction that a character could not be repeated on successive trials. The position of the task-relevant character within a pair was randomly determined on each trial. The participants responded with their left index finger (on the “C” key) to indicate “even” or “consonant” and their right index finger (on the “M” key) to indicate “odd” or “vowel”.

The participants received a practice set of 9 switch blocks, each with 16 trials, before entering the experimental phase. This consisted of two sets of 15 blocks, one set for each RSI, each block consisting of 16 trials. The RSI was 150 or 1200 ms, and remained constant for a given set. The order of the RSIs was counterbalanced across participants. The stimulus was displayed until a response was registered.

2.3. Procedure and design

All participants were tested individually. Upon arrival, participants were asked to rate their mood on a 9×9 Pleasure×Arousal grid (Russell, Weiss, & Mendelsohn, 1989) with values ranging from –4 to 4. Heart rate (HR) and systolic and diastolic blood pressure (SBP and DPB) were collected from the non-dominant arm with an OSZ 3 Automatic Digital Electronic Wrist Blood Pressure Monitor (Spiedel and Keller). One hour following the administration of TYR (corresponding to the beginning of the 1 h-peak of the plasma concentration; Glaeser, Melamed, Growdon, & Wurtman, 1979) or placebo, participants rated again their mood before having HR, SBP and DBP measured for the second time. Immediately after, participants were asked to perform the task-switching paradigm measuring cognitive flexibility which took about 30 min. After completing it, participants again rated their mood before having HR, SBP and DBP measured for the third time.

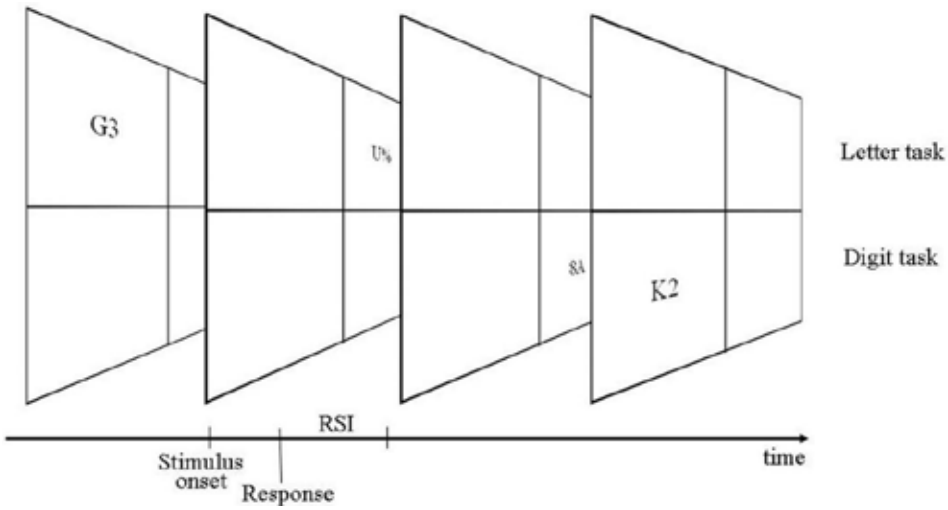

Figure 1. Illustration of the sequence of events. A stimulus is comprised of two characters, as described in the text. On consecutive trials, stimuli appear in adjacent quadrants rotating clockwise in the four quadrants of the square. One pair of adjacent quadrants is assigned to the letter task

(the upper two, in the example), and the other pair to the digit task. As a consequence, the task changes predictably every second trial. The response-stimulus interval (RSI) was either 150 ms or 1200 ms.

2.4. Statistical analysis

Mood (i.e., pleasure and arousal scores), HR, BPS and BPD were analyzed separately by means of repeated-measures analyses of variance (ANOVAs) with condition (Placebo vs. TYR) and effect of time (first vs. second vs. third measurement) as within-subjects factor. The effect of TYR on cognitive flexibility was assessed by means of 2×2×2 repeated-measures ANOVAs with condition (Placebo vs. TYR). Task Repetition (i.e., repetition vs. alternation of task) and RSI (150 vs. 1200) as within-subject factors[1].We adopted a significance level of $p<0.05$ for all statistical tests.

3. Results

3.1. Task-switching performance

Table 1 provides an overview of the outcomes for reaction times (RTs) and percentage of errors (PEs). RTs revealed a significant main effect of Task Repetition, $F(1,21)=112.63$,$p<0.001$, $\eta^2p=0.84$; and of RSI, $F(1,21)=19.53$, $p<.001$, $\eta^2p=0.48$. These two main effects were involved in two-way interaction, $F(1,21)=20.67$, $p<0.001$, $\eta^2p=0.50$, and in a three-way interaction involving condition, $F(1,21)=4.45$, $p=0.047$, $\eta^2p=0.18$.

Fisher LSD post-hoc tests showed that switching costs differed significantly between placebo and TYR for the long RSI, $p=0.009$, SEd

[1] We also examined effects of cross-talk (i.e., whether the currently irrelevant, unattended symbol of the two-symbol stimulus compound was related to the task or neutral) and congruency (i.e., whether the currently irrelevant, unattended symbol of the two-symbol stimulus compound was signaling the same response as the relevant symbol or not). The only effect we observed was that participants were faster when the unattended symbol of the two-symbol stimulus compound was neutral (711 ms) than when it was related to the task (811 ms), $F(1,21) = 274.60$, $p < 0.001$, $\eta2p = 0.92$. Importantly, neither factor was involved in any interaction involving Condition and/or RSI, $Fs\leq2.90$, $ps\geq.10$, so that they were not considered further.

(standard error of the mean difference) =10.24, 95% CI=(8.30, 50.90), but not for the short RSI, p=0.927, SEd=10.24, 95% CI=(−22.25, 20.35). Hence, TYR promotes cognitive flexibility (i.e., less switching costs), but only for the long RSI (see Figure 2 and Table 1).

In the error analysis, a significant main effect of Task Repetition was observed,$F(1,21)=50.99$, $p<0.001$, $\eta^2p=0.71$, due to fewer errors when the task was repeated than alternated. Condition was not involved in any significant effect, Fs ≤2.171, ps≥0.13.

Table 1. Mean response latencies (in ms), error rates (in percent), and switching costs (alternation – repetition) for placebo and TYR conditions. Standard errors in parentheses.

Variables (SE)	*Placebo*		*Tyrosine*	
SOA	150	1200	150	1200
Repetition				
Reaction Times	699 (23)	664 (21)	684 (25)	657 (19)
Error Rates (%)	3.8 (0.6)	3.5 (0.6)	4.1 (0.7)	3.1 (0.6)
Alternation				
Reaction Times	940 (36)	858 (35)	926 (35)	822 (29)
Error Rates (%)	8.8 (1.3)	9.1 (1.2)	8.5 (1.3)	8.2 (1.3)
Switch Costs				
Reaction Times	241 (25.5)	194* (19.5)	242 (25.3)	165* (17.1)
Error Rates (%)	5.1 (0.9)	5.6 (0.9)	4.4 (1.0)	5.0 (1.0)

Significant group difference; * $p < 0.05$.

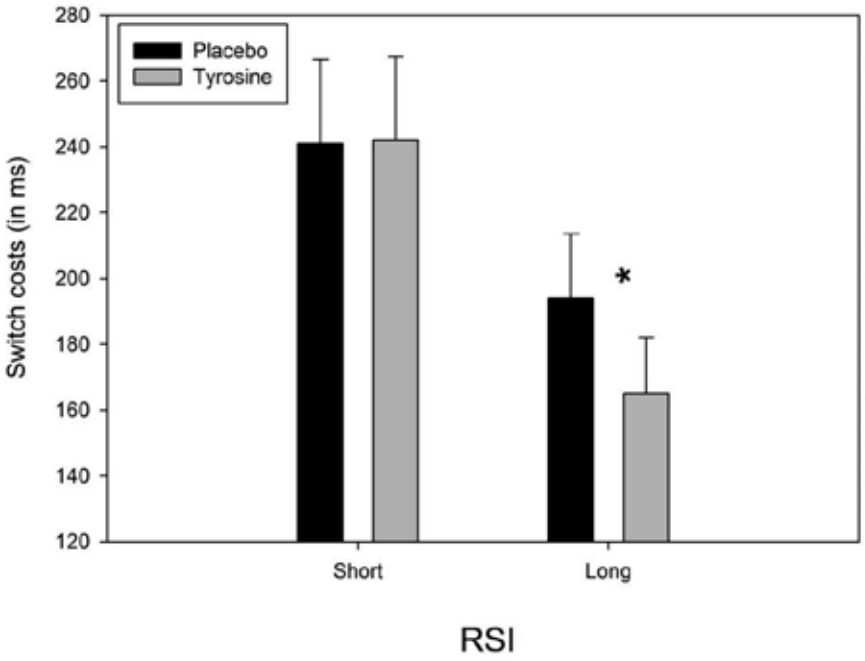

Figure 2. Mean switching costs (calculated as the RT difference between Task Repetition and Alternation) +SEMs (standard error of the means), as a function of condition (Placebo vs. TYR) and the response-stimulus interval (RSI) (150 and 1200)

Table 2. Mean heart rate values (HR; in beats per minute), systolic (SBP) and diastolic (DBP) blood pressure (in mmHg), and mood and arousal scores for placebo and TYR conditions. Standard errors in parentheses.

	T1		T2		T3	
	Placebo	*TYR*	*Placebo*	*TYR*	*Placebo*	*TYR*
HR	72 (2)	72 (2)	72 (2)	69 (2)	64 (2)	66 (2)
SBP	114 (2)	112 (3)	115 (3)	112 (3)	113 (3)	109 (2)
DBP	68 (2)	68 (2)	69 (2)	66 (2)	69 (2)	70 (2)
Mood	1.4 (0.3)	1.4 (0.3)	1.6 (0.2)	1.8 (0.2)	1.4 (0.3)	1.4 (0.3)
Arousal	0.4 (0.3)	0.3 (0.4)	0.4 (0.3)	0.6 (0.3)	-0.1 (0.3)	0.1 (0.3)

3.2. Physiological and mood measurements

Table 2 provides an overview of the outcomes for physiological and mood measurements. ANOVAs showed a main effect of timing only for HR, F=12.099, p<0.001, indicating that heart rate decreased with the duration of the experiment (72 vs. 71 vs. 66). However, HR, BPD, BPS, pleasure and arousal, did not significantly change after the intake of TYR, Fs ≤2.171, ps≥0.13. This suggests that we can rule out an account of our results in terms of physiological and mood changes.

4. Discussion

Our findings show that TYR, the precursor of DA, modulates cognitive flexibility as measured by a task-switching paradigm. Participants showed smaller switching costs after the intake of TYR than of a neutral placebo when the preparation interval to switch was long, but not when it was short. This implies that TYR impacts the residual, but not the preparatory, component of switching costs. An effect on the preparatory

component might be due to either the speed of task-set retrieval and implementation, or the efficiency to maintain the prepared task set, or some combination of these processes[2].TYR might have supported these processes by improving sustained attention. This should have been visible as an effect of TYR on the short RSI (reflecting the preparatory component), which however was not obtained. Even though we need to be careful in interpreting a null effect, the absence of a reliable impact of TYR on the preparatory task-switching component might thus be taken to suggest that TYR has little effect on processes underlying the retrieval, implementation, and maintenance of task sets. As these functions are commonly attributed to the frontal dopaminergic pathway, we speculate that this pathway does not belong to the main targets of TYR-induced increases of DA.

In contrast, the residual component of task-switching costs is likely to reflect the online resolution of conflict induced by inertia or stimulus-triggered reactivation of the old task set. The significant effect of TYR on the residual component can thus be taken to reflect TYR-induced support of processes underlying such conflict-resolving processes. Given the available evidence that TYR supplementation has an acute beneficial effect on multitasking (Thomas et al., 1999), the updating and monitoring of working memory (Colzato et al., 2013a), and response inhibition (Colzato et al., 2014b), this might be taken to imply a stronger impact of TYR on

[2] De Jong, Berendsen, and Cools (1999) proposed an alternative account of residual switching costs in terms of goal neglect. According to this account, such costs may be due to occasional failures to engage in advance preparation, which lengthen RTs . Thus, one may argue that the smaller switching costs observed in the TYR condition when the preparation interval was long are due to improved sustained attention, and thus, to a reduced incidence of trials that fall in the slowest portion of the RTs distribution. To rule out this possibility, we further examined the data of the long RSI by means of a RT distribution analysis (RT bin analysis; De Jong, Liang, & Lauber, 1994). For each level of Condition (placebo and TYR) and Task Repetition (repetition vs. alternation of the task), the distribution of correct RTs was rank-ordered into quintiles (20% bins) and submitted to an ANOVA with three within-subjects factors: Condition, Task Repetition and Bin. For both repetition and alternation trials, we did not observe any difference between placebo and TYR in terms of RTs distributions, $F<1$, $p=0.84$.

the nigrostriatal dopaminergic pathway, which is assumed to be involved in switching to novel information, updating, and inhibition (Cools, 2008, Cools & D'Esposito, 2010; van Schouwenburg et al., 2010).

Previous neuroimaging studies investigating the effect of preparatory processes and residuals switching costs did not find switch-specific activations in the preparation phase (e.g., Brass & von Cramon, 2002, 2004; Braver, Reynolds, & Donaldson, 2003; Dove, Pollmann, Schubert, Wiggins, & von Cramon, 2000; Luks, Simpson, Feiwell, & Miller, 2002) but revealed strong activation in the left inferior frontal junction (IFJ) for residual switching costs (Brass & von Cramon, 2004). It is thus possible that TYR supplementation affects the activation of the left IFJ during task switching. Indeed, a direct pharmacological manipulation of DRD2 stimulation has found that fronto-striatal connectivity under bromocriptine was slightly increased for rule switches compared to rule repetitions (Stelzel, Fiebach, Cools, Tafazoli, & D'Esposito, 2013). Moreover, it would be interesting to know whether TYR affects tonic and/ or phasic DA and the functioning of D1-class vs. D2-class receptors in the striatum, given the important roles of these receptors type in cognitive flexibility.

As suggested by Robbins and Arnsten (2009), there is evidence that noradrenergiccoeruleo-cortical projections are involved in different forms of cognitive flexibility, whenever attention must be shifted from one perceptual dimension to another (Birrell & Brown, 2000; Dias, Robbins, & Roberts, 1996). Even though TYR is the precursor of both DA and NE, another study from our lab suggests that it was DA that was responsible for our results. In this study, we had participants perform a global-local task-switching paradigm after intake of an oral dose of 80 mg propranolol (a central and peripheral beta-adrenergic antagonist) or placebo in a randomized, double-blind, counterbalanced cross-over design (Steenbergen, Sellaro, de Rover, Hommel, & Colzato, 2015). We failed to find any significant impact of propanolol on switching costs and congruency effects. One may claim that elevated NE levels resulted in better attention after TYR supplementation, and that this might have improved performance (i.e., less switching costs). However, this consideration is not supported by the observation that the α2 adrenoceptor agonist clonidine (150 µg, oral

dose) has no effect on temporal or spatial attention (Nieuwenhuis, van Nieuwpoort, Veltman, & Drent, 2007).

We suggest that TYR administration selectively counteracts DA depletion, a process in which performance levels decline corresponding to the decrease DA function in the brain: When exposed to physical stress or a cognitively challenging task, the rate of DA synthesis rises (Lehnert, Reinstein, Strowbridge, & Wurtman, 1984; Mahoney, Castellani, Kramer, Young, & Lieberman, 2007). In order to meet the situational demands more DA is synthesized from TYR and L-DOPA. Once these chemical forerunners abate, DA synthesis get sparse, causing less DA availability and accordingly decrements in performance (Muly, Szigeti, & Goldman-Rakic, 1998; Goldman-Rakic, Muly, & Williams, 2000). Under these circumstances, TYR may provide the resources necessary to allow DA synthesis to carry on and DA to remain at a level that allows optimal performance (Wurtman, Hefti, & Melamed, 1980). Indeed, TYR supplementation has been found to stimulate DA production in actively firing neurons only (Lehnert, Reinstein, Strowbridge, & Wurtman, 1984; Fernstrom & Fernstrom, 2007). In contrast, when the rate of DA synthesis is low, TYR supplementation amounts to providing unnecessary extra resources from which to synthesize DA, which should not impact DA level or performance.

Taken together, the available observations provide converging evidence for the idea that the amino-acid TYR is a promising cognitive enhancer that facilitates cognitive flexibility.

Chapter seven

Tryptophan promotes charitable donating

Steenbergen, L., Sellaro, R., & Colzato, L.S. (2014). Tryptophan promotes charitable donating. *Frontiers in Psychology, 5*, 1451. doi: 10.3389/fpsyg.2014.01451

Abstract

The link between serotonin (5-HT) and one of the most important elements of prosocial behavior, charity, has remained largely uninvestigated. In the present study, we tested whether charitable donating can be promoted by administering the food supplement L-Tryptophan (TRP), the biochemical precursor of 5-HT. Participants were compared with respect to the amount of money they donated when given the opportunity to make a charitable donation. As expected, compared to a neutral placebo, TRP appears to increase the participants' willingness to donate money to a charity. This result supports the idea that the food we eat may act as a cognitive enhancer modulating the way we think and perceive the world and others.

1. Introduction

"Every good act is charity. A man's true wealth hereafter is the good that he does in this world to his fellow", Molière once said. Indeed, charitable donating is an essential component of prosocial behavior and a key determinant of social reliability (Milinski, Semmann, & Krambeck, 2002).

Pharmacological studies in rats and humans suggest that the neurotransmitter serotonin (5-HT) plays a crucial role in promoting prosocial behavior (Crockett, 2009). Indeed, as pointed out by Siegel and Crockett (2013), serotonergic levels tend to be negatively correlated with antisocial behaviors such as social isolation and aggression, and tend to be positively correlated with prosocial behaviors such as cooperation and affiliation. Prosocial behavior can be reduced by lowering 5-HT levels through tryptophan depletion (Wood, Rilling, Sanfey, & Bhagwagar, 2006; Crockett, Clark, Tabibnia, Lieverman, & Robbins, 2008; Crockett, 2009) and enhanced through administering the food supplement L-Tryptophan (TRP), the biochemical precursor of 5-HT (Colzato et al., 2013) or through administering selective serotonin reuptake inhibitors (Knutson et al., 1998; Tse & Bond, 2002).

Here, for the first time, we investigated whether the administration of the essential amino acid TRP, contained in food such as fish, eggs, soy, and milk, can promote charitable donating. TRP supplementation is known to increase plasma TRP levels and to influence brain 5-HT synthesis (Markus, Firk, Gerhardt, Kloek, & Smolders, 2008). We expected to find a beneficial effect of TRP on charitable donating because donating was found to selectively activate the subgenual–septal area (Moll et al., 2006), which shares reciprocal anatomical connections with raphe nuclei (Drevets, 2001), the principal release center of 5-HT in the brain. Hence, it is likely that the activation of the subgenual–septal area is modulated through serotonergic projections—which we aimed to target by the supplementation of TRP.

2. Method

2.1. Participants

Thirty-two healthy international south European students (mean age = 21.8; 4 male, 28 female; mean Body Mass Index = 21.5, range 17.8–30.8) 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. Participants were screened via a phone call by the experiment leader before inclusion, using the mini international neuropsychiatric interview (MINI; Sheehan et al., 1998). The MINI is a short, structured, interview of about 15 minutes that screens for several psychiatric disorders and drug use, often used in clinical and pharmacological research (Sheehan et al., 1998; Colzato & Hommel, 2008; Colzato, Hertsig, van den Wildenberg, & Hommel, 2010). Participants were randomly assigned to two experimental groups. Sixteen participants (2 male, 14 female) were exposed to an oral dose (powder) of 0.8 grams of TRP (supplied by AOV International Ltd.)—which roughly corresponds to the amount of TRP contained in 3 eggs–and 16 (2 male, 14 female) to 0.8 grams of microcrystalline cellulose (Sigma-Aldrich Co. LLC), a neutral placebo. These doses were always dissolved in 200 ml of orange juice. Following Markus et al. (2008) and Colzato et al. (2013) women using contraception were tested when they actually used the contraception pill. On each experimental morning, participants arrived at the laboratory at 9:30 am. Participants had been instructed to fast overnight (eating was not allowed after 11:00 pm); only water or tea without sugar was permitted. In addition, subjects were not allowed to use any kind of drugs before or during the experiment, or to drink alcohol from the day before their participation until their completion of the study.

Written informed consent was obtained from all subjects; the protocol and the remuneration arrangements of 10 Euros were approved by the local ethical committee (Leiden University, Institute for Psychological Research).

2.2. Apparatus and procedure

All participants were tested individually. Upon arrival, following Colzato et al. (2013), participants were asked to rate their mood on a 9 × 9 Pleasure × Arousal grid (Russell, Weiss, & Mendelsohn, 1989) with values ranging from –4 to 4. Heart rate (HR; in beats per minute) and systolic and diastolic blood pressure (SBP and DBP) were collected from the non-dominant arm with an OSZ 3 Automatic Digital Electronic Wrist Blood Pressure Monitor (Spiedel & Keller). One hour following the administration of TRP (corresponding to the beginning of the 1 hour-peak of the plasma concentration; Markus et al., 2008) or placebo, participants again rated their mood, before having HR, SBP, and DBP measured for the second time.

Next, after having performed an unrelated computer-based attentional blink task that requires the detection of two targets in a rapid visual on-screen presentation, which took about 30 minutes, participants were presented with the donating task. After the donating task, participants again rated their mood, before having HR, SBP, and DBP measured for the third time.

2.2.1. Donating task

Following van IJzendoorn and colleagues (2011), participants were not informed beforehand that the donating task was part of the experiment. Donating behavior was measured by the amount of money the participant donated. After having received 10 Euros (one 5-Euronote, two 1-Euro coins, and 6 Fifty-cent coins) for their participation in the study, participants were left alone and asked whether they were willing to donate part of their financial reward to charity. Four money boxes (Unicef, Amnesty International, Greenpeace, and World Wildlife Fund) had been positioned on the table. All money boxes were filled with four Fifty-cent coins in order to enhance credibility (see van IJzendoorn, Bakermans-Kranenburg, Pannebakker, & Out, 2010; van IJzendoorn, Huffmeijer, Alinks, Bakermans-Kranenburg, & Tops, 2011, for a similar task).

Hence, the donating task was standardized, without the presence of an experimenter, and with a fixed amount of money in a fixed number of notes and coins. The donating task used in the van IJzendoorn et al.

(2011) study was similar in terms of participants donating their own money to a real charity but it differed in terms of having the choice between four different charities compared to solely Unicef. Donated money was transferred to the bank accounts of the charities after data collection.

2.3. Statistical Analysis

Heart rate, systolic and diastolic blood pressure, mood, and arousal were analyzed separately by means of repeated-measures analyses of variance (ANOVAs) with effect of time (first vs. second vs. third measurement) as within-subjects factor and with group (Placebo vs. TRP) as between-group factor. A *t*-test for independent groups was performed to assess differences between the two groups (Placebo vs. TRP) in the amount of money donated. Effect magnitudes were assessed by calculating Cohen's d (Cohen, 1988). A significance level of $p < 0.05$ was adopted for all statistical tests.

3. Results

3.1. Participants

No significant differences were found among group with respect to age (21.7, SD = 1.4 vs. 21.8, SD = 2.9, for the placebo and TRP group respectively), $t(30) = 0.15$, $p > 0.88$, and sex, $\chi 2$ (1, $N = 32$) = 0.00, $p = 1.00$.

3.2. Donating Task

As expected, participants donated significantly more euros to the charities in the TRP condition (1.00, SD = 0.79) than in the placebo condition (0.47, SD = 0.59), $t(30) = 2.14$, $p < 0.05$, Cohen's $\delta = 0.78$.

3.3. Physiological and Mood Measurements

Analysis of variances revealed that HR, DBP, SBP, pleasure, and arousal did not significantly change after the intake of TRP, *F's* < 1 (see Table 1).

Table 1. Mean HR (in beats per minute), SBP and DBP (in mmHg), and pleasure and arousal scores for the TRP and placebo groups as measured on each timepoint. Standard deviations are shown in parentheses.

	T1		T2		T3	
	TRP	Placebo	TRP	Placebo	TRP	Placebo
HR	75 (13.1)	78 (10.9)	71 (8.5)	72 (9.4)	68 (11.2)	69 (9.2)
SBP	114 (8.1)	110 (12.8)	109 (10.3)	104 (11.2)	112 (9.7)	106 (11.8)
DBP	71 (8.5)	68 (10.4)	66 (7.2)	63 (9.0)	70 (9.4)	64 (10.6)
Pleasure	0.9 (1.6)	0.9 (1.4)	1.0 (1.6)	1.4 (1.4)	1.2 (1.6)	1.2 (1.4)
Arousal	0.5 (1.6)	0.6 (1.8)	0.5 (1.5)	1.1 (1.5)	0.2 (1.3)	0.4 (1.8)

4. Discussion

The present study is the first demonstration that charitable donating can be enhanced by serotonin-related food supplements. We argued that TRP supplementation, and the resulting boost in 5-HT should be beneficial for the participants' willingness to donate money to a charity.

One may wonder how this novel finding relates to the observations of Barraza, McCullough, Ahmadi, and Zak (2011) and Van IJzendoorn, Huffmeijer, Alinks, Bakermans-Kranenburg, and Tops (2011) that the neuropeptide oxytocin (OT) also increases charitable donation. Serotonergic terminals, mainly originating from the dorsal and median raphe nuclei of the brainstem, project to the paraventricular nuclei (Larsen, Hay-Schmidt, Vrang, & Mikkelsen, 1996), where the neurons release OT. So, comparable effects on prosocial behavior of TRP and OT are conceivable if one considers the functional and anatomical interactions between serotonergic and oxytocinergic systems. Further, the administration of the serotonergic agonist fenfluramine to healthy subjects increases plasma OT levels (Lee, Garcia, van de Kar, Hauger, & Coccaro, 2003). Thus, it may be likely that the willingness to donate money to a charity is modulated by the effect that 5-HT exerts on OT levels.

More research is needed to extend and replicate our preliminary findings with a bigger sample size. Follow-up studies should correlate the amount of money donated with plasma levels of TRP. Finally, to evaluate the effect of the TRP administration on the brain, it would be interesting to investigate whether TRP supplementation is associated with increased blood oxygenation level dependent (BOLD) changes in the fronto-mesolimbic networks, which are associated with charitable donating (Moll et al., 2006).

The present study is the first to show that TRP promotes charitable donating, an important element of prosocial behavior. Our results support the materialist approach that "you are what you eat" (Feuerbach, 1862 as cited in Feuerbach, 1960)—the idea that the food one eats has a bearing on one's state of mind. The food we eat may thus act as a cognitive enhancer that modulates the way we deal with the "social" world. In particular, the supplementation of TRP, or TRP-containing diets, may support the prosocial behavior of charity that Molière was concerned about.

Chapter eight

Tryptophan supplementation modulates social behavior: a review

Steenbergen, L., Jongkees, B.J., Sellaro, R., & Colzato, L.S. (2016). Tryptophan supplementation modulates social behavior: a review. *Neuroscience and Biobehavioral Reviews, 64,* 346-358.

Abstract

Tryptophan (TRP), the precursor of serotonin (5-HT), is one of the most investigated amino-acids. TRP supplementation can increase 5-HT levels in the brain and for this reason numerous studies have investigated whether administration of TRP can positively influence social behavior that relies on serotonergic function. Here we review the available studies on TRP, to clarify if and under what circumstances TRP supplementation might modulate social behavior. TRP supplementation seems to improve control over social behavior in patients and individuals suffering from disorders or behaviors associated with dysfunctions in serotonergic functioning. In contrast, in healthy humans TRP supplementation seems to promote social behavior. Although more research is needed to disentangle and understand the relations between individual differences, TRP effectivity, 5-HT functioning, social interactions, and context, we conclude TRP can be a promising tool for modulating social behavior.

1. Introduction

Social interactions pervade our daily lives. Although 'social behavior' is a very broad term encompassing many different actions, one can distinguish between two major, albeit non-exhaustive categories: on the one hand there is prosocial behavior, which has been defined as voluntary acts intended to help or benefit others, for example by helping or donating (Bar-Tal, 1976; Staub, 1978; Eisenberg, 1982; Brief & Motowidlo, 1986; Penner, Dovidio, Piliavin, & Schroeder, 2005). On the other hand there is antisocial behavior, which has been defined as voluntary acts intended to harm or disadvantage others, for example through aggression and dysfunctional impulsivity (e.g. Kavussanu, Seal, & Philips, 2006; Sage, Kavussanu, & Duda, 2006). A different way of classifying social behavior uses the interpersonal circle model of behavior (Moskowitz, 1994; 2010), according to which behavior can be classified along two dimensions, namely agreeable-quarrelsome and dominant-submissive. The dimension of agreeableness and quarrelsomeness bears resemblance to prosocial and antisocial behavior, with prosocial and agreeable behavior typically serving to affiliate with others, whereas antisocial and quarrelsome behavior typically serves to distance the person from others. Notably, neither behavior is exclusively 'dominant' or 'submissive', which comprises an orthogonal dimension.

Interestingly, increased serotonin (5-HT) levels in the brain have been linked to social behaviors such as affiliation and cooperation (for reviews see Crockett, 2009; Kiser, Steemer, Brainchi, & Homberg, 2012). In contrast, research has shown social behaviors such as aggression and irritability, as well as certain disorders, are related to disturbances in serotonergic functioning, although the GABA-ergic, dopaminergic and glutaminergic systems may be involved as well (Williams, Brignell, Randall, Silove, & Hazell, 2013; Selvaraj, Arnone, Cappai, & Howes, 2014; Lesch , Araragi, Waider, van den Hove, & Gutknecht, 2012; for reviews see Young & Leyton, 2002; Miczek, Fish, Joseph, & De Almeida, 2002; Kiser, Steemer, Brainchi, & Homberg, 2012). For example, 5-HT dysfunctions have been found to be associated with antisocial, impulsive, and violent criminal behaviors (Brown, Ballanger, Minichiello, & Goodwin, 1979; Virkkunen &

Närvänen 1987; Virkkunen et al., 1994; Liao, Hong, Shih, & Tsai, 2004; Coccaro, Fanning, Phan, & Lee, 2015).

Given this association between 5-HT function and social behavior, there is the possibility that modulating 5-HT could lead to positive changes in social behavior. One method of modulating 5-HT is administering its precursor tryptophan (TRP). TRP is an essential amino-acid that is derived from the diet, as the human body cannot produce TRP itself. Importantly, TRP contributes to brain protein synthesis and can increase 5-HT synthesis in rats (Yuwiler, 1973) and humans (Bowers, 1970; Eccleston, Ashcroft, & Crawford, 1970). For this reason, numerous studies have investigated whether administration of TRP can positively influence social behavior that relies on serotonergic function (Crockett, 2009; Kiser, Steemer, Brainchi, & Homberg, 2012).

However, findings from TRP studies have not been completely unequivocal and numerous factors—such as individual differences and social context—might determine the effect of TRP on social behavior. In this review, we will summarize the available studies on TRP and social behavior with the aim of illustrating equivocal findings, and, where possible, highlight consensus among studies. Afterwards, we list potential modulators of response to TRP supplementation. In doing so, we hope the present review may stimulate future studies to take into consideration the unresolved inconsistencies as well as the possible modulator factors when designing and analyzing experiments involving TRP. Before reviewing any studies, we will first elaborate on how TRP supplementation influences 5-HT function.

Mechanism of action

After ingesting TRP, its plasma levels increase (Yuwiler et al., 1981) and the synthesis of 5-HT in the brain can be doubled (Young & Gauthier, 1981). Effects of TRP on 5-HT synthesis mainly occur because of the enzyme tryptophan-hydroxylase (TPH), involved in the first step of TRP to 5-HT conversion and responsible for regulating the rate at which TRP is transformed into 5-HT (Young & Gauthier, 1981; Sheehan, Tharyan, McTavish, Campling, & Cowen, 1996; Silber & Schmitt, 2010). TPH is already saturated at a dose of 3 grams (gr) of TRP, which results in doubling of the

rate of 5-HT synthesis (Young & Gauthier, 1981). However, lower doses are used as well, which presumably do not fully saturate TPH and therefore increase but not necessarily double 5-HT synthesis (Young & Gauthier, 1981; Sheehan, Tharyan, McTavish, Campling, & Cowen, 1996).

In contrast to other large neutral amino-acids (LNAAs) such as valine, leucine, tyrosine, isoleucine, and phenylalanine, TRP is the amino-acid that is least found in protein (Wu, 2009). Thus a diet rich in protein will lead to smaller increases in TRP plasma levels than in the plasma levels of other LNAAs (for a detailed explanation see le Floc'h, Otten & Merlot, 2011). Furthermore, all LNAAs have to be transported through the blood brain barrier (BBB) by the same transport system. As such, the LNAAs compete for transport across the BBB, which limits uptake of TRP in the brain (Oldendorf & Szabo, 1976; Fernstrom, 1990; 2013). As a result of this, brain TRP and 5-HT levels could actually decline when TRP is consumed along with other LNAAs (Fernstrom & Faller, 1978, see also Figure 1). The intake of pure TRP, however, leads to a significant increase in plasma TRP levels and the TRP:LNAA ratio at approximately 60 minutes after administration. Peak plasma and TRP:LNAA levels are reached 2 hours after intake and remain elevated for at least 7-12 hours (Yuwiler et al., 1981; Volavka et al., 1990; Markus, Firk, Gerhardt, Kloek, & Smolders, 2008).

As reported by Hiratsuka et al. (2013), doses up to 5 gr of TRP per day do not cause any known adverse metabolic effects. One study reported side-effects of TRP intake such as dizziness and epigastric pain when administering doses of 3 gr daily for 3 weeks, although these complaints were also observed before the start and during the run-in placebo week of the study (Thomson et al., 1982). In a study in which 3 gr TRP daily was administered to participants for 12 weeks, one patient reported diarrhea as a side-effect of TRP intake (van Praag, Korf, Dols, & Schut, 1972). These studies are two examples of cases in which long-term use of moderate doses of TRP resulted in side-effects. The aforementioned side-effects are more likely to occur when higher doses are used (i.e. 70-200 mg/kg; for a review see Fernstrom, 2012). The number and variety of reported side-effects increases when looking at higher doses taken over longer periods (e.g. 6 gr daily for 3 months; Steinberg, Annable, Young, & Liyanage, 1999). Such high doses might not be recommendable not only because of such

side-effects, but also because the TPH enzyme is likely to already be saturated by a dose of TRP up to 3 gr (Young & Gauthier, 1981), suggesting doses higher than this are unlikely to provide further enhancement of 5-HT function. Lastly, side-effects may occur when TRP is taken in combination with a drug that also enhances 5-HT functioning (e.g. certain antidepressants). These side-effects include tremor, nausea, drowsiness, and dizziness (Fernstrom, 2012). In rare cases, serotonergic functioning can be stimulated too much (e.g. when combining TRP with 5-HT drugs) and "serotonin syndrome" occurs. Symptoms of this syndrome include delirium, myoclonus, hyperthermia, and coma (Fernstrom, 2012).

Regarding the cognitive mechanism underlying effects of TRP on social behavior, a prevalent hypothesis is that TRP—through its effect on the serotonergic system—might bias processing of emotional information. Low 5-HT function has been associated with an increase in aversive processing (Cools, Roberts, & Robbins, 2008), that is, low 5-HT levels are related to a bias in attention towards punishment (Chamberlain et al., 2006b; Cools, Robinson, & Sahakian, 2008) and distractors with a negative emotional load (Murphy, Smith, Cowen, Robbins, & Sahakian, 2002), and away from happy facial expressions (Murphy, Smith, Cowen, Robbins, & Sahakian, 2002). Conversely, enhanced 5-HT levels—achieved via repeated administration of the selective serotonin reuptake inhibitor (SSRI) citalopram or TRP supplementation—are associated with reduced fear recognition (Harmer, Mackay, Reid, Cowen, & Goodwin, 2006) and intensity rating (Gibson et al., 2014), increased recognition of happy faces (Murphy, Longhitano, Ayres, Cowen, & Harmer, 2006) and intensity rating (Gibson et al., 2014), and reduced attentional vigilance towards negative words (Murphy, Longhitano, Ayres, Cowen, & Harmer, 2006). However, not every study has demonstrated a selective bias towards positive information with high 5-HT levels and towards negative information with low 5-HT levels. For example, Attenburrow et al. (2003) showed intake of TRP increased the recognition of both happiness and fear. Nevertheless it is clear from these studies that the serotonergic system is closely related to the processing of emotional information. Indeed, Harmer (2008) and Harmer, Goodwin and Cowen (2009) suggested that decreasing TRP levels may decrease 5-HT synthesis and turnover, resulting in a negative bias in automatic processing

of information. Such a bias could potentially promote negative social behaviors like aggression. In contrast, TRP supplementation, similar to antidepressants that raise 5-HT levels (Harmer, 2008), might cause 'positive re-biasing in information processing', resulting in more attentiveness to positive stimuli. It is possible such a bias could promote more positive social behaviors such as affiliation and cooperation.

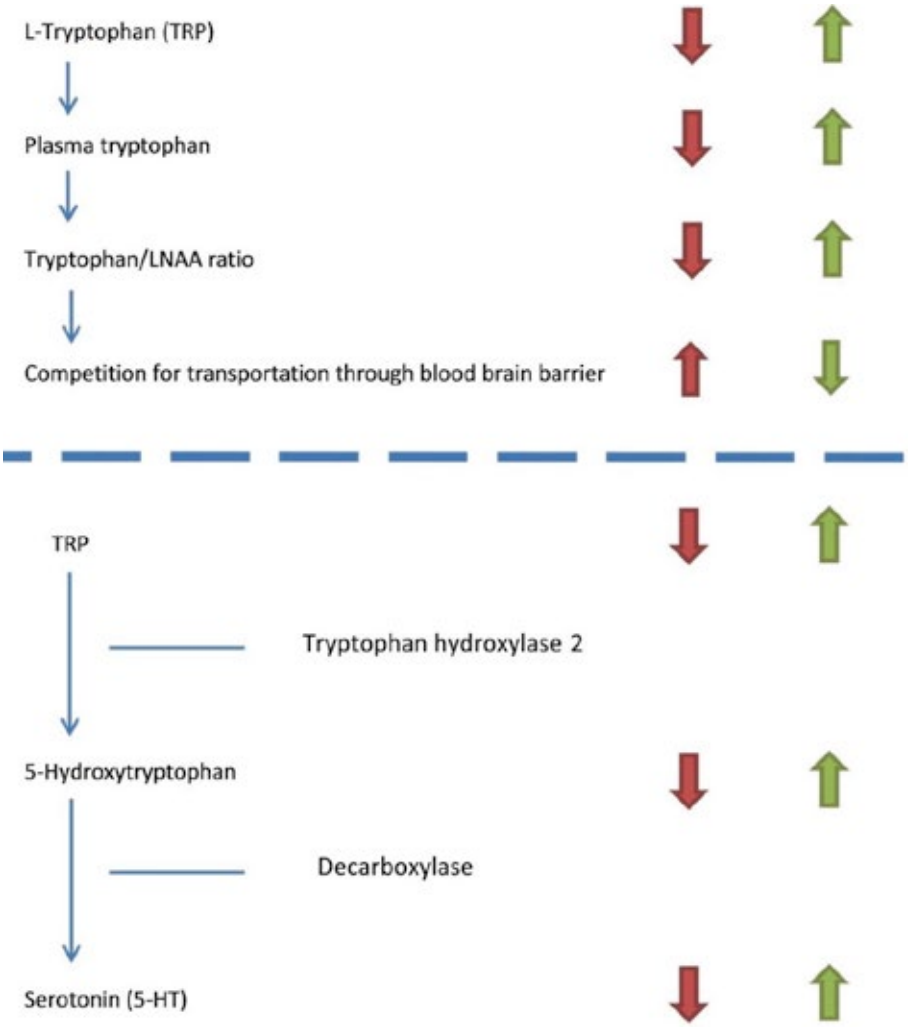

Figure 1. Schematic representation of the effect of acute tryptophan (TRP) depletion (red arrows) and supplementation (green arrows) on TRP to

serotonin (5-HT) conversion. Processes above the dashed line take place before travelling through the blood brain barrier, processes below the dashed line after transportation through the blood brain barrier. LNAA = large neutral amino acids.

2. Literature Overview

Previous reviews have extensively focused on the effect of TRP *depletion* on cognitive functions (Mendelsohn, Riedel, & Sambeth, 2009), mood, and social behavior (Ruhé, Mason, & Schene, 2007; Young & Leyton, 2002; Young, 2013). The general finding in these reviews is that low 5-HT can decrease mood and increase aggressive and antisocial behavior, although results are still equivocal and vary somewhat between studies. In contrast, the goal of the current paper is to review studies on TRP *supplementation* and its effects on social behavior.

Studies reviewed here were found using the keywords "tryptophan", "supplementation OR loading", "social" and "behavior" in Web of Science. In addition, forward and backward citations were studied to look for additional studies not directly found through Web of Science. Studies selected for this review had to be studies in humans including at least one TRP supplementation condition and measuring social behavior outcomes.

The available studies on TRP supplementation can be divided into two major domains of research. One line of research has focused on humans with psychiatric disorders and/or showing antisocial behaviors associated with decreased or dysfunctional 5-HT availability in the brain (hereafter, referred to as "clinical populations"). Results of these studies suggest that TRP can serve as a potential treatment or supplement to treatment for clinical symptoms associated with suboptimal or dysregulated 5-HT levels, e.g. in order to promote inhibition of antisocial behavior such as aggression, impulsivity, etc. A different line of research has focused on healthy humans with supposedly normal 5-HT levels. These studies suggest that TRP has promising potential for promoting social behavior, in particular prosocial and agreeable behavior. These two lines of research are used to structure our overview. We start with studies on TRP and clinical populations, followed by studies on healthy individuals.

Characteristics and main outcomes of the reviewed studies are presented in Table 1.

Table 1. Overview of studies on the effect of tryptophan supplementation on antisocial and prosocial behavior. When available, the mean age is reported alongside the range. TRP = Tryptophan, LNAA = Large neutral amino acids, 5HT = serotonin, * = study directly measuring social behavior as opposed to indirect measures such as self-reports or questionnaires.

Authors and year	Design	Sample	Supplement	Dose	Measure	Psychological outcome	Physiological outcome
Hogenelst, Schoevers & aan het Rot, 2015	Double blind, placebo controlled, counterbalanced cross-over, 7-day wash-out period	N = 40 (27 female), mean age 31.5	TRP	1 g 3 times a day for 14 days	Self-reports	Quarrelsomeness (at home) ↑, trend agreeableness (at home) ↓, negative affect ↓. Placebo first: positive affect ↑, negative cognitions ↓	n/a
Moskowitz et al., 2011	Double blind, placebo controlled, cross-over	Study 1: N = 98 (48 female) Study 2 (individuals selected for high irritability): N = 39 (19 female)	TRP	1 g 3 times a day for 9 days	Self-reports	Study 1: no effect on spin Study 2: spin in low agreeable subjects ↓	n/a
Morand, Young & Ervin, 1983	Double blind, placebo controlled, cross-over, 1 week wash-out	N = 12 male schizophrenic patients convicted for person-related crimes, mean age 30.0	TRP	N = 6: 4 g vs. N= 6: 8 g daily for 4 weeks	Self-reports and reports by ward staff	4 gr: 15% ↓ in depressive symptoms, 10% ↓ in hostility, 8 g: measures not possible, overall: 30% ↓ in incidents when on TRP	n/a

Volavka et al., 1990	Double blind, placebo controlled, between-subject, 1 month baseline observation	N = 20 (8 female) psychiatric inpatients aged 19-56	10 g chocolate bars containing 0.5 g TRP	6 g for 3 weeks	Reports by ward staff and biochemical analyses	No effect on aggressive incidents but need for injections of antipsychotics and sedatives ↓	TRP:LNAA ratio ↑ 2 hours after TRP
*Bjork et al., 2000	Double blind, within subject, latin squared (T-, T+, food restricted) including baseline	N = 12 agrressive (mean age 27.9) and 12 non-aggressive men (mean age 31.1)	Amino acid drink with TRP	10.3 g of TRP added to a drink containing 15 amino acids	Computer tasks, self-reports and biochemical measures	Aggressive responding ↓ in aggressive men, trend towards ↑ aggressive responding in non-aggressive men	↑ plasma free TRP in non-aggressive men after ingestion of TRP
Finn et al., 1998	Double blind, between-subject, including baseline	N = 48 males, mean age 21.9	Amino acid drink with TRP	2.3 g of TRP added to 100 g LNAAs, acute	Self-reports, biochemical measures	Significant negative correlation between TRP plasma levels and hostile mood. Stronger association between changes in plasma TRP and changes in hostility in subjects with high levels of pre-existing hostile traits, and in subjects with high vs. low antisocial traits.	Plasma TRP ↑

*Pihl et al., 1995	Double blind, between-subject	N = 90 males aged 18-34, mean age 24.0	Amino acid drink with TRP	2.3 vs. 10.3 g of TRP added to 100 g of amino acids, acute	Computer tasks, biochemical measures of alcohol	10.3 g: Lower shock intensity compared to TRP depletion	n/a
*Cleare & Bond, 1995	Double blind, between-subject, including baseline	N = 24 males high trait aggression, mean age 32.0, N = 24 males low trait aggression, mean age 33.0	Amino acid drink with TRP	10.3 g of TRP added to 100 g of amino acids, acute	Computer tasks, self-reports, physiological- and biochemical measures	No effects on behavioral task. High trait aggression: ↓ aggressiveness on angry-peaceful, quarrelsome-affable, hostile-friendly and annoyed-composed variables. Low trait aggression: drowsiness ↑	Mean plasma TRP ↑, blood 5-HT ↑
*Marsh et al., 2002	Double blind, within subject, counterbalanced (T- and T+) with fasting control day at the end	N = 12 females aged 18-36, mean age 26.2	Amino acid drink with TRP	2.3 g of TRP added to 100gr LNAAs, acute	Computer tasks, self-reports and biochemical measures	Aggressive responding ↓	plasma TRP ↑
*Smith et al., 1986	Double blind, between-subject	N = 36 males aged 18-25	Amino acid drink with TRP	2.3 vs. 10.3 g of TRP added to 100 g of amino acids, acute	Computer tasks, biochemical measures	No effects on aggression (shock duration and intensity)	↑ free and total plasma TRP

*Bjork et al., 1999	Double blind, within subject, latin squared (T-, T+, food restricted) including baseline	N = 8 males, mean age 32.6	Amino acid drink with TRP	10.3 g of TRP added to a drink containing 15 amino acids	Computer tasks	No significant effects of TRP administration on aggressive responding in high-provocation situations	n/a
*Nantel-Vivier et al., 2011	Double blind, placebo controlled, between-subject	N = 23 males aged 10	Chocolate milkshake containing TRP	500mg acute	Computer tasks	Decision time ↓ during high provocation, optimal responding as function of the level of provocation. Trend towards less impulsiveness, ↑ perspective taking and better distinction of happiness and fear	n/a
Nemzer et al., 1986	Double blind, placebo controlled, latin-square cross-over, including baseline measures	N = 14 (3 female) aged 7-12, mean age 9.4	TRP	100mg/kg daily for 1 week	Teacher and parent reports and biochemical analyses	Improvement in parent ratings of impulsivity and concentration	TRP serum levels ↑

aan het Rot et al., 2006	Double blind, placebo controlled, counterbalanced cross-over, 6-day wash-out period	N (selected for high quarrelsomeness) = 39 (19 female), mean age 32.1	TRP	1 g 3 times a day for 15 days	Self-reports	Quarrelsome behavior ↓, perceived dominance ↑, agreeableness (men only) ↑ and dominance (men only) ↑. Only when placebo first: Pleasantness of, and positive, affect, perceived agreeableness ↑.	n/a
Moskowitz et al., 2001	Double blind, placebo controlled, counterbalanced cross-over, 2-day wash-out period	N = 98 (48 female) aged 18-67	TRP	1 g 3 times a day for 12 days	Self-reports	Dominance ↑, arousal ↓(females only). Only when placebo first: quarrelsomeness ↓	n/a
*Cerit et al., 2015	Double blind, placebo controlled, between-subject, pre- and post-assessment	N = 47 (23 female) aged 18-35, mean age 20.3	TRP	2.8 g per day for 6 days	Self-reports and computer tasks	Rejection of very unfair offers ↑	n/a
*Colzato et al., 2013	Double blind, placebo controlled, between-subject	N = 40 (36 female), mean age 19.4	TRP	800mg acute	Self-reports, computer task and physiological measures	Money transferred to recipient ↑	Heart rate ↓

*Steenbergen, Sellaro & Colzato, 2014	Double blind, placebo controlled, between-subject	N = 32 (28 female), mean age 21.8	TRP	800mg acute	Self-reports, donating and physiological measures	Money donated to charity ↑	No changes

2.1. Clinical populations: inhibiting antisocial behavior

Many clinical conditions are associated with reduced or dysfunctional 5-HT levels. Hence, it is reasonable to assume that TRP supplementation may improve certain clinical symptoms by altering 5-HT availability. One of the most prominent disorders related to decreased 5-HT functioning is depression (Coppen, 1967; Albert, Benkelfat, & Descarries, 2012). Indeed, in addition to increasing the effectiveness of antidepressants such as monoamine oxidase inhibitors and tricyclic antidepressants (for a review see Young, 1991), TRP may be effective as an antidepressant alone (for reviews see Shaw, Turner & Del Mar, 2002; Silber & Schmitt, 2010). However, as pointed out by Silber & Schmitt (2010), the variance between the available studies with regard to dosage, study design, sample size, and sample population makes that there is still little consensus in terms of the effectiveness of TRP in treating depression. For example, TRP has no additional effectiveness when compared to placebo in severely depressed patients (Chouinard, Young, Bradwejn, & Annable, 1983; Shaw, Turner, & Del Mar, 2002), but does have positive effects in mild to moderately depressed patients (Thomson et al., 1982; Shaw, Turner, & Del Mar, 2002). Despite the strong connection between depression and social behavior (e.g. Leader & Klein, 1996; Bosc, 2000), to the best of our knowledge, no studies have addressed whether and to what extent TRP supplementation can affect social behavior in depressed patients. However, a study by Hogenelst, Schoevers, and aan het Rot (2015) did address the idea that TRP supplementation may be beneficial for individuals at risk of developing depression, such as those who have a family history of depression, whose social functioning is altered relative to control individuals (Watters et al., 2013; Mannie, Harmer, & Cohen, 2007). Importantly, Mannie, Harmer and Cohen (2007) suggested decreased social functioning, as observed in these individuals, may be a result of impairments in the processing of emotional stimuli. This is in line with the idea that decreased 5-HT functioning may lead to a negative bias in information processing (Cools, Roberts, & Robbins, 2008) and that antidepressants that raise 5-HT levels cause a re-biasing towards positive information processing (Harmer, 2008). Following

this idea, Hogenelst, Schoevers, & aan het Rot (2015) conducted a double-blind study to investigate whether individuals with a family history of depression would show more agreeable and less quarrelsome behavior after a TRP supplementation intervention (1 gr of TRP or placebo, 3 times a day, for 14 days). In contrast to the expectations, however, the intervention had the opposite effect: TRP supplementation led to increased quarrelsome behavior and diminished agreeable behavior. Notably, the findings of Hogenelst, Schoevers and Aan het Rot (2015) only applied to interactions at home and, as such, it is not clear whether these results generalize to other social contexts. Furthermore, quarrelsomeness is sometimes regarded as a mild form of reactive aggression (Moskowitz, 2010) that, at least in animals, is aimed at enhancing social status, coherence and territorial control and is positively related to serotonergic activity (de Boer, Caramaschi, Natarajan, & Koolhaas, 2009). Hence, the authors speculated the increase in quarrelsome behavior may indicate achieving more control over social interactions at home (Hogenelst, Schoevers, & aan het Rot, 2015), instead of promoting social behavior per se. Consistent with the idea that TRP may improve control over social interactions, Moskowitz, Zuroff, Aan het Rot, and Young (2011) found 1 gr of TRP taken 3 times a day to lower interpersonal spin (i.e., the large fluctuation in interpersonal behaviors around the interpersonal circumplex across situations) and, thus, to increase social behavior consistency, in individuals with elevated trait irritability and low trait agreeableness. This suggests that individuals with high propensity to anger and/or problems with bonding with others because of their difficulties with the control of interpersonal behavior might benefit from TRP supplementation.

Another prominent psychiatric disorder that has been associated with reduced 5-HT levels is schizophrenia (Meltzer et al., 2003). A study on treating patients with schizophrenia by supplementing them with TRP dates back several decades ago (Morand, Young, & Ervin, 1983). This treatment was reported to help reduce the frequency of aggressive incidents in schizophrenic patients convicted for interpersonal crimes (Morand, Young, & Ervin, 1983). Another study on female patients showed that TRP (6 gr per day) did not affect the frequency of occurrence of aggressive or violent

incidents, however it did significantly reduce the need for antipsychotic or sedative injections to control their aggression (Volavka et al., 1990).

Other studies have addressed the role of 5-HT functioning in modulating aggressive, violent, and criminal behaviors, which have previously been associated with 5-HT dysfunctions and reduced 5-HT levels (e.g. Kruesi et al., 1992; Brown, Ballanger, Minichiello, & Goodwin, 1979; Virkkunen & Närvänen, 1987; Virkkunen et al., 1994; Liao, Hong, Shih, & Tsai., 2004; Coccaro, Fanning, Phan, & Lee, 2015). Given that TRP can enhance 5-HT functioning, TRP might have a positive influence on these antisocial behaviors. Indeed, Bjork and colleagues (2000) showed that in aggressive men, after TRP supplementation (10.3 gr TRP added to an amino acid drink) higher plasma TRP levels were associated with less aggressive responses to provocation as assessed by a modified version of the point subtraction aggression paradigm (Cherek, Schnapp, Moeller, & Dougherty, 1996). In a study by Finn, Young, Pihl, and Ervin (1998), it was found that changes in plasma TRP levels as a result of TRP administration (2.3 gr of TRP added to 100 gr of other LNAAs) negatively correlated with hostile mood: increase in TRP levels was associated with less hostility, whereas TRP level reduction was associated with higher hostility. Interestingly, these correlations were found to be stronger for males with high trait levels of hostility and aggression. Further evidence that TRP availability can affect aggressive responses comes from a study by Pihl et al. (1995). In this study aggressive behavior was indexed via the intensity of the shocks people were willing to deliver to another individual after having received themselves shocks with intensities either below their pain threshold (i.e., low provocation condition) or above it (i.e., high provocation condition). It was found that an increase in TRP levels led participants to reduce the intensity of the shocks delivered to the alleged partner. However, such an outcome was only observed for the low provocation condition and when TRP depletion was compared to TRP administration (10.3 gr added to 100 gr of LNAAs) but not when compared to a balanced drink (2.3 gr TRP added to 100 gr LNAAs). In another study, TRP supplementation (10.3 gr added to 100 gr of amino acids) was found to reduce self-report ratings of angriness, quarrelsomeness, hostility, and annoyance, but only for males with high trait levels of aggression (Cleare & Bond, 1995). Lastly, Marsh and

colleagues (2002) found, using a similar design (2.3 gr of TRP added to 100 gr of amino acids) but including only females, that TRP administration significantly reduced aggressive responses in an aggression-provoking task (Cherek, Schnapp, Moeller, & Dougherty, 1996) when compared to a control condition (i.e., a low monoamine diet). Overall, these findings suggest TRP may modulate aggressive behavior, although effects may strongly depend on gender and personality characteristics (e.g. trait levels of aggression). The lack of consideration of these factors may explain why other studies failed to observe any effect of TRP on aggression (Smith, Pihl, Young, & Ervin, 1986; Bjork, Dougherty, Moeller, Cherek, & Swann, 1999),

TRP supplementation also seems to be beneficial for male children with a history of physical aggression and behavior regulation difficulties such as ADHD. Using a double-blind procedure, Nantel-Vivier et al. (2011) compared two groups of boys with a history of elevated physical aggression with regard to provocative, impulsive, and affiliative behavior, perspective taking, and emotion recognition. One group received an acute 0.5 gr dose of TRP, while the other group received a neutral placebo. All children then played a game against the computer, although they thought they were playing against another person. In this game, participants have to press the space bar as fast as they can upon seeing a cue. When being the fastest of the two, points can be earned and the participant is allowed to choose how many points they want to deduct from the other person and transfer to a neutral 'bank'. However, when the participant themselves are the slowest one, either no, a few, or a lot of their own points can be deducted by the computer, resulting in no, low, or high provocation conditions, respectively. Results showed that, compared to the placebo group, children in the TRP condition showed optimal adjustment of responding corresponding to the degree of provocation. That is, compared to baseline, boys in the TRP condition decided faster and took away more points from the computer when the boys themselves were highly provoked, making the game more fair. No differences were found in the no or low provocation conditions. In addition, the TRP group showed less impulsive behavior, a trend towards greater perspective taking, and was better able to distinguish between facial expressions of fear and happiness (Nantel-Vivier et al., 2011). Similarly, several decades ago Nemzer, Arnold, Votolato, and McConnell

(1986) showed parents reported an improvement in behavior (i.e., lower impulsivity and higher concentration) of their children with ADHD after one week of 100mg/kg TRP per day, as assessed by the Conners Parent's Questionnaire (Conners, 1970) and the Quay-Peterson scale (Quay & Peterson, 1967). Since 5-HT may play a role in ADHD (Oades, 2008) and ADHD is associated with antisocial behavior (Richards et al., 2015), TRP supplementation might be of interest for attention deficit disorder with hyperactivity (ADHD).

In sum, TRP may be used to alleviate psychiatric and neurological disorders and antisocial behaviors associated with suboptimal or dysfunctional serotonergic levels. It may help patients and individuals at risk for decreased social functioning to gain more control over social interactions (Moskowitz, Zuroff, Aan het Rot, & Young, 2011) and to show less impulsive, antisocial behavior. Indeed, the reported findings are in line with the idea of a key role of 5-HT in inhibiting responses to stimuli such as provocation (Soubrié, 1986; Spoont, 1992; Young, 2013), possibly by inducing a bias towards positive instead of negative stimuli. However, it is worth noting that effects of TRP on measures related to social behavior in clinical populations are not yet straight-forward and predictable, as evidenced by counterintuitive results in individuals at risk for depression. It would be valuable for future studies to investigate whether results are more consistent when considering social context, the behavior of interaction partners, and the myriad of modulating factors discussed later in the section "Factors modulating TRP effectivity".

2.2. Healthy humans: promoting behavior

In this section we describe studies suggesting that TRP can have promising implications for healthy individuals in promoting social behavior. A placebo-controlled study by aan het Rot, Moskowitz, Pinard, and Young (2006) reported the intake of TRP (1 gr, 3 times a day for 15 days) lessened quarrelsome and enhanced agreeable behavior and perceptions of agreeableness. In addition, a placebo-controlled study by Moskowitz, Pinard, Zuroff, Annable, and Young (2001) investigated the effect of 12 days of TRP supplementation (1 gr). TRP augmented dominant behavior

independent of treatment order, an effect also found in monkeys (for a review see Watanabe & Yamamoto, 2015). Further, TRP reduced quarrelsome behavior but only when given after the placebo treatment. The authors argued this might be explained by the possibility that effects of TRP on cognitions and social behaviors prolonged even after TRP intake had stopped and placebo intake was started. Indeed, the authors suggested that it is possible that TRP changed reciprocal interactions of participants and their acquaintances. This highlights the need for baseline measures of behavior, as we will propose later, to detect any changes following TRP intake.

TRP has also been shown to modulate behavior in economic decision-making tasks such as the ultimatum game (Güth, Schmittberger, & Schwarze, 1982) and the trust game (Camerer & Weigelt, 1988; Berg, Dickhaut, & McCabe, 1995). These games exemplify important concepts related to social behavior, including empathy, fairness, and altruism (Ebstein, Israel, Chew, Zhong, & Knafo, 2010). For instance, the ultimatum game taps into the trade-off between decisions motivated by fairness versus selfishness. In this game, participants are usually asked to make a proposal with regard to a distribution of money among the participant and another player, and/or to accept or reject a proposal by another (fake) participant. If the proposal is not accepted, both players receive nothing. A study by Cerit, Schuur, de Bruijn, and van der Does (2015), in which participants received 2.8 gr of TRP or placebo for six days, suggested no significant effect of TRP on behavior in the ultimatum game. However, an additional analysis in which seven participants who accepted all offers post-intervention were excluded showed an increase in rejections of very unfair offers in the TRP group compared the placebo group. As pointed out by the authors, this outcome seems to challenge idea that TRP can promote prosocial behavior, although it may be explained by the fact that on the testing day participants did not consume TRP. Specifically, according to the authors, this may have caused a relative depletion as compared to previous days, thereby inducing an outcome that one would expect as a consequence of TRP depletion (Crockett, Clark, Tabibnia, Lieverman, & Robbins, 2008). However, a fact questioning this possibility is that, within the same study, TRP supplementation was also found to reduce the

physiological response to stress (i.e., cortisol level; for consistent results, see also Cerit, Jans, & van der Does, 2013). Indeed, cortisol responses are strongly related to the automatic processing of emotional information (Ellenbogen, Carson, & Pishva, 2010), with lower cortisol responses indicating less reactivity to stressors. Therefore, the lower cortisol response to stressors following TRP, as found in the study by Cerit et al (2013; 2015) is might indicate TRP supplementation induced a positive bias in information processing, which does not fit with the relative depletion account advocated by the authors.. An alternative interpretation of the finding that TRP increased instead of reduced rejection rates is related to the idea that reciprocity is important for cooperation, which consists of a combination of altruistic rewarding and altruistic punishment (i.e., imposing sanctions on others who violate norms; Fehr & Fischbacher, 2003). As such, if one considers the rejection of unfair offers to reflect altruistic behavior, i.e. altruistic punishment, then an increase of rejections after TRP can in fact represent a form of prosocial behavior.

Finally, research has found TRP can promote interpersonal trust as measured in a trust game—a task that quantifies the extent to which one participant (the trustor) trusts another participant (the trustee), as indicated by money transferred from trustor to trustee (Camerer & Weigelt, 1988). In line with the idea that TRP supplementation might promote agreeable, prosocial behavior, after the ingestion of 0.8 gr TRP, participants transferred significantly more euros to the trustee than after intake of the placebo, an indication of increased interpersonal trust (Colzato et al., 2013). Consistent with these findings, acute TRP supplementation (0.8 gr) has also been found to promote charitable donating by almost doubling the amount of money participants donated to charity, as compared to the placebo condition (Steenbergen, Sellaro, & Colzato, 2014).

All in all these results suggest that TRP supplementation, resulting in enhanced 5-HT functioning, has promising potential to promote positive social, i.e. agreeable, prosocial behavior.

3. Factors modulating TRP effectivity

As mentioned in the Introduction, TRP effects on 5-HT synthesis and functioning seem to depend on a variety of factors, such as the competition between LNAAs (see "Mechanism of action" section), neuronal activity, enzymatic activity, genetic variability, gender, age, and the amount of TRP contained in one's diet (Young, 2013). These factors potentially explain part of the variability in TRP effectivity, both within and between individuals.

It has been shown that, at least in animals, the intake of TRP significantly decreases the firing rate of serotonergic neurons (Trulson & Jacobs, 1976). Interestingly, this is also the case for the administration of selective 5-HT reuptake inhibitors, which are supposed to increase 5-HT availability in the synapse (Fischer, Jocham, & Ullsperger, 2015). Altering serotonergic levels via TRP supplementation is most likely to influence the rate of 5-HT release when neurons are firing at a high rate (Trulson & Jacobs, 1976). This leads to the possibility that effects of TRP administration might be most effective in circumstances under which the firing rate of 5-HT neurons is increased, for instance when showing a high level of behavioral arousal (Young, Pihl, & Ervin, 1988; Young, 2013), which, at least in animals, has been found to determine the amount of release of 5-HT (Rueter, Fornal, & Jacobs, 1997). However, in the aforementioned study by Pihl et al. (1995), in which arousal levels were supposedly increased by delivering shocks to the participants before being confronted with an aggression-provoking task, effects of TRP were only observed in the low arousal condition (i.e., when the intensity of the shocks was below the pain threshold) but not in the high arousal condition (i.e., when the intensity of the shocks was above the pain threshold). This suggests that the supposed relationship between arousal levels and TRP efficacy may apply only to situations in which arousal levels are moderately high.

As the TPH enzyme is essential to converting TRP into 5-HT, it is important to consider the conversion of TRP takes place in two locations via two different types of enzyme: the gut (TPH1) and the brain (TPH2) (Walther et al., 2003). Since 5-HT cannot pass the BBB whereas TRP can, all available 5-HT in the brain depends on the conversion of TRP to 5-HT by

TPH2 after TRP has passed the BBB. Hence, if the TPH1 enzyme in the gut is very active, more TRP is converted there and less will be available to pass through the BBB and be converted into 5-HT in the brain. Thus TRP might have less impact on social behavior in individuals with highly active TPH1 enzyme.

Another source of variability in the effectiveness of TRP supplementation may be vitamin B and D availability. Indeed, activation of the TPH2 enzyme, involved in the first step of the conversion of TRP into 5-HT in the brain (Walther et al., 2003; Gutknecht, Kriegebaum, Waider, Schmitt, & Lesch, 2009), depends on vitamin D hormone availability (Haussler, Jurutka, Mizwicki, & Norman, 2011; Hsieh et al., 2013; Patrick & Ames, 2014). Also, the decarboxylase enzyme involved in the last step of the conversion of TRP in 5-HT needs vitamin B6 (pyridoxine) as a cofactor in order to convert 5-HTP into 5-HT. Accordingly, even though vitamin B6 is not a precursor of 5-HT, it can be considered a rate-limiting factor in the final step of 5-HT synthesis (Calderón-Guzmán et al., 2004; Deac et al., 2015). For these reasons, it is often advised to take vitamin B and D supplements along with TRP. It is possible TRP might have reduced effectivity in individuals with deficient vitamin B and D levels.

Furthermore, variations in genes associated with serotonergic functioning might contribute to inter-individual variability in response to TRP. Two examples of relevant genes are the TPH gene and the serotonin transporter gene (5-HTTLPR). In the TPH gene, the A-C polymorphism seems to play an important, functional role. Although the exact role of this gene in the activity of TPH is unclear, A-carriers (A2051C) have lower levels of 5-HIAA, the main metabolite of 5-HT, as compared to C-carriers (Chen et al., 2010), suggesting reduced 5-HT transmission. In addition, A-carriers (A218C and A779C) show higher levels of aggression and explicit anger (Manuck et al., 1999; Reuter & Hennig, 2005). These findings suggest two contrasting hypotheses regarding the potential effect of this polymorphism on TRP effectivity. On the one hand, A-carriers demonstrate elevated levels of aggression and explicit anger, suggesting much room for improvement following TRP supplementation. Alternatively, their reduced 5-HT activity might actually lead to less impact of TRP based on the hypothesis that TRP is especially effective when the firing rate of serotonergic neurons is high.

Currently it is not yet clear if and in which direction this polymorphism predicts response to TRP supplementation and more research would be valuable to answer this question.

The second functionally relevant polymorphism is in the 5-HTTLPR gene, which can have either long (l) or short (s) alleles. In homozygotic carriers of the "l" allele, expression of the serotonin transporter (5-HTT) is higher and the reuptake of 5-HT is almost double as compared to heterozygous or homozygous carriers of the "s" allele (Heils et al., 1996). This increased reuptake of 5-HT suggests l-carriers have less 5-HT activity than s-carriers, but, counterintuitively, s-carriers seem to be the ones who have lower 5-HT function (Bethea et al., 2004). Correspondingly, they demonstrate increased risk for depression (Caspi et al., 2003) and higher levels of trait anxiety (Lesch et al., 1996). It has been proposed the counterintuitive effect of transport availability on serotonergic activity may be due to the polymorphism's effect on early brain development (Bethea et al., 2004), leading to adaptions persisting into adulthood. Since s-carriers have reduced 5-HT function, it is possible they are more reactive to 5-HT manipulations (Cerit, Jans, & van der Does, 2013) and benefit more from TRP supplementation than l-carriers. However, as is the case with the TPH polymorphism, it is also possible reduced 5-HT activity might actually lead to less effect of TRP, as TRP might be most effective in neurons with high firing rates. In contrast, one study showed TRP reduced the physiological response to social stress in s-carriers, but not l-carriers (Cerit, Jans, & van der Does, 2013). Thus, it seems that s-carriers may benefit more from TRP supplementation than l-carriers. Furthermore, it is important to note there is an A-G polymorphism in the "l" allele of the 5-HTTLPR gene, leading to the distinction between an "l_A" and "l_G" variant, with the "l_G" variant being functionally similar to the "s" allele (Hu et al., 2006). This raises the possibility that both the "s" and "l_G" allele may be associated with stronger responses to TRP supplementation. Hence, disregarding the A-G polymorphism in the "l" allele might lead to an underestimation of the 5-HTTLPR gene's influence on TRP supplementation. For this reason we strongly recommend future studies to consider both these polymorphisms, to explain variability in TRP response within samples and across studies.

Another potential determinant of TRP effects is inter-subject and inter-sample variability in several factors. For example, variation in body mass index (BMI) might lead to different substance concentration levels when the same dose is given to all participants. Interestingly, only one of the above discussed studies included individualized dosages (Nemzer, Arnold, Votolato, & McConnell, 1986). It would be interesting to investigate whether individual differences in TRP effectivity might perhaps be explained by an individual's BMI. This would promote the use of individualized dosages (e.g. a dosage of X mg per kg of bodyweight instead of the same dosage for everyone), thereby increasing the chance to demonstrate consistent and replicable findings with TRP. Moreover, gender might modulate the efficacy of TRP supplementation, since 5-HT synthesis seems to be lower in females than in males (Nishizawa et al., 1997; Sakai et al., 2006). Consistent with this finding, TRP depletion lowered mood in women but not in men (Ellenbogen et al., 1996). Furthermore, age can significantly influence both serotonergic functioning and (pro)social behavior. For instance, ageing has been related to decreases in 5-HT availability, receptors, transporters, and enzymes (Fidalgo, Ivanov, & Wood, 2013). In addition, at least in animals, aging and age-related diseases are associated with unbalanced TRP metabolism (van der Goot & Nollen, 2013). However, empathy and prosocial behavior have been found to improve with increasing age (Sze, Gyurak, Goodkind, & Levenson, 2012), although as pointed out by the authors themselves, a cross-sectional design cannot exclude the possibility that reported age differences in empathy and prosocial behavior may in fact be explained by historical factors or contemporary social roles instead of age per se. For instance, in this study, study members of the older cohort grew up just after the second world war, and their experiences of being in need and experiencing distress in those times might have contributed to enhanced empathy and more prosocial behavior towards others in need. Additionally, old age might be associated with reduced self-sufficiency and, as a consequence, increased dependency on more prosocial behavior towards others. In sum, more longitudinal research that also takes into account contemporary social roles is needed to disentangle and understand the relation between TRP, 5-HT, ageing, and social behavior.

Lastly we would like to stress the importance of baseline levels of social behaviors or related measures. This suggestion is based on the finding of Crockett, Clark, Hauser, and Robbins (2010), who showed citalopram only affected moral judgments in those who reported higher baseline empathy levels. Given that one mechanism by which TRP could act is through the biasing of information processing towards positive stimuli, this suggests that in individuals with low 5-HT, the initial bias towards negative stimuli might be greatest and hence they could benefit most from an increase in 5-HT. However, evidence for a relation between TRP efficacy and initial 5-HT state is still controversial (Silber & Schmitt, 2010). Related to the previous point, extensive research is needed to investigate the possible long-term effects of TRP. This issue is particularly important when placebo-controlled within-subjects designs are implemented, as it may help to set the appropriate distance between two or more critical sessions (e.g., placebo and TRP; for evidence suggesting the importance of this issue, see e.g., the results reported by Moskowitz, Pinard, Zuroff, Annable, & Young, 2001).

4. Conclusion

As the biochemical precursor of 5-HT, TRP has the potential to enhance serotonergic function in the brain. There is promising utility of TRP supplementation for patients or individuals suffering from disorders or behaviors related to dysfunctions in the 5-HT system, as TRP might help improve control over negative social behavior such as aggression, although studies on this issue are still limited. There is also promising potential of TRP for promoting positive social behavior such as agreeableness, sharing, helping, donating in healthy humans. This suggests TRP supplementation may be a useful tool to enhance social functioning in inexpensive and efficient ways.

TRP, through stimulating 5-HT synthesis, possibly acts by inducing a positive bias in information processing, leading to more attentiveness to positive stimuli and, as a consequence, less negative (e.g. aggressive), social behavior. This suggests the effect of TRP on social behaviors may be strongest for individuals with low baseline 5-HT functioning, as their initial

bias towards negative stimuli might be greatest. However, evidence for this relation between TRP efficacy and initial 5-HT state is still controversial (Silber & Schmitt, 2010).

It is important to acknowledge that, aside from inducing a bias in information processing, the modulating effect of TRP on social behavior might also be mediated by other pathways. For instance, TRP administration and increases in brain TRP are also associated with better quality of sleep and better mood (for a review see Silber & Schmitt, 2010), which might impact behavior in several ways. The relationship between TRP and quality of sleep is not surprising if one considers that 5-HT is also the precursor of melatonin, which plays an important role in regulating the sleep-wake cycle (Richardson, 2005). Furthermore, TRP is reckoned to have a mild sedative effect, possibly due to the increase in melatonin production associated with the increase in 5-HT levels (Ruddick, 2006; Bravo et al., 2012). Such a relation may explain, for example, the positive effects that TRP can have on impulsive behavior (Silber & Schmitt, 2010).

The relation between TRP and mood may represent an alternative pathway through which TRP can affect social behavior. As pointed out by Young (2013), given that increases in 5-HT may have positive effects on mood, and as better mood is typically associated with more positive social interactions, the effects of TRP in promoting social behavior may just reflect the consequence of better mood following TRP intake, though the opposite may be true as well.

Interestingly, improved sleep and mood are related to reduced stress and better coping abilities (e.g. Markus et al., 2000). When experiencing stress, people tend to behave and process information in ways that are less resource demanding (Starcke & Brand, 2012). As taking into account the mental states of others can be considered resource demanding (e.g., Epley, Keysar, Van Boven, & Gilovich, 2004), a stressed individual may tend towards behaviors and processes that are more egocentric or self-centered (i.e., to behave less prosocially; but see von Dawans, Fischbacher, Kirschbaum, Fehr, & Heinrichs, 2012) – a possibility that, however, may apply only to men, as females seem to be able to express accurate social responses during stress; Tomova, von Dawans, Heinrichs, Silani, & Lamm, 2014). Therefore, one may argue that, at least in men, TRP may improve

social behavior by reducing stress. However, the lack of a straightforward relation between TRP, stress and social behavior makes this possibility highly speculative.

Taken together, the aforementioned observations cannot allow one to rule out that at least some of the effects of TRP administration on social behavior might in fact be a result of enhanced sleep and mood, and/or reduced stress. This warrants further investigations in order to understand the potential role of these factors in mediating TRP effects on social behavior.

Another important consideration pertains to the fact that TRP can be metabolized not only through the 5-HT pathway but also through the kynurenine (KYN) pathway. In fact, outside the central nervous system, only one percent of dietary TRP is converted into 5-HT. That is, the majority of TRP is catabolized along the KYN pathway (Russo et al., 2003). In the first step of this metabolic way, TRP is transformed into KYN. Next, KYN is converted to a series of metabolites, such as 3-hydroxykynurenine and quinolinic acid (for a detailed explanation of this oxidative pathway, see Russo et al., 2003). Interestingly, KYN can pass the blood brain barrier and lead to the production of neuroactive metabolites that modulate glutamatergic and cholinergic signaling (Capuron & Miller, 2011), suggesting TRP effects are not mediated solely by 5-HT. This might be particularly true for females suffering from irritable bowel syndrome, as they show an increase of TRP catabolism along the KYN pathway, which contributes to the abnormal 5-HT functioning in this syndrome (Fitzgerald et al., 2008). Hence, taking into account individual differences in TRP metabolism (e.g. the amount of TRP metabolized via the KYN pathway vs. via the 5-HT pathway) may provide important insights into the effectivity of TRP in modulating social behavior.

We would like to point out that in laboratory studies such as the ones discussed in this review, social behavior is typically measured by attitudes, behavioral indicators of helping or self-reported intent to help, aggressiveness, etc. However, more direct behavioral measures such as charitable donating (Steenbergen, Sellaro, & Colzato, 2014) and aggressive incidents (Morand, Young, & Ervin, 1983) are sometimes used as well. Aside from the possibility that attitudes assess socially desirable responses and

not behavior per se, the variability in measures used to assess social behavior makes the reviewed studies hard to compare. We would therefore like to call for more direct measures of social behavior to be used in future studies on TRP supplementation, as this may help gaining a better understanding on how TRP can affect social behavior in real-life situations, outside the lab. In addition, other measures of prosocial behavior that have not yet been investigated in relation to TRP, such as how much time people are willing to spend with others (Farrelly, Moan, White, & Young, 2015), could be considered as well.

Although more research is needed to disentangle and understand the relation between individual differences, TRP effectivity, 5-HT functioning, and social contexts and interactions, we conclude TRP can be an effective method of modulating social behavior.

Chapter nine

A randomized controlled trial to test the effect of multispecies probiotics on cognitive reactivity to sad mood

Steenbergen, L., Sellaro, R., van Hemert, S., Bosch, J.A., & Colzato, L.S. (2015). A randomized controlled trial to test the effect of multispecies probiotics on cognitive reactivity to sad mood. *Brain, Behavior, and Immunity, 48,* 258-264. doi:10.1016/j.bbi.2015.04.003

Abstract

Recent insights into the role of the human microbiota in cognitive and affective functioning have led to the hypothesis that probiotic supplementation may act as an adjuvant strategy to ameliorate or prevent depression. Heightened cognitive reactivity to normal, transient changes in sad mood is an established marker of vulnerability to depression and is considered an important target for interventions. The present study aimed to test if a multispecies probiotic containing Bifidobacterium bifidum W23, Bifidobacterium lactis W52, Lactobacillus acidophilus W37, Lactobacillus brevis W63, Lactobacillus casei W56, Lactobacillus salivarius W24, and Lactococcus lactis (W19 and W58) may reduce cognitive reactivity in non-depressed individuals. In a triple-blind, placebo-controlled, randomized, pre- and post-intervention assessment design, 20 healthy participants without current mood disorder received a 4-week probiotic food-supplement intervention with the multispecies probiotics, while 20 control participants received an inert placebo for the same period. In the pre- and post-intervention assessment, cognitive reactivity to sad mood was assessed using the revised Leiden index of depression sensitivity scale. Compared to participants who received the placebo intervention, participants who received the 4-week multispecies probiotics intervention showed a significantly reduced overall cognitive reactivity to sad mood, which was largely accounted for by reduced rumination and aggressive thoughts. These results provide the first evidence that the intake of probiotics may help reduce negative thoughts associated with sad mood. Probiotics supplementation warrants further research as a potential preventive strategy for depression.

1. Introduction

The intestine and the brain are intimately connected via the brain-gut axis, which involves bidirectional communication via neural, endocrine and immune pathways (Grossman, 1979; Grenham, Clarke, Cryan, & Dinan, 2011; Mayer, 2011; Mayer, Naliboff, & Craig, 2006). In recent years it has become increasingly evident that this communication also involves interactions with the intestinal microbiota, which release immune activating and other signaling molecules that may play an important role in regulating the brain and subsequent behavior (Mayer, 2011; Cryan & Dinan, 2012; Foster & McVey Neufeld, 2013). For example, the microbiota produce neuroactive substances and their precursors (e.g., tryptophan) which can reach the brain via endocrine and afferent autonomic pathways (Desbonnet et al., 2008, 2010). Also, bacterial products, such as the gram-negative endotoxins, can influence mood and cognitive functions via indirect (e.g., immune activation) and direct (e.g., Toll-like receptors on glial cells) mechanisms (Lehnardt et al., 2003; Krabbe et al., 2005; Ait-Belgnaoui et al., 2012; McCusker & Kelley, 2013).

These novel insights have fueled the hypothesis that modification of microbial ecology, for example by supplements containing microbial species (probiotics), may be used therapeutically to modify stress responses and symptoms of anxiety and depression (Logan & Katzman, 2005; Cryan & O'Mahony, 2011). While most of this research is relatively recent, and predominantly involves animal and pre-clinical human studies, the results appear in support of this hypothesis (Logan & Katzman, 2005; Cryan & Dinan, 2012; Foster & McVey Neufeld, 2013; Tillisch, 2014; Savignac, Tramullas, Kiely, Dinan, & Cryan, 2015). For instance, Bravo et al. (2011) observed a reduction in anxious and depressive behavior after feeding healthy mice with *Lactobacillus rhamnosus* JB-1. Similarly, Desbonnet et al. (2010) observed a reduction in depressive-like behaviors in adult rats after feeding them with *Bifidobacterium infantis* 35624. This reduction was comparable to the effects of administering the antidepressant citalopram (Desbonnet et al., 2010). Probiotic studies in humans are still scarce, but the available data are

promising. For example, Benton, Williams and Brown (2006) found in a non-clinical sample that a 3-week intervention with probiotics-containing milk drink (i.e., *Lactobacillus casei* Shirota) improved mood scores compared to participants who received a placebo intervention. Improvement in mood was only observed for participants who showed elevated symptoms of depression at baseline. In another pre-clinical study it was demonstrated that participants who were given a mixture of probiotics containing *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 showed significantly less psychological distress than matched controls (Messaoudi et al., 2011). Furthermore, Rao et al. (2009) demonstrated that patients with chronic fatigue syndrome, which is often comorbid with anxiety disorders, reported significantly less anxiety symptoms after ingestion of a daily dose of *L. casei* Shirota for 2 months, as compared to a placebo group. On the basis of these and other results it has been suggested that probiotics may serve as adjuvant or preventive therapy for depression (for reviews see Logan & Katzman, 2005; Cryan & Dinan, 2012; Foster & McVey Neufeld, 2013; Tillisch, 2014).

These novel discoveries come at an opportune time. The increasing incidence of depression is alarming and development of preventive measures has been identified as a priority (World Health Organization, 2012). According to cognitive theories of depression, cognitive reactivity plays a central role in the development, maintenance, and recurrence of depression and therefore is a relevant target for interventions (Beck, 1967, Kovacs & Beck, 1978; Abramson, Metalsky, & Alloy, 1989; Haaga, Dyck, & Ernst, 1991; Scher, Ingram, & Segal, 2005; Ingram, Mirand, & Segal, 2006). Cognitive reactivity refers to the activation of dysfunctional patterns of thinking that are triggered by subtle changes in mood, such as ruminative (e.g., recurrent thoughts about possible causes and consequences of one's distress), aggressive (e.g., to think about hurting others or oneself), hopelessness (e.g., loss of motivation and expectations about the future), and/or suicidal thoughts (e.g., to think that one's death is the only way to end the suffering). Such dysfunctional cognitive responses are assumed to stem from latent negative beliefs that become reactivated during low mood (Beck, 1967).

The degree to which these dysfunctional thoughts are activated seems to be critical in determining whether sad mood will be a transient state or will become protracted, increasing the risk of developing clinical depression (Beck, 1967; Kovacs & Beck, 1978 Abramson, Metalsky, & Alloy, 1989; Haaga, Dyck, & Ernst, 1991; Scher, Ingram, & Segal, 2005; Ingram, Mirand, & Segal, 2006). Indeed, cognitive reactivity is considered one of the most predictive vulnerability markers of depression (Beck, 1967; Segal, Gemar, & Williams, 1999; Segal et al., 2006; Moulds et al., 2008). Among these dysfunctional thought patterns, rumination seems to be particularly relevant (Nolen-Hoeksema, Morrow, & Fredrickson, 1993; Kuehner & Weber, 1999; Nolen-Hoeksema, 2000; Spasojevic & Alloy, 2001; Moulds et al., 2008). For instance, Moulds et al. (2008) showed that recovered and never-depressed individuals mainly differ in the degree of activation of ruminative thoughts when experiencing sad mood. Evidence strongly suggesting a causal role of cognitive reactivity in depression onset is provided by a recent study of Kruijt et al. (2013), who showed that higher cognitive reactivity precedes and predicts the episode of depression: never-depressed individuals with high scores on cognitive reactivity were more likely to develop a clinical depression during the subsequent two years, as compared to individuals with lower scores (see also van der Does, 2005, for a review). These associations were independent of a range of confounding factors including baseline mood, life events, and family history of mood disorders (Kruijt et al., 2013). Thus, interventions targeting cognitive reactivity may offer a promising approach to prevent and/or to reduce the incidence of depression-related disorders in the population.

In light of the preceding discussion, the present study aimed to complement previous findings by assessing the possible beneficial effect of probiotics on cognitive reactivity to sad mood, a vulnerability marker for depression. To this end, healthy individuals without any current mood disorder underwent a 4-week intervention period, during which they were supplied with either probiotics or an inert placebo. We tested the effect of multispecies probiotics containing different stains and species of the genera *Lactobacillus*, *Lactococcus* and *Bifidobacterium* (see methods for further details). These genera have been found to be effective in ameliorating anxious and depressive symptoms (Benton, Williams, Browns, 2006; Rao et

al., 2009; Yamamura et al., 2009; Desbonnet et al., 2010; Bravo et al., 2011; Messaoudi et al., 2011).

Importantly, studies have shown that multispecies probiotics (i.e., combining different strains of specific genera) can have increased effectiveness through an additive effect of specific strain properties such as colonization of different niches, enhanced adhesion and induction of an optimal pH range, as compared to mono-species supplements (Timmerman et al., 2004; Chapman, Gibson, & Rowland, 2011). Each bacterial strain of the multispecies probiotics used in this study has been found to improve epithelial barrier function both when tested separately and in combination (Van Hemert & Ormel, 2014). However, some probiotics may compete with each other in terms of functionality and therefore the assumption that combinations of different strains may have additive effects needs verification on a preparation by preparation basis.

Before and after the intervention, perceived cognitive reactivity to transient changes in sad mood was measured by means of the revised Leiden Index of Depression Sensitivity (LEIDS-r; van der Does & Williams, 2003), which has been shown to be predictive of depression in multiple longitudinal studies (van der Does, 2005; Kruijt et al., 2013). It was hypothesized that the probiotics intervention would lower the activation of negative thoughts that accompany sad mood, i.e., it would decrease cognitive reactivity as measured by the LEIDS-r.

2. Method

2.1. Participants

Forty non-smoking young adults, with no reported cardiac, renal, or hepatic conditions, no allergies or intolerance to lactose or gluten, no prescribed medication or drug use, and who reported to consume no more than 3–5 alcohol units per week participated in the study. All participants were screened via a phone interview by the experiment leader before inclusion. During the phone interview, the Mini International Neuropsychiatric Interview (M.I.N.I.; Sheehan et al., 1998) was administered too. The M.I.N.I.

is a short structured interview, taking about 15 min, which screens for several psychiatric disorders (Sheehan et al., 1998, Colzato & Hommel, 2008; Colzato et al., 2013). Participants with no psychiatric or neurological disorders, no personal or family history of depression or migraine were considered suitable to take part in the study. Participants were equally and randomly assigned to receive a 4-week intervention of either placebo or probiotics. Twenty participants (3 male) with a mean age of 19.7 years (SD = 1.7) and a mean body mass index (BMI) of 21.5 (SD = 2.0) were assigned to the placebo condition, and twenty participants (5 male) with a mean age of 20.2 years (SD = 2.4) and a mean BMI of 22.6 (SD = 2.2) were assigned to the probiotics condition (see Table 1). Female participants were not controlled for the menstrual cycle. No information was provided about the different types of intervention (probiotics vs. placebo) or about the hypotheses concerning the outcome of the experiment. All participants believed they were supplied with probiotic supplementation. When informed about the different conditions during the debriefing, none of the participants brought up the deception. Written informed consent was obtained from all participants and the protocol was approved by the local ethical committee (Leiden University, Institute for Psychological Research).

Table 1. Demographic characteristics for the Placebo and Probiotics groups. Standard deviations are shown within parentheses

	Placebo	Probiotics
N(M:F)	20(3:17)	20(5:15)
Age (years)	19.7(1.7)	20.2(2.4)
Body Mass Index (BMI)	21.5(2.0)	22.6(2.3)

2.2. Apparatus and procedure

A blind at three levels (group allocator, participants, outcome assessor), placebo-controlled, randomized, pre- and post-intervention assessment design was used to investigate the effect of multispecies probiotic

intervention on cognitive reactivity to sad mood, as well as reported symptoms of depression and anxiety in healthy young students. Participants received a 4-week food supplementation intervention of either placebo or probiotics. In the probiotics intervention participants were provided with 28 sachets (one for each day of intervention), each containing 2 g freeze-dried powder of the probiotic mixture Ecologic®Barrier (Winclove probiotics, The Netherlands). Ecologic®Barrier (2.5 × 109 CFU/g) contains the following bacterial stains: *Bifidobacterium bifidum* W23, *Bifidobacterium lactis* W52, *Lactobacillus acidophilus* W37, *Lactobacillus brevis* W63, *L. casei* W56, *Lactobacillus salivarius* W24, and *Lactococcus lactis* (W19 and W58). In the placebo intervention, participants were provided with 28 sachets, each containing 2 g freeze-dried powder of the carrier of the probiotic product: maize starch and maltodextrins. The placebo was indistinguishable from the probiotics sachets in color, taste, and smell, but contained no bacteria. The bacteria in Ecologic Barrier have been identified by using 16S rRNA sequencing and the results have been compared with the bacterial nucleotide database of the National Center for Biotechnology Information (NCBI). The viability of the probiotic bacteria was checked both by the producer and by an independent lab (Institut für Mikroökologie GmbH, Herborn, Germany, specialized in microbial analysis, ISO15189 certificated) by determining the number of colony forming units. 1 g of the product was mixed well with 9 ml of a physiological salt solution (0.9% NaCl in ddH_2O). This mixture was tenfold serial diluted in the same physiological salt solution, and 50 µl of each dilution was plated on Mann Rogosa Sharpe (MRS) + 0.5% cysteine agar plates. The plates were incubated anaerobically for 48–72 h at 37 °C. The number of colonies was counted and the total number of colony forming units was calculated based on the dilution and the number of colonies. The batch used for the present experiments contained $>2.5 \times 10^9$ CFU/g, whereas the placebo contained $<1 \times 10^4$ CFU/g. Rehydration of freeze-dried lactic acid bacteria in milk, water and physiological salt solution has been shown to result in equal survival rates (de Valdez et al., 1985). Stability studies, whereby the number of colony forming units was determined every three months, showed that the freeze-

dried product is stable for at least 1.5 years when stored at 25 °C with 60% relative humidity.

At the pre- and post-intervention assessments, participants filled out a questionnaire to assess cognitive reactivity to sad mood and questionnaires that assessed symptoms of depression and anxiety. E-prime 2.0 software system (Psychology Software Tools, Inc., Pittsburgh, PA) was used to present the questionnaires and to collect participants' responses, which were to be given using the computer mouse. After having filled out the questionnaires, participants performed two social cognitive tasks tapping into reactions to fairness (ultimatum game) and interpersonal trust (trust game) unrelated to the purposes of the present study (data not reported here). In each session, the complete test battery lasted about 20 min.

At the end of the pre-intervention assessment, participants were provided with the 28 sachets of powder (containing either the inert placebo or the multispecies probiotics) for the 4-week intervention. Participants were instructed, using their own supplies, to dissolve the powder in water or lukewarm milk and to drink it in the evening before going to bed. Compliance was facilitated by reminding the participants via a text message sent by the experimenter.

2.2.1. Questionnaires

The LEIDS-r (van der Does & Williams, 2003) is a self-report questionnaire with 34 items that assesses to what extent dysfunctional thoughts are activated when experiencing mild dysphoria (i.e., it measures cognitive reactivity to sad mood, also referred to as vulnerability to depression). LEIDS-r scores have been found to predict depression incidence in multiple longitudinal studies and to correlate with depression risk factors, such as depression history (Moulds et al., 2008), genetic markers of depression (Antypa & van der Does, 2010), and reaction to tryptophan depletion (Booij & van der Does, 2007). Before answering the items, participants were asked to take a few minutes to imagine how they would feel and think if they were to experience a sad mood and then to indicate, on a 5-point Likert scale ranging from 0 (i.e. 'not at all') to 4 ('very

strongly'), the extent to which each statement applied to them. It was emphasized that the statements applied to the situations when "it is certainly not a good day, but you don't feel truly down or depressed". The scale consists of six subscales that measure vulnerability with respect to:

- Aggression (e.g.,When I feel down, I lose my temper more easily);
- Hopelessness/Suicidality (e.g., When I feel down, I more often feel hopeless about everything; When I feel sad, I feel more that people would be better off if I were dead);
- Acceptance/Coping (e.g., When I am sad, I feel more like myself);
- Control/Perfectionism (e.g., I work harder when I feel down);
- Risk aversion (e.g., When I feel down, I take fewer risks);
- Rumination (e.g., When I feel sad, I more often think about how my life could have been different).

Hopelessness and Acceptance/Coping both consist of 5 items, with a maximum score of 20 per subscale, whereas the other scales comprise 6 items with a maximum score of 24 per subscale. The LEIDS-r total score is derived by adding up the scores from each subscale, resulting in total scores ranging from 0 to 136. Internal consistency (Cronbach's alpha; α) is 0.89 for the LEIDS total score, and ranges between 0.62 (Acceptance/Coping) and 0.84 for the subscales (Hopelessness/Suicidality; Antypa & Van der Does, 2010; Williams et al., 2008).

The Beck Depression Inventory II (BDI-II; Beck, Steer, Ball, & Ranieri, 1996) is a widely used 21-itemmultiple-choice self-report questionnaire with high internal consistency (α = .91; Beck, Steer, Ball, & Ranieri, 1996), which assesses the existence and severity of current (past 2 weeks) depressive symptoms. The study used the Dutch translation validated by Van der Does (2002b). The BDI–II has been found to be a valid indicator of depression and show good diagnostic discrimination (Dozois, Dobson, & Ahnberg, 1998). Participants were presented with items related to symptoms of depression and asked to choose, for each item, the statement that best described how they have been feeling during the past 2 weeks (including the current day). Items are rated on a 4-point scale ranging from

0 to 3 in terms of severity. The total score is calculated by adding-up all items, hence scores range between 0 and 63 (0–13: minimal depression, 14–19: mild depression, 20–28: moderate depression and 29–63: severe depression; van der Does, 2002a).

The Beck Anxiety Inventory (BAI) (Beck, Epstein, Brown, & Steer, 1988) is a 21-item self-report questionnaire with high internal consistency (α = .90; Beck & Steer, 1993), which assesses the existence and severity of anxiety symptoms. A validated Dutch translation was used (Bouman, 1994). Participants are presented with items describing common symptoms of anxiety (such as numbness and tingling, sweating not due to heat, and fear of the worst happening) and asked to rate, on a 4-point Likert scale (0, not at all, 1, mildly, 2, moderately, 3, severely), how much they have been bothered by each symptom over the past week. Total scores are obtained by summing all items, with values ranging between 0 and 63 (as suggested by Beck & Steer (1993); 0–9: normal anxiety; 10–18: mild-moderate; 19–29: moderate-severe and 30–63: severe anxiety).

2.3. Statistical analyses

For each questionnaire, the mean scores (total and/or partial) were calculated and submitted to a repeated measures analysis of variance (ANOVA) with time (pre- vs. post-intervention) as within-subjects factor and group (placebo vs. probiotics) as between-subjects factor. All alpha levels were set at p = .05. Tukey HSD post hoc tests were performed to clarify mean differences in case of significant interactions.

In addition to standard statistical methods, we calculated Bayesian (posterior) probabilities associated with the occurrence of the null [$p(H_0|D)$] and alternative [$p(H_1|D)$] hypotheses, given the observed data. Bayesian inference allows making inferences about both significant and non-significant effects by providing the exact probability of their occurrence. The probabilities range from with 0 (i.e., no evidence) to 1 (i.e., very strong evidence; see Raftery, 1995). To calculate Bayesian probabilities we used the method proposed by Wagenmakers (2007) and Masson (2011). This method uses Bayesian information criteria (BIC), calculated using a simple transformation of sum-of-squares values generated by the standard

ANOVA, to estimate Bayes factors and generate $p(H_0|D)$ and $p(H_1|D)$, assuming a "unit information prior" (for further details, see Kass & Wasserman, 1995; see also Jarosz & Wiley, 2014).

Due to a technical problem, one participant, assigned to the placebo group, did not fill out the pre-intervention BAI questionnaire. No other data were missing.

3. Results

3.1. Randomization

Table 1 presents the participant characteristics by group (probiotics versus placebo). No significant group differences were observed for age [$t(38) = -0.76$, $p = 0.45$], BMI[$t(38) = -1.64$, $p = 0.11$], and gender distribution [χ^2 (1, $N = 40$) = 0.63, $p = 0.43$].

Table 2 gives a summary of pre- and post-intervention scores on the LEIDS-R, BDI and BAI in the placebo and probiotics groups.

As anticipated on basis of participant selection, ANOVA performed on the BDI–II total score revealed no main effect of time [$F(1,38) = .41$, $p = .52$, $p(H_0|D) = .84$], group [$F(1,38) = 1.1$, $p = .31$, $p(H_0|D) = .78$], nor a time by group interaction [$F(1,38) = .41$, $p = .52$, $p(H_0|D) = .84$]. Similarly, for the BAI scores no effect was observed for time [$F(1,37) = 2.30$, $p = .14$, $p(H_0|D) = .66$], group [$F(1,37) = 0.226$, $p = .64$, $p(H_0|D) = .85$], or for the interaction between the two factors [$F(1,37) = 0.064$, $p = .80$, $p_s(H_0|D) = .86$]. Thus, the two groups of participants (placebo and probiotics) were comparable in terms of depression and anxiety scores at baseline and follow-up. Importantly, participants did not show any sign of depression and anxiety in either sessions: only minimal/mild scores were observed at both time points for the BDI–II (the mean scores were 8.53, SD = 4.47, and 8.17, SD = 5.30, for the pre- and post-intervention assessment, respectively) and BAI (the mean scores were 11.77, SD = 7.32, and 10.55, SD = 7.20, the pre- and post-intervention assessment, respectively; see also Table 2).

Table 2. Mean pre- and post-intervention scores and standard error of the means (shown in parentheses) on the LEIDS-r, BDI and BAI in the Placebo and Probiotics groups. * = significant treatment effect differences between pre- and post-intervention assessments.

		Pre-intervention	Post-intervention
LEIDS-r			
Aggression	Placebo	8.80 (0.94)	8.45 (0.98)
	Probiotics**	8.68 (0.94)	6.25 (0.98)
Control	Placebo	7.65 (0.80)	6.70 (0.82)
	Probiotics	7.25 (0.83)	5.80 (0.82)
Hopelessness	Placebo	5.60 (0.85)	4.70 (0.74)
	Probiotics	4.75 (0.85)	4.0 (0.74)
Risk Aversion	Placebo	9.50 (0.93)	9.25 (0.87)
	Probiotics	10.00 (0.93)	7.95 (0.87)
Rumination	Placebo	11.75 (0.90)	11.85 (0.93)
	Probiotics***	11.20 (0.90)	8.25 (0.93)
Acceptance	Placebo	1.40 (0.34)	1.35 (0.37)
	Probiotics	0.90 (0.34)	1.10 (0.37)
Total	Placebo	44.70 (3.24)	42.30 (3.51)
	Probiotics***	42.75 (3.24)	33.35 (3.51)
BDI	Placebo	9.10 (1.00)	9.10 (1.19)
	Probiotics	7.90 (1.00)	7.25 (1.19)

BAI	Placebo	12.21 (1.70)	11.21 (1.69)
	Probiotics	11.35 (1.66)	9.95 (1.65)

* p < .05, ** p < .01., *** p < .001.

3.2. Probiotic treatment and cognitive reactivity

ANOVAs revealed significant time by group interactions for the LEIDS-r total score [$F(1,38) = 6.05$, $p = .019$, $\eta_p^2 = 0.137$, MSE = 40.468, $p(H_1|D) = .79$], aggression [$F(1,38) = 4.94$, $p = .032$, $\eta_p^2 = .115$, MSE = 4.255, $p(H_1|D) = .65$], and rumination [$F(1,38) = 12.16$, $p = .001$, $\eta_p^2 = .242$, MSE = 3.826, $p(H_1|D) = .98$]. Tukey HSD post hoc tests performed to disentangle the interactions revealed that participants who received a 4-week placebo intervention showed comparable scores pre- versus post-intervention (total score: $p = .63$, $p(H_0|D) = .70$; aggression: $p = .95$, $p(H_0|D) = .80$; rumination: $p = 1.0$,$p(H_0|D) = .82$; see Table 2). In contrast, participants who received a 4-week probiotics intervention scored significantly lower at post-intervention compared to the pre-intervention (total score: $p < .001$, $p(H_1|D) > .99$; aggression: $p = .004$, $p(H_1|D) > .99$; rumination: $p < .001$, $p(H_1|D) > .99$; see Table 2). Thus, our results show that the intake of multispecies probiotics for a 4-week period significantly reduced overall cognitive reactivity to depression and in particular aggressive and ruminative thoughts.

4. Discussion

The aim of the current study was to investigate the effect of a multispecies probiotic intervention on cognitive reactivity in healthy individuals not currently diagnosed with a mood disorder. As mentioned in the introduction, cognitive reactivity is an important vulnerability marker of depression; the content and the type of thoughts that are activated when an individual experiences sad mood predicts whether the sad mood will be transient or will persist, and predicts the development of clinical depression (Beck, 1967, Kovacs & Beck, 1978; Abramson, Metalsky, & Alloy,

1989; Haaga, Dyck, & Ernst, 1991; Scher, Ingram, & Segal, 2005; Ingram, Mirand, & Segal, 2006). We found that a 4-week multispecies probiotic intervention reduced self-reported cognitive reactivity to sad mood, as indexed by the LEIDS-r (van der Does & Williams, 2003; van der Does, 2005; Kruijt et al., 2013). Further analyses showed that the strongest beneficial effects were observed for the aggression and rumination subscales, indicating that in the probiotics supplementation condition participants perceived themselves to be less distracted by aggressive and ruminative thoughts when in a sad mood. Notably, studies have shown that the tendency to engage in ruminative thoughts is sufficient to turn mood fluctuations into depressive episodes, and that individuals who typically respond to low mood by ruminating about possible causes and consequences of their state have more difficulties in recovering from depression (Nolen-Hoeksema, Morrow, & Fredrickson, 1993; Kuehner & Weber, 1999; Nolen-Hoeksema, 2000; Spasojevic & Alloy, 2001; Moulds et al., 2008). Further, the activation of aggressive thoughts has been associated with suicidal ideation and attempts (Oquendo, Currier, & Mann, 2006; Mann et al., 2008). In sum, the present results indicate, for the first time, that probiotics intervention can influence cognitive mechanisms that are known to determine vulnerability to mood disorders.

The present sample consisted of healthy individuals with minimal to mild baseline scores on both the BAI and the BDI, and it is not surprising therefore that the beneficial effect of probiotics intervention was selective for cognitive reactivity to depression and not for self-report symptoms of depression or anxiety. This observation is consistent with the findings reported by Benton, Williams, and Brown (2006), who found that improvements in mood after probiotics administration only occurred in participants who showed elevated symptoms of depression at the baseline. Importantly, the selection of a nonclinical sample of participants provided the opportunity to test specifically the possible beneficial effects of probiotics intervention on cognitive reactivity, i.e., not confounded by ongoing depressive symptomatology. Further longitudinal studies in high-risk or clinical groups are necessary to confirm potentially clinically relevant effects. Given that the transition from persistent changes in mood to the

development of a depressive episode can be months or longer, such studies may need to extend past the current 4-week period.

While the present study did not set out to test specific biological mechanisms that could underlie possible beneficial cognitive effects, the extant literature does allow for a number of hypotheses testable in future studies. For example, it has been proposed that intestinal microbiota increase plasma tryptophan levels, and hereby potentially facilitate serotonin turnover in the brain (Desbonnet et al., 2008, 2010). Interestingly, cognitive reactivity to sad mood has been associated with serotonin concentrations, with higher scores correlating with lower serotonin levels (Booij & van der Does, 2007; Wells et al., 2010; see also Firk & Markus, 2009). However, other pathways are plausible as well. For instance, it has been proposed that an increased intestinal permeability can induce depressive symptoms (Ait-Belgnaoui et al., 2012), possibly by endotoxin activated inflammatory pathways or via direct activation of glial and neural cells that carry Toll-like receptors and are hereby responsive to a wide range of microbial products (McCusker & Kelley, 2013). Given that certain probiotics have been found to improve the epithelial barrier function and hereby decrease permeability (Van Hemert, Verwer, & Schütz, 2013), this mechanism might account for the beneficial effects of probiotics on cognitive reactivity. Follow-up probiotics studies could explore this possibility, for example by using the lactulose/mannitol ratio in urine to evaluate intestinal permeability (Teixeira et al., 2014). Animal studies have further suggested that gut-to-brain signals are transmitted via the vagus nerve (ter Horst & Postema, 1997; Tillisch et al., 2013). For example, a study in mice has shown that the supplementation of probiotics has a beneficial effect on anxious and depressive behavior, but only with an intact vagus nerve (Bravo et al., 2011). In humans the vagus nerve reaches, via the locus coeruleus and the raphe nuclei (the principal sources of serotonin released in the brain), the anterior cingulate cortex (ACC) and the prefrontal cortex (PFC; Thayer & Lane, 2007), in particular the mPFC (Mayer, Naliboff, & Craig., 2006) – i.e., one of the brain regions associated with the processing of affective and social information (Adolphs, 2001). Stimulation of the vagus nerve has already been described as a successful method to

treat patients suffering from depression (Nemeroff et al., 2006). Interestingly, Tillisch and colleagues (2013) have found that 4-week intake of a fermented probiotic milk product by healthy women was associated with altered activity of brain regions (e.g., primary interoceptive and somatosensory cortices, and precuneus) that control central processing of emotion and sensation. Therefore, it would be of interest to explore whether the treatment of depressive disorders would further benefit by combining probiotic supplementation with stimulation of the vagus nerve.

The present study has a few limitations that deserve discussion. First, we did not include dietary measures and did not control for consumption of other probiotic products or fermented foods (e.g., yogurt). Hence we cannot exclude that the consumption of probiotics was accompanied by spontaneous dietary changes that may have indirectly accounted for the effect. Second, compliance was facilitated by text message reminders, but not further confirmed e.g., by stool bacterial analysis. However, prior studies which used partly the same bacterial strains have shown presence of the strains in stool samples of healthy volunteers (Koning et al., 2008). A third limitation of the present study is that it tested a predominantly female sample, and generalizability to males is uncertain therefore.

Finally, it is worth noting that our assessment only relied on self-reported cognitive reactivity that, although established as a psychometrically reliable index of cognitive reactivity and found to be predictive of the development of depressive symptoms and depressive disorder (van der Does, 2005; Kruijt et al., 2013), would be considered to provide only indirect information on actual cognitive reactivity at times of low mood. Future studies may therefore expand these observations by experimentally inducing negative mood and/or by including ambulatory measurements, e.g., using experience sampling techniques, to evaluate possible beneficial effects of probiotics.

To conclude, the present study demonstrated, for the first time, that a 4-week multispecies probiotic intervention has a positive effect on cognitive reactivity to naturally occurring changes in sad mood in healthy individuals not currently diagnosed with a depressive disorder. More specifically, the probiotic intervention reduced aggressive and ruminative

thoughts in response to sad mood. These findings provide information on a cognitive mechanism that may be responsible for the positive mood effects of probiotic supplementation (Benton, Williams, & Brown, 2006, Rao et al., 2009, Messaoudi et al., 2011; Logan & Katzman, 2005; Tillisch, 2014). Future studies should investigate the neurobiological underpinnings of these observed effects and test the applicability of the current findings to high-risk and clinical populations.

Conclusion

Cognitive enhancement (i.e., any means aimed at enhancing cognitive performance) has gained great attention over the past years, as the economic problems of the welfare system have boosted interest in procedures and activities that make welfare more affordable for society. Especially with regard to the aging population, there is the need to enhance vitality and healthy aging in order to keep people autonomous. In addition, societies seem to become more individualistic and emphasize the idea that an individual is the director of his or her own life. As such, interest in procedures and activities that help express individual needs as well as to minimize weaknesses and further support strengths has increased rapidly. The present dissertation aimed to not only demonstrate which methods are promising ways to enhance cognition, but also to get a better understanding of the underlying mechanisms of how enhancement methods (e.g., brain stimulation, the intake of food supplements, or playing videogames) can affect cognition and behavior in healthy humans. That is, in order to reach interesting levels of enhancement and in order to be able to eventually possibly apply this to the general public, clear ideas about the mechanisms underlying the cognitive functions one aims to improve are required. Cognitive enhancement is generally aimed at improving executive functions including attentional control, inhibitory control, working memory, and cognitive flexibility, but can be aimed at improving social cognition as well. That is, social cognition and social behavior stem from numerous cognitive processes (e.g. attention), and can therefore be targeted by cognitive enhancement. In this thesis, enhancing effects on both cognitive and social functioning, and the respective underlying mechanisms were therefore discussed.

Brain stimulation techniques allow researchers to infer causal relations between the stimulated neurotransmitter or brain area and the behavioral outcome. An example is transcutaneous vagus nerve stimulation (tVNS), which stimulates the GABA-ergic and noradrenergic systems as well as increases activation in the thalamus, prefrontal cortex and insula. tVNS

provides a relatively safe, healthy, and easy tool to investigate and possibly enhance the functioning of these systems, which are assumed to play a crucial role in action cascading and vicarious ostracism. Although active tVNS led to enhanced action cascading performance (Chapter 1), suggesting a possible causal role for GABA and norepinephrine in action cascading, it did not affect prosocial helping behavior in the Cyberball game (Chapter 2). One major problem with brain stimulation techniques is that, given the sometimes promising findings, the brain-training industry is bringing these techniques to the commercial market. However, the underlying mechanisms are often not well understood, and applying these techniques commercially can actually have detrimental effects on cognition (e.g. working memory, Chapter 3). It is therefore important that the scientific community becomes more active in warning consumers for the possible dangers of using such techniques, and evaluating the far-reaching claims made by the brain training industry. Another very important issue that warrants further research is the question whether, and under what circumstances, brain stimulation techniques like tDCS are actually able to modulate cognitive function in the first place. With regard to tDCS specifically, there is ongoing discussion about whether tDCS can target specific brain areas and affect behavior associated to that area (such as the dorsolateral prefrontal cortex and working memory, Horvath, Forte, & Carter, 2015a, 2015b, but see Antal et al., 2015). That is, it is well known that there are various factors affecting the effectivity of tDCS. To mention a few examples, it has been shown that electrode montage and drift (e.g. Woods, Bryant, Sacchetti, Gervits, & Hamilton, 2015), genetic differences (Nieratschker, Kiefer, Giel, Krüger, & Plewnia, 2015), anatomical differences (e.g. Datta, Truong, Minhos, Parra, & Bikson, 2012) , and even hair thickness (Horvath, Forte, & Carter, 2015b) can affect tDCS effectivity, which in itself may also interfere with a wide range of cognitive functions (for extensive reviews see Tremblay et al., 2014, Horvath, Forte, & Carter, 2015a, 2015b, Antal et al., 2015; Sellaro, Nitsche, & Colzato, in press). For now, given the wide range of variability in experimental protocols (e.g. with regard to stimulation parameters, the implemented task, individual differences, etc.), it is difficult to converge on the idea that tDCS unequivocally modulates cognitive performance.

Besides applying actual devices or techniques, certain 'lifestyles' that in itself train certain cognitive processes can enhance cognitive performance as well. For example, first person shooter video game playing is associated with enhanced performance with regard to the prioritizing and cascading of actions (Chapter 4). Playing these games most likely allows for cognitive-control improvements as these games are not just about pressing a button at the right moment, but require the players to develop different action control strategies to rapidly react to fast moving visual and auditory stimuli, and to flexibly adapt their behavior to an ever-changing context. Interestingly, this resembles complex daily life situations, such as multitasking conditions, in which we are required to inhibit a planned, ongoing response and to rapidly adapt our behavior (e.g., to execute a different response). Even though this has promising potential, further studies are needed to investigate how much experience with such videogames is needed to obtain enhancing effects, and to investigate for how long these effects last. More acute beneficial effects on cognitive performance and social behavior in healthy humans are observed after the intake of food supplements such as GABA, tyrosine and tryptophan. GABA enhances action cascading performance both when an action has to be stopped and changed towards an alternative one simultaneously, and when one is given more time to stop the first action (Chapter 5). This is especially important with regard to our ever-changing and demanding environment, in which we have to efficiently cascade and prioritize actions. In addition, tyrosine improves cognitive flexibility in terms of pro-active task switching (Chapter 6), again very important with regard to our everyday lives. Looking at social cognition, tryptophan supplementation was found to stimulate charitable donating (Chapter 7). A review of the available studies on tryptophan supplementation (Chapter 8) suggests that tryptophan re-biases attention away from negative stimuli and towards more positive ones. These studies support the idea that the food we eat modulates the synthesis of certain neurotransmitters, which affects the way we perceive and act upon the world. This idea is further supported by the existence of the "gut-brain axis", where communication involves interactions with the intestinal microbiota, which for example release immune activating molecules. Supporting the intestinal microbiota by taking probiotic food

supplements may be used to modify the stress response and consequent symptoms of anxiety and depression, which can reduce cognitive reactivity to sad mood and make people less vulnerable to develop depression (Chapter 9).

In sum, the present dissertation provides further evidence for the idea that brain stimulation, video gaming, and food supplements provide promising tools in enhancing cognitive performance and social behavior. Moreover, this dissertation attempted to gain further insight into the underlying mechanisms that can explain the observed effects. These findings have important societal and economic implications and go hand-in-hand with the ideological individualistic trend in society. More research is needed in order to gain better insights into the underlying mechanisms and the role of individual differences (in for example genetic predispositions, gender, age, etc.) in modulating the observed effects. But the discussed techniques do have promising potential not only in possibly delaying cognitive decline in elderly, but also enhancing social functioning and mental well-being in healthy humans. Similarly, the risk of behavioral problems and pathology in children might be reduced by training (i.e., enhancing) them – which likewise implies considerable savings for our welfare systems.

On a final note, the studies discussed in this thesis do not only have important implications for society in terms of the aging population and costs of welfare, but also at a more personal level. For example, students nowadays tend to apply sometimes dangerous methods to work more efficiently and to be better able to focus and study (e.g. by taking drugs such as methylphenidate or stimulating their brains using commercially available devices). Although these methods have beneficial effects for some individuals, they can be detrimental for and do serious harm to others. What is concerning about this is that people, sometimes even scientists, apply these methods without having any knowledge about the underlying mechanisms. In this thesis, based on the mechanisms involved in these cognitive processes, more healthy and safe ways to enhance cognitive performance are provided. Again, although future studies are needed to gain more insight into the underlying mechanisms, (some of) the methods discussed in this dissertation may eventually be applied to the general

public. Nevertheless, keeping in mind the competitive nature of today's society and combining this with the natural human tendency to always grow, develop, and learn more, and always demand more from ourselves, we should exert caution not to be overenthusiastic, and ask ourselves where to draw the line. In the end, the use of cognitive enhancers could increase the pressure of always being the best and in control, to work harder, longer, and more intensively and so it could, in fact, end up actually worsening the very problem it was intended to solve.

Samenvatting

‘Cognitive enhancement’ is het gebruik van elke methode of techniek met als doel het verbeteren van cognitieve prestaties. Dit onderzoeksveld heeft de laatste jaren een snelle groei doorgemaakt, wat wellicht te verklaren is door de economische problemen die het huidige welvaartssysteem met zich meebrengt. Deze problemen hebben gezorgd voor een sterke toename van de interesse in activiteiten en methoden die het welvaartssysteem betaalbaarder maken. Met name vanwege de toenemend ouder wordende bevolking is er de behoefte om vitaliteit en gezond ouder worden te stimuleren, en zo langer zelfstandig te kunnen blijven. Daarnaast lijkt het zo dat de samenleving steeds individualistischer wordt, wat het idee dat een individu zijn eigen leven dirigeert benadrukt. Als gevolg hiervan is er een groeiende interesse in procedures en activiteiten die helpen individuele behoeften uit te drukken, zwakheden te minimaliseren en sterke eigenschappen te benadrukken. Dit proefschrift is erop gericht niet alleen te demonstreren welke methoden veelbelovend zijn in het verbeteren van de cognitie, maar ook op het beter begrijpen van de onderliggende mechanismen van hoe bepaalde methoden (zoals het elektrisch stimuleren van het brein, het spelen van videospellen of het innemen van voedingssupplementen) cognitie en gedrag van gezonde mensen kunnen verbeteren. Duidelijke ideeën en theorieën met betrekking tot de onderliggende mechanismen is namelijk nodig om de methoden die mogelijk leiden tot interessante verbeteringen toe te kunnen passen in andere disciplines. Het verbeteren van cognitieve prestaties is over het algemeen gericht op het verbeteren van executieve functies zoals inhibitiecontrole, het werkgeheugen, het reguleren van de aandacht, en cognitieve flexibiliteit, maar kan ook gericht zijn op het verbeteren van de sociale cognitie. Immers, sociale cognitie en sociaal gedag worden aangestuurd door een groot aantal cognitieve processen (zoals bijvoorbeeld aandacht), en vallen daarom ook onder ‘cognitive enhancement’. In dit proefschrift worden daarom positieve effecten op zowel cognitief als sociaal functioneren, en de onderliggende mechanismen, bediscussieerd.

Hersenstimulatietechnieken zorgen ervoor dat onderzoekers

causale relaties tussen het gestimuleerde neurotransmittersysteem of hersengebied en de gedragsuitkomst kunnen onderzoeken. Een voorbeeld hiervan is 'transcutane vagus nerve stimulatie' (tVNS), waarbij, met behulp van een kleine electrode in het oor, de GABA en noradrenerge systemen, alsmede de thalamus, prefrontale cortex, en insula, kunnen worden gestimuleerd. tVNS is een relatief gezonde, veilige en makkelijke techniek om deze systemen, waarvan wordt gedacht dat ze een cruciale rol spelen in het prioriteren van acties en het beleven van plaatsvervangende sociale uitsluiting, te onderzoeken en mogelijk te verbeteren. Hoewel tVNS leidde tot een verbeterd vermogen om acties te prioriteren (Hoofdstuk 1), wat suggereert dat GABA en noradrenaline mogelijk een causale rol spelen in multitasking, had tVNS geen effect op helpend gedrag bij sociale uitsluiting in de Cyberball game (Hoofdstuk 2). Eén van de grootste problemen met hersenstimulatietechnieken is dat, mede door de veelbelovende bevindingen, de 'brain-training' industrie deze technieken naar de commerciële markt brengt. De onderliggende mechanismen van de geobserveerde effecten worden vaak nog niet goed begrepen, en het commercieel toepassen van deze technieken kan zelfs negatieve effecten hebben op cognitie (bijv. het werkgeheugen, Hoofdstuk 3). Het is daarom van groot belang dat de wetenschappelijke gemeenschap actiever wordt in het waarschuwen van consumenten voor de mogelijke gevaren die het gebruik van deze technieken met zich meebrengt. Daarnaast is het belangrijk dat de verstrekkende claims die de 'brain-training' industrie soms maakt worden geëvalueerd, hoewel dit niet ten koste moet gaan van het onderzoeken en verklaren van de onderliggende mechanismen die tot verbeterende effecten leiden.

Naast het toepassen van bepaalde apparaten of technieken, kunnen bepaalde leefstijlen ook zorgen voor een verbetering van bepaalde cognitieve processen. Het spelen van 'first person shooter' (FPS) videospellen bijvoorbeeld, is geassocieerd met betere prestaties als het gaat om het prioriteren van acties (Hoofdstuk 4). Het spelen van deze spellen zorgt waarschijnlijk voor verbeteringen in de cognitieve controle omdat deze spellen niet alleen gaan om het drukken van de juiste knop op het juiste moment, maar spelers ook verschillende controlestrategieën moeten ontwikkelen en toepassen om zo snel te kunnen reageren op snel

bewegende visuele en auditieve stimuli. Naast dit alles moeten spelers zich ook flexibel te kunnen aanpassen aan de steeds veranderende context van het spel. Interessant genoeg weerspiegelt dit dagelijkse situaties, waarin we moeten multi-tasken, acties moeten inhiberen en steeds opnieuw snel ons gedrag moeten aanpassen aan de omgeving. Hoewel deze bevindingen veelbelovend zijn is verder onderzoek nodig om te onderzoeken hoeveel ervaring met deze videospellen precies nodig is, en hoe lang de positieve effecten ervan duren.

Meer acute positieve effecten op cognitieve prestaties en sociaal gedrag in gezonde mensen worden geobserveerd na de inname van voedingssupplementen zoals GABA, tyrosine en tryptofaan. GABA bevordert het prioriteren van acties wanneer een eerste actie gestopt moet worden en tegelijkertijd moet worden vervangen door een tweede, maar ook wanneer de eerste actie al (bijna) volledig geïnhibeerd is en dan moet worden vervangen door een tweede (Hoofdstuk 5). Dit is met name belangrijk met betrekking tot de altijd veranderende en veeleisende omgeving waarin we leven, waarin we steeds efficiënt bepaalde acties prioriteit moeten geven. In toevoeging hierop verbetert tyrosine de cognitieve flexibiliteit wanneer het gaat om proactief wisselen tussen twee taken (Hoofdstuk 6), wat opnieuw heel belangrijk is met betrekking tot ons dagelijks leven. Wanneer we kijken naar sociale cognitie, wordt bijvoorbeeld gevonden dat tryptofaan ervoor zorgt dat mensen meer geld geven aan een goed doel, wat als een vorm van prosociaal gedrag wordt gezien (Hoofdstuk 7). Een review over de beschikbare studies met betrekking tot het innemen van het voedingssupplement tryptofaan en het effect daarvan op sociale cognitie en consequent gedrag (Hoofdstuk 8), suggereert dat tryptofaan ervoor zorgt dat de aandacht weggetrokken wordt van negatieve stimuli en juist gestuurd wordt naar meer positieve stimuli. Deze studies onderbouwen het idee dat het voedsel dat we eten de aanmaak van bepaalde neurotransmitters kan beïnvloeden, wat weer effecten heeft op de manier waarop we de wereld waarnemen en hoe we daarop reageren. Dit idee wordt verder ondersteund door het bestaan van de “brein-darm as”, waarbinnen communicatie grotendeels bestaat uit interacties van de microbiota in de ingewanden, die bijvoorbeeld immuun activerende moleculen loslaten. Het ondersteunen van de microbiotische

samenstelling door probiotische voedingssupplementen te nemen zou kunnen worden gebruikt om de stressreactie in het lichaam, en daaropvolgende symptomen van angst en depressie, te verlagen. Dit zorgt vervolgens voor een verlaging van de cognitieve reactiviteit (de mate waarin men dysfunctionele gedachtepatronen activeert ten gevolge van een slechte stemming; Hoofdstuk 9).

Samenvattend levert dit proefschrift meer bewijs voor het idee dat hersenstimulatie, het spelen van videospellen en het nemen van voedingssupplementen veelbelovende methoden zijn voor het verbeteren van cognitieve prestaties en sociaal gedrag. Verder geeft dit proefschrift inzicht in de onderliggende mechanismen die de geobserveerde effecten zouden kunnen verklaren. Hoewel meer onderzoek nodig is om meer inzicht te verkrijgen in de onderliggende mechanismen en de rol van individuele verschillen (in bijvoorbeeld genetische aanleg, sekse, leeftijd, etc.) in het beïnvloeden van de geobserveerde effecten, hebben de bevindingen belangrijke maatschappelijke en economische implicaties. Niet alleen kunnen de technieken mogelijk de cognitieve achteruitgang in ouderen vertragen, maar ook kunnen ze het sociaal functioneren en het mentale welzijn van gezonde mensen verbeteren. Op een gelijke manier kan het risico op gedragsproblemen en pathologie bij kinderen wellicht worden verlaagd, wat eveneens besparingen op het welvaartssysteem impliceert.

Tot slot hebben de besproken studies in dit proefschrift niet alleen belangrijke implicaties voor de samenleving in termen van de vergrijzende populatie en geassocieerde zorgkosten, maar ook op een meer persoonlijk niveau. Studenten passen tegenwoordig bijvoorbeeld soms gevaarlijke methoden toe om zo meer efficiënt te kunnen werken en zich beter te kunnen focussen op hun studie (bijvoorbeeld door het nemen van drugs zoals methylfenidaat of door hun hersenen te stimuleren met commercieel beschikbare apparaten). Hoewel deze methoden positieve effecten hebben voor sommige individuen, kunnen ze serieus nadelige effecten hebben voor anderen. Wat hier het meest zorgwekkend aan is, is dat mensen, soms zelfs wetenschappers, deze methoden toepassen zonder ook maar enige kennis te hebben over de onderliggende mechanismen. In dit proefschrift, gebaseerd op de onderliggende mechanismen betrokken bij de cognitieve

processen, zijn meer gezonde en veilige methoden om cognitieve prestaties te verbeteren besproken. Hoewel, opnieuw, toekomstige studies nodig zijn om meer inzicht te krijgen in de onderliggende mechanismen, kunnen (sommige van) de methoden besproken in dit proefschrift in de toekomst wellicht worden toegepast buiten de wetenschap. Hoe dan ook moeten we niet vergeten dat het competitieve karakter van de huidige samenleving, gecombineerd met de natuurlijke neiging om altijd te willen groeien en ontwikkelen, meer te willen leren, en altijd meer van onszelf te eisen, ons ook overenthousiast kan maken voor deze technieken. We moeten onszelf afvragen waar we de grens willen trekken. Uiteindelijk kan het gebruik van methoden om cognitie te verbeteren er namelijk voor zorgen dat de druk om de beste te zijn, om altijd controle te hebben, om harder, langer en intensiever te werken, toeneemt. En hierdoor maakt het het probleem dat het zou moeten oplossen uiteindelijk wellicht alleen maar erger.

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Curriculum Vitae

Laura Steenbergen was born on August 4, 1991 in Gouda, the Netherlands. In 2009 she obtained her pre-university level high school diploma from the St.-Antoniuscollege in Gouda, after which she studied Psychology at Leiden University. During her bachelor, Laura worked as a voluntary research assistant for several projects, including research on the effect of tryptophan on interpersonal trust and the imaging of dopaminergic nuclei. She received her bachelor's degree in 2012. During her master's Laura worked as a research assistant investigating the effect of bilingual education on cognitive flexibility. After a six-month research internship on the effect of cocaine on creativity and emotional processing at Maastricht University, she received her master of science in Psychology (research) in June 2014 (cum laude/with honors). Since July 2014, Laura has worked as a PhD student at Leiden University under the supervision of Dr. Lorenza Colzato and Dr. Roberta Sellaro. As part of her doctoral research, Laura has spent one month in the Actionlab of Prof. dr. Christian Beste at the Technical University of Dresden. The results of her doctoral work are described in this dissertation.

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