Investigating the human locus coeruleus-norepinephrine system in vivo : discussions on the anatomy, involvement in cognition and clinical applications

Tona, K.

Cover Page

Universiteit Leiden

Author: Tona, K.
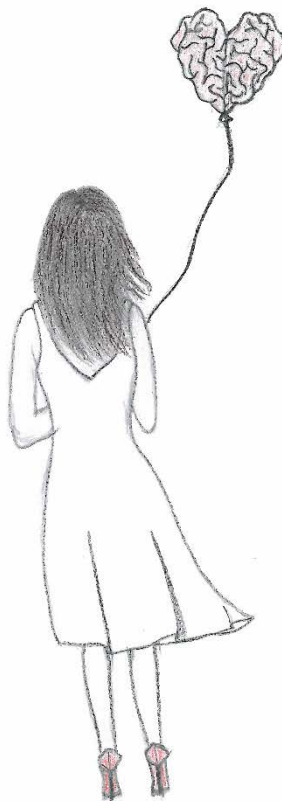**Title**: Investigating the human locus coeruleus-norepinephrine system in vivo : discussions on the anatomy, involvement in cognition and clinical applications
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Chapter 6

Noradrenergic regulation of cognitive flexibility: No effects of stress, transcutaneous vagus nerve stimulation and atomoxetine on task-switching in humans

Abstract

Cognitive flexibility allows us to adaptively switch between different responsibilities in important domains of our daily life. Previous work has elucidated the neurochemical basis underlying the ability to switch responses to a previously non-reinforced exemplar, and to switch between attentional sets. However, the role of neuromodulators in task switching, the ability to rapidly switch between two or more cognitive tasks afforded by the same stimuli, is still poorly understood. We attempted to fill this gap by manipulating norepinephrine (NE) levels using a stress manipulation (Study 1a, N=48; between-group design), transcutaneous vagus nerve stimulation (tVNS) at two different intensities (Study 1b, N=48; sham-controlled between-group design), and a pharmacological manipulation (Study 2, N=24, double-blind crossover design), all of which increased salivary cortisol measures. Participants repeatedly switched between two cognitive tasks (classifying a digit as high/low (task 1) or as odd/even (task 2)), depending on the preceding cue. On each trial, a cue indicated the task to be performed. The cue-stimulus interval (CSI) was varied to manipulate the time to prepare for the switch. Participants showed typical switch costs, which decreased with the time available for preparation. None of the manipulations modulated the size of the switch costs or the preparation effect. Task-switching performance reflects a complex mix of cognitive control and bottom-up dynamics of task-set representations. Our findings suggest that NE does not affect either of these aspects of cognitive flexibility.

General Introduction

Cognitive flexibility, the ability to learn associations between stimuli, actions and outcomes and to quickly adapt ongoing behavior to salient changes in the environment, is very important for human survival (Kehagia et al., 2010). It allows us to "juggle" between different responsibilities in important domains of our daily life, and allows species to face new and unexpected conditions in the environment, including threatening conditions (Canas, Quesada, Antoli, & Fajardo, 2003). Cognitive flexibility is a multifaceted construct. Two examples of lower-order cognitive flexibility are basic reinforcement learning and reversal learning—responding to a previously non-reinforced exemplar within the same dimension. These cognitive functions are critically dependent on environmental signals or feedback, allowing us to flexibly learn and unlearn goal-directed behaviors (Kehagia et al., 2010). Two examples of higher-order cognitive flexibility are extra-dimensional attentional set shifting and task switching. Extra-dimensional set-shifting concerns the ability to adapt behavior flexibly following feedback, but pertains to broader stimulus dimensions rather than a specific exemplar. Task switching is a purer form of cognitive flexibility because it is uncontaminated by learning and feedback processing (Kehagia et al., 2010). Instead, people rely on implicit or explicit cues that indicate frequent shifts between two or more tasks afforded by the same stimuli; for example to classify a digit as odd–even or as high–low with a left or right button press (Fischer, Plessow, & Kiesel, 2010; Monsell & Mizon, 2006; Monsell, Sumner, & Waters, 2003).

Previous literature shows that neuromodulators such as dopamine, serotonin and NE modulate several forms of cognitive flexibility. Dopamine modulation in the striatum and prefrontal cortex is critical for basic reinforcement learning and the integration of negative feedback during reversal learning, while serotonin appears to play an important role in inhibiting perseverative responding after reversal of cue–outcome contingencies (Kehagia et al., 2010; Matias, Lottem, Dugué, & Mainen, 2017; Walker, Robbins, & Roberts, 2009). In contrast, NE plays a crucial role in extra-dimensional set shifting and adaptive updating of beliefs about the environment (Janitzky et al., 2015; Jepma et al., 2018; Lapiz & Morilak, 2006; Pajkossy, Szollosi, Demeter, & Racsmany, 2018; Sales, Friston, Jones, Pickering, & Moran, 2019; Tait et al., 2007). Building on such findings, Kehagia and colleagues (2009, 2010) proposed that NE may also be critical for task switching: flexibly shifting between task sets on the basis of trial-to-trial instruction cues. However, surprisingly little research has investigated the role of NE in task switching (Steenbergen, Sellaro, de Rover, Hommel, & Colzato, 2015; Wolff et al., 2018). Here we addressed this question by examining task-switching performance after manipulating activity of the locus coeruleus-norepinephrine (LC-NE) system using stress induction, transcutaneous auricular vagus nerve stimulation (tVNS), and administration of the drug atomoxetine.

The relationship between stress and LC activation is well-documented, and the LC is an important component of the central stress circuitry (for reviews see Aston-Jones, Rajkowski, Kubiak, & Alexinsky, 1994; Itoi & Sugimoto, 2010; Sara & Bouret, 2012). Most environmental stressors increase the spontaneous discharge rate of the LC. Several brain areas involved in the typical stress response, including the central nucleus of the amygdala and paraventricular nucleus of the hypothalamus, provide inputs to the LC. Corticotropin-releasing hormone is an important mediator of this stress-induced LC activation and ensuing effects throughout the brain, which prepare the organism for a rapid and appropriate behavioral response to the stressor. Indeed, blockade of noradrenergic beta receptors diminishes the effect of stress on emotional memory (Cahill & McGaugh, 1998; Kroes et al., 2016) and global brain state (Hermans et al., 2011). In the present research, we used an effective, standardized protocol for experimental stress induction in humans (Schwabe & Schachinger, 2018).

The vagus nerve is the longest nerve in our body and communicates the state of the viscera to the brain and vice versa. Importantly, the vagus nerve projects to the nucleus tractus solitarius which in turn projects directly and indirectly to the LC, the main source of NE in the brain (Berridge & Waterhouse, 2003). External stimulation of the vagus nerve (VNS, used usually to suppress epileptic seizures) can be achieved either invasively, with a surgical procedure involving vagus nerve stimulator implantation within the chest cavity; or transcutaneously, with an iPod-like device delivering electrical impulses to the auricular branch of the vagus nerve, which is situated close to the surface of the skin of the outer ear. Animal studies have found that VNS increased the firing rate of NE neurons in the LC (Dorr & Debonnel, 2006; Raedt et al., 2011; Roosevelt et al., 2006), and increased extracellular NE levels in the prefrontal cortex (Follesa et al., 2007), basolateral amygdala (Hassert et al., 2004), and cerebrospinal fluid (Martlé et al., 2015). Importantly, this increase in NE levels occurred in a dose-dependent manner, and returned to baseline after termination of VNS (Raedt et al., 2011; Roosevelt et al., 2006). Although there is no direct evidence that tVNS has similar effects on the LC-NE system, functional magnetic resonance imaging studies in healthy humans have demonstrated that tVNS elicits widespread changes in cortical and brainstem activity (Frangos et al., 2014; Kraus et al., 2007). Other recent work has shown that tVNS modulates hormonal (Fischer et al., 2018; Warren et al., 2019) and psychophysiological (Fischer et al., 2018; but see Warren et al., 2019) indices of noradrenergic function in human subjects. In the current research, we examined the effects on cognitive flexibility of tVNS at two levels of intensity.

Although the above-mentioned methods of tVNS and stress induction allow examination of NE effects on cognition, psychopharmacological manipulation provides a more robust method for directly manipulating NE levels and for establishing a causal role for NE. To this end, we also manipulated brain-wide NE levels via administration of the NE transporter blocker atomoxetine, a compound usually prescribed to treat attention-deficit hyperactivity disorder (Pringsheim, Hirsch, Gardner, & Gorman, 2015; Sharma & Couture, 2014). In cortical areas, the NE transporter is responsible for the reuptake of not

only NE but also dopamine from the synaptic cleft (Devoto & Flore, 2006). Thus, atomoxetine increases both central NE and cortical dopamine levels (Bymaster et al., 2002; Koda et al., 2010). Finally, in human subjects atomoxetine administration has been shown to affect NE biomarkers such as alpha-amylase (Chamberlain et al., 2007; Warren, van den Brink, et al., 2017).

We studied higher-order cognitive flexibility using a task switching paradigm (Monsell et al., 2003) in which the task to be performed on each trial was indicated by a cue presented at the start of the trial. This paradigm distinguishes between trials on which the task changes (" switch trials") and trials on which the task stays the same ("repeat trials"). The finding of interest is that reaction time is longer, and error rate greater, on switch trials (the "switch cost"); and as the cue–stimulus interval is prolonged—allowing more opportunity for advance preparation—the switch cost is reduced (the "preparation effect"). Switch costs are attributable to a combination of the time required for resolving interference from residual activation of the previous, no-longer relevant task set ("task-set inertia") and of the time required for retrieving the newly cued task set ("task-set reconfiguration"; "task-set reconfiguration"; "task-set reconfiguration"; "task-set reconfiguration"; Monsell et al., 2003). The cognitive flexibility required to switch between tasks depends on the dynamic transformation of neural task-set representations from trial to trial (Qiao, Zhang, Chen, & Egner, 2017; Yeung, Nystrom, Aronson, & Cohen, 2006). In order to ensure that the observed switch costs and preparation effect would accurately reflect this type of cognitive flexibility instead of a cue-repetition effect, we used two cues per task, which allowed us to avoid direct cue repetitions between trials (Logan & Bundesen, 2003; Monsell & Mizon, 2006).

In Study 1 we tested three groups of participants. All participants performed the task on two separate occasions in which either tVNS or sham stimulation was applied according to a single-blind counterbalanced design. Two of the groups received common, medium-intensity (0.5 mA) tVNS. One of those groups underwent also a stress induction procedure, the other group underwent a control procedure. Comparison of those two groups allowed us to examine the effect of stress (in the context of tVNS) on task switching, as reported under 'Results Study 1a: Effects of tVNS and stress'. The third group received tVNS (versus sham) at a higher intensity (1.0 mA). Comparison of this group with the medium-intensity/no-stress group allowed us to examine the relatively unknown dose-dependent effects of tVNS. This can be seen as an initial step toward establishing a linear or curvilinear relationship between tVNS intensity and cognitive flexibility, or other aspects of cognitive task performance (Dietrich et al., 2008; Frangos et al., 2014; Ghacibeh, Shenker, Shenal, Uthman, & Heilman, 2006; Hulsey et al., 2017). These results are reported under 'Results Study 1b: effects of tVNS intensity'. In Study 2 we examined the effect of our psychopharmacological manipulation on task switching, using a double-blind placebo-controlled cross-over design.

Note that we do not claim that stress induction, tVNS and atomoxetine selectively affect NE levels. Stress causes a myriad of adaptive neurochemical changes. VNS can affect levels of dopamine and serotonin, although these effects may require chronic stimulation

or reflect indirect effects of the change in NE levels (Manta, El Mansari, Debonnel, & Blier, 2013; Martlé et al., 2015). And as mentioned above, atomoxetine also increases cortical dopamine levels. We also do not intend to suggest that the three manipulations affect the LC-NE system in a similar way. However, if task switching is crucially dependent on activity of the LC-NE system, one would expect effects of (some of) these manipulations on task switching performance. To foreshadow the results, we did not find such effects.

STUDY 1

Methods

Participants
Seventy-two Dutch native-speaking volunteers (18-29 years old; mean age: 21.4) participated in this study. All had normal or corrected-to-normal vision. To avoid menstrual cycle effects on cortisol responses (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999), only male participants were included. Exclusion criteria were: neurological or psychiatric disorder, bradycardia, cardiac arrhythmia, cardiovascular disease, psychoactive medication or drug use, active implants (e.g., cochlear implant), and skin disorder such as eczema. Participants were asked to refrain from alcohol intake within 24 hours prior to the study, and to refrain from excessive exercise, caffeine and heavy meals within 3 hours prior to the study. To ensure that the participants were blind to the active stimulation/sham condition, prior participation in other tVNS studies was an additional exclusion criterion. All participants gave written informed consent prior to their participation, and, based on their preference, were compensated with 24 euros or course credits. The study was approved by the ethics committee of the Institute of Psychology at Leiden University.

Design
tVNS was applied according to a single-blind, sham-controlled, cross-over design. The study consisted of two sessions, scheduled one week apart at the same time of the day. To control for circadian fluctuations in cortisol levels (Lupien, Maheu, Tu, Fiocco, & Schramek, 2007), all sessions were conducted between 12.00 and 6.00 pm. Participants received either tVNS or sham stimulation, in a counterbalanced order. Participants were randomly assigned to one of three groups (each with N = 24):

- medium-intensity tVNS (0.5 mA)
- medium-intensity tVNS (0.5 mA) and stress induction
- high-intensity tVNS (1.0 mA)

The study design and procedure were exactly the same for all groups, with the following exceptions. The intensity of tVNS differed between the medium- and high-intensity groups. Additionally, in the stress group, stress was induced using a socially evaluated cold pressor test (SECPT), while the other two groups received a control treatment that involved a similar procedure but without social, psychological and physical stress induction (see below).

Task

Experimental phase

To assess cognitive flexibility, we used an unpredictable cued task-switching paradigm (Monsell et al., 2003), implemented in E-Prime (Psychology Software tools, Pittsburgh, PA). Participants were asked to classify a digit (1-4, 6-9) as high/low (task 1) or as odd/even (task 2), depending on the preceding cue. The cue was a colored circle (pink or yellow) or an outline shape (diamond or square, see Figure 1), displayed on a grey background. The diamond-shaped and pink-colored cues indicated that task 1 should be performed, while the square-shaped and yellow-colored cues indicated that task 2 should be performed. The probability of a task switch was 25%.

Digits were displayed in a black Courier New 24-pt font with a height of 1 cm centered on the cue of side 3 cm, which was displayed in the center of the screen (Figure 1). Each trial started with a blank screen for 750 ms, followed by a fixation cross of 500 ms. After a preparation interval of 150 ms or 800 ms, the digit appeared in the center of the cue until the participant's response. A response triggered the immediate onset of the next trial, unless a wrong key was pressed, in which case an error message appeared for 2 sec to allow the participant to recover before onset of the next trial.

To unconfound the effects of task switching and cue change (Monsell & Mizon, 2006), the cue changed on every trial, alternating between shape and color. The cue-stimulus interval (CSI) was manipulated in such a way that the participants had little time for advance preparation (CSI=150 ms) or more time (CSI=800 ms). Participants performed the tasks by pressing the letters "Q" (odd, high) or "P" (even, low) on a keyboard with their left and right index fingers. They were instructed to respond as quickly as possible while minimizing the number of errors and to use the available preparation time effectively.

The experiment consisted of 768 trials that were presented in six blocks of 128 trials. Each block started with four warm-up trials and consisted of trials with only a long or a short CSI. Blocks with long and short CSI alternated in an ABABAB order for half of the participants and a BABABA order for the other half of the participants. This factor was stratified with treatment order to ensure that the order of the CSIs across blocks was orthogonal to the order of treatments (tVNS/sham in first session for Study 1 and placebo/atomoxetine in first session for Study 2). In between the blocks, there were subject-paced breaks with a maximum duration of 15 sec, and at the end of each block participants received written feedback about their mean RT and error rate for that block. Participants were challenged to beat this performance in the remaining blocks. The task-switching experiment lasted approximately 30 minutes.

Practice phase

Prior to the actual experiment, participants received extensive practice to ensure that they learnt the cue-stimulus mapping well. The practice task consisted of four blocks and lasted approximately 20 min. During the first block, participants learnt the first task by

practicing 32 trials of the odd-even task with the digit displayed inside the appropriate cue. During the second block, participants practiced 32 trials of the high-low task, using the same procedure. In the third block, participants performed 64 trials with a long CSI and random switches between task 1 and task 2. The last block consisted of 64 trials with a short RSI and random switches between task 1 and task 2.

To ensure proper learning of the cue-task mapping, the cue-task mapping was displayed in the top-right corner of the screen throughout the practice phase. Additionally, before the initiation of the third practice block, the experimenter ran through the learned tasks together with the participant, to make sure the cue-task mappings were properly represented. To mitigate learning effects, the practice phase of session 2 was half as long as the practice phase of session 1.

Data analysis

We performed repeated-measures ANOVAs with mean correct RT and accuracy (% errors) as dependent variables, treatment (tVNS/sham), CSI (150 ms/800 ms), trial type (switch/repeat) and task (odd/even or high/low) as within-subject independent variables, and group (stress vs no-stress) and treatment order as between-subject variables (Study 1a), or tVNS intensity (0.5/1.0 mA) and treatment order as between-subject variables (Study 1b). Treatment order and task were factors of no interest, so we do not report any statistical terms involving these factors. The following trials were excluded from the analyses: all practice trials, warm-up trials, trials following errors, incorrect trials and trials with RTs longer than 2 sec.

Data was analyzed with IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, N.Y., USA). A significance level of $p<.05$ was adopted for all statistical tests. Significant results were followed by t-tests to clarify the direction of the effect. Greenhouse-Geisser correction was used whenever the assumption of sphericity was violated.
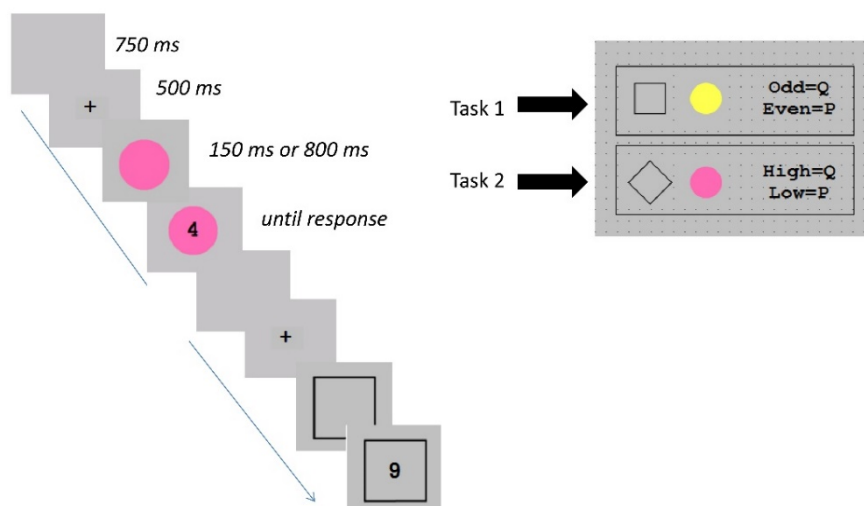

Figure 1: Illustration of the task switching paradigm. The same paradigm was used in all the studies reported in this manuscript.

Procedure

The timeline of each experimental session is illustrated in Figure 2. The tVNS or sham stimulation started before the practice phase of the task switching experiment. After the practice, which took about twenty minutes, the participant underwent an assessment of their mood. Then the stress induction (or control procedure) took place, followed by the actual task switching paradigm.
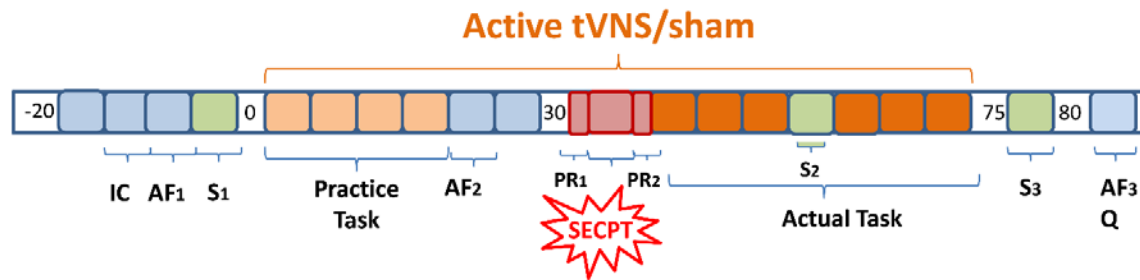

Figure 2. Timeline of procedures in Study 1. IC= informed consent, $S_{(1,2,3)}$ = saliva measurement, AF $_{(1,2,3,)}$ = Affect Grid, PR $_{(1,2)}$ = pain rating, Q= after-effect questionnaire

tVNS

We used a tVNS device to stimulate the vagus nerve (NEMOS®, Cerbomed, Erlangen, Germany). The device was switched on for a period of 75 minutes, with a frequency of 25 Hz, and stimulation intensity set at 0.5 mA for the medium-intensity groups or 1.0 mA for the high-intensity group. The stimulation followed a pattern set by the manufacturer, alternating between 30 seconds on and 30 seconds off. In one of the sessions the participant received tVNS on the left concha, at the inner side of the ear where the afferent auricular branch of the vagus nerve can be stimulated. In the other session, participants received sham stimulation on the left ear lobe. Administering sham stimulation in this manner has proven to produce no significant activation in the brainstem and cortex (Kraus et al., 2013).

At the end of each session, an after-effect questionnaire was used to assess possible side effects of tVNS and how the participants experienced the stimulation (Sellaro et al., 2015). The participants were asked to indicate on a 5-point scale the extent to which specific possible tVNS side effects/experiences applied to them. The included complaints were: headache, neck pain, uncomfortable feeling, nausea, muscle constructions, tingling sensation, burning feeling under electrodes, and open question "other" where participants could mention complains missing from the list.

Mean ratings of uncomfortable feeling were 2.0 for the 0.5 mA (sham: 1.6) versus 2.1 for the 1.0 mA (sham: 1.7) group, and 1.9 (sham: 1.9) for the stress group respectively. Scores for tingling feeling were 2.7 for the 0.5 mA (sham: 2.4) versus 3.1 for the 1.0 mA (sham: 3.4) group, and 3.1 for the stress group (sham: 2.1). Finally, scores of feeling of

burning sensation were 2.3 for the 1.0 mA (sham: 1.7) group but below 2.0 for the other groups. Ratings for other scales were all below 2.0.

Stress Induction

To manipulate stress, we used the socially evaluated cold pressor test (SECPT), which combines induction of physiological (cold pressor) and psychological (negative social evaluation) stress. The SECPT has been shown to be a robust stressor, leading to increased levels of the stress hormone cortisol (Schwabe, Haddad, & Schachinger, 2008).

The participant was instructed to submerge his right hand until the wrist into a bucket of ice water (4°C), to not move this hand, and to keep it in the water until he could not bear it anymore. Although no time restriction was revealed to the participant, in line with ethical and safety restrictions, he was told by the experimenter to take his hand out of the water after three minutes. To ensure induction of psychological stress, instructions were given in a strict manner by an experimenter who was wearing a white coat, and the participant was evaluated by a committee of two members while having his hand in cold water. The evaluation included visual observation, mock notes and negative feedback by the committee members and simultaneous videotaping of the participant. The participant was told that the camera was recording his facial expressions, which would be evaluated at a later time point.

In the control condition, the same procedure was followed but the participant submerged his hand in room temperature water (22-25 °C) and the evaluators did not induce any psychological/social stress. To be consistent, the same instructions were given to the participant as during the stress manipulation but not in a strict manner. Although the participant was told to keep his hand in the water until he could not bear it anymore and no time restriction was revealed to him, the experimenter asked him to take his hand out of the water when the three minutes had passed. Throughout the control condition, the control social evaluators avoided looking at the participant, and acted in a neutral manner. Also, it was clear that the camera that was in the room was turned off and pointing towards the wall instead of the participant.

Validation of stress manipulation

To test the effectiveness of the stress manipulation, we assessed psychological and physiological distress by means of subjective pain ratings and salivary cortisol levels. Pain ratings were collected immediately before and after the stress induction. Participants indicated on a visual scale ranging from 0-100 how much pain they felt at the moment. Changes in pain perception were assessed by subtracting the values reported after the SECPT/control manipulation from those reported immediately before. The difference values for the stress and non-stress group were compared using independent *t*-tests.

To assess cortisol levels, saliva samples were collected at three time points (see Figure 2): at baseline, soon after the stress or control manipulation and after completion of the task. Samples were assayed for cortisol using a competitive enzyme-linked immunosorbent assay, according to the manufacturer's instructions (IBL, Hamburg, Germany). All

samples of a given participant were assayed simultaneously. For additional information about saliva sample collection and processing methods see (Warren et al., 2019; Warren, van den Brink, et al., 2017). Cortisol data were analyzed using a 3x2 mixed ANOVA with time point (first vs. second vs. third measurement) as a within-subjects factor and group (stress vs. no stress) as between-subjects factor.

Results Study 1a: Effects of tVNS and stress

Effects of stress on subjective pain and salivary cortisol
Stress led to increased pain perception and elevated salivary cortisol levels (cortisol levels are reported in μg/dl unit). Exposure to the SECPT increased mean subjective pain ratings (stress group: 29.65, no-stress group: 3.79, $t(32) = 7.94$, $p < 0.001$). The stress-induction procedure increased cortisol levels over time, as compared to the control procedure, yielding a significant main effect of stress ($F(1,42) = 4.45$, $p = .041$), and a time point x stress interaction, ($F(2,84) = 5.540$, $p = .017$ after Greenhouse-Geisser correction). While baseline cortisol levels did not differ between the groups (stress group mean: 0.39, no-stress group mean: 0.32; $t(45) = 0.82$, $p = .42$), cortisol levels were significantly higher in the stress group at 15 minutes and 70 minutes after the end of stress induction (stress group mean: 0.41, no-stress group mean: 0.22; $t(43) = 3.38$, $p = 0.002$; and stress group mean: 0.37, no-stress group mean: 0.18; $t(29) = 3.14$, $p = 0.004$, respectively).

Reaction times
Mean reaction times (RTs) are presented in Figure 3. Participants showed a typical switch cost of 60 ms ($F(1,44) = 39.8$, $p < .001$; switch 786 ms, repeat 726 ms), which decreased with the time available for preparation ($F(1,44) = 14.45$, $p < .001$). The switch cost was smaller in long CSI blocks (39 ms) than in short CSI blocks (79 ms). There was also a main effect of CSI ($F(1,44) = 160$, $p < .001$). These are well-established findings in task switching research.

Treatment ($F(1,44) < 1$, $p = .97$) and stress ($F(1,44) < 1$, $p = .73$) did not have a main effect on RT (stress 749 ms, no stress 763 ms), and there was no significant interaction between treatment and stress ($F(1,44) = .24$, $p = .62$). Importantly, the switch cost and the preparation effect were not modulated by tVNS ($F(1,44) = 1.55$, $p = .22$; $F(1,44) = 3.19$, $p = .08$) or stress ($F(1,44) = 2.39$, $p = .13$; $F(1,44) = 1.19$, $p = .28$) and there were no interactions between those factors and other factors of interest.

Accuracy
Percentages of errors are presented in Figure 3. Participants showed a typical switch cost of 4.2% ($F(1,44) = 67.23$, $p < .001$; switch: 91.2%, repeat: 95.4%), which decreased with the time available for preparation ($F(1,44) = 12.75$, $p = .001$). The switch cost was smaller in long CSI blocks (3.1%) than in short CSI blocks (5.1%). There was also a main effect of CSI ($F(1,44) = 17.25$, $p < .001$).

Treatment ($F(1,44) < 1$, $p = .97$) and stress ($F(1,44) = 0.48$, $p = .55$) did not have a main effect on accuracy. There was no significant interaction between treatment and stress ($F(1,44) < 1$, $p = .67$). The switch cost and the preparation effect were not modulated by

tVNS ($F(1,44) < 1$, $p = .58$; $F(1,44) < 1$, $p = .71$) or stress ($F(1,44) < 1$, $p = .82$; $F(1,44) < 1$, $p = .41$), and there were no interactions between these factors and other factors of interest.

Exploratory analysis: effect of stress in sham condition only

It is possible that stress did not affect task switching performance because it was manipulated in the context of tVNS—perhaps tVNS somehow suppressed the effect of stress (Lerman et al., 2019; Tobaldini et al., 2019). To examine this scenario, we carried out an ANOVA comparing the stress and non-stress groups (0.5 mV), including only data from the sham condition. With regard to RT, stress did not have a main effect ($F(1,44) < 1$, $p = .59$), and did not modulate the switch cost ($F(1,44) = 2.75$, $p = .10$) and the preparation effect ($F(1,44) < 1$, $p = .33$). Similarly, with regard to accuracy, stress did not have a main effect ($F(1,44) = 0.37$, $p = .55$), and did not modulate the switch cost ($F(1,44) < 1$, $p = .89$) or the preparation effect ($F(1,44) = 3,38$, $p = .07$). There was a significant stress x CSI interaction ($F(1,44) = 4.65$, $p = .037$), with the effect of CSI on overall accuracy (i.e., regardless of trial type) being smaller in the stress group (mean: 0.6%) than in the non-stress group (mean: 1.8%). This result was driven by a difference in accuracy at the long CSI. Interpretations of this finding must remain tentative, given the post hoc nature of this analysis, the large number of statistical tests performed in the present studies, and the fact that the RT data did not show a similar interaction.

Results study 1b: effects of tVNS intensity

Effects of tVNS on mood

Elsewhere we show that for a subset of the participants in this study, from whom we collected and analyzed saliva measurements tVNS treatment (0.5 mA) significantly increased salivary levels of cortisol and α-amylase compared to sham stimulation (Warren et al., 2018), thus confirming the effect of tVNS. Here we examine the effects of tVNS on mood. Mood was assessed at three time points (Figure 2) using the Affect Grid (Russell, Weiss, & Mendelsohn, 1989), a quick means of assessing affect along the dimensions of pleasure-displeasure and arousal-sleepiness. Pleasure and arousal scores were analyzed separately by means of repeated-measures ANOVAs with time point (first vs. second vs. third measurement) and treatment (tVNS vs. sham) as within-participants factors, and tVNS intensity (0.5/1.0 mA) and treatment order as between-subject factors. There was an effect of time point on pleasure ($F(2,88) = 7.77$, $p = 0.001$), with pleasure decreasing over time, but there was no effect of treatment or tVNS intensity on arousal ($F(1,44) = 1.78$, $p = 0.18$; $F(1,44) = 0.44$, $p = 0.51$) and pleasure ($F(1,44) = 0.46$, $p = 0.60$; $F(1,44) = 0.006$ $p = 0.94$).

Reaction times

Mean RTs are presented in Figure 3. Participants showed a typical switch cost of 66 ms ($F(1,44) = 49.5$, $p < .001$; switch 760 ms, repeat 694 ms), which decreased with the time available for preparation ($F(1,44) = 35.7$, $p < .001$). The switch cost was smaller in long CSI blocks (37 ms) than in short CSI blocks (96 ms). There was also a main effect of CSI ($F(1, 44) = 177.4$, $p < .001$).

Treatment ($F(1,44) = 2.8$, $p = .10$) and tVNS intensity ($F(1,44) = 3.2$, $p = .08$) did not have a main effect on RT, but the means indicated faster responses for the high-intensity group (0.5 mA: 763 ms, 1.0 mA: 690 ms). There was no significant interaction between treatment and tVNS intensity ($F(1,44) = 1.3$, $p = .25$). Importantly, the switch costs and the preparation effect were not modulated by treatment or tVNS intensity and there were no interactions between these factors and other factors of interest.
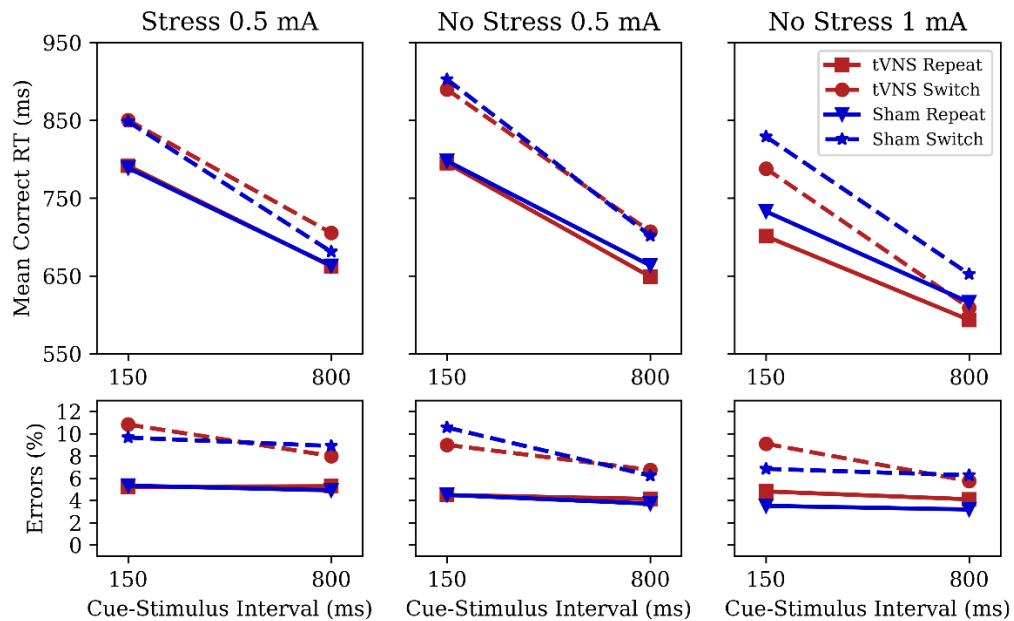

Figure 3: Effects of stress, tVNS (vs sham) and tVNS intensity on mean correct RT (top) and error rate (bottom).

Accuracy

Participants' accuracy scores showed a typical switch cost of 3.5% ($F(1,44) = 50.43$, $p < .001$; switch 92.5%, repeat 96.0%), which decreased with the time available for preparation ($F(1,44) = 15.12$, $p < .001$). The switch cost was smaller in long CSI blocks (2.5%) than in short CSI blocks (4.6%). There was also a main effect of CSI ($F(1,44) = 24.53$, $p < .001$). Treatment ($F(1,44) = 1.60$, $p = .21$) and tVNS intensity ($F(1,44) = .46$, $p = .50$) did not have a significant main effect on accuracy. Finally, there was no significant interaction between treatment and tVNS intensity ($F(1,44) < 2.87$, $p = .09$).

Exploratory analysis: tVNS at 1.0 mA intensity causes speed-accuracy tradeoff

Although the omnibus ANOVA did not yield significant main effects of treatment and tVNS intensity, the right panel of Figure 3 suggests that tVNS at 1.0 mA intensity decreased RTs at the expense of more errors. To examine the robustness of this finding, we carried out an ANOVA that included only the 1.0 mA group. While high-intensity tVNS did not affect the switch cost and the preparation effect ($F(1, 22) = 1.89$, $p = .18$; $F(1, 22) < 1$, $p = .55$, respectively), it had a main effect on RT ($F(1,22) = 5.30$, $p = .03$),

indicating that overall responses were faster in the tVNS condition (673 ms) than in the sham condition (708 ms).

Regarding accuracy, while high-intensity tVNS did not affect the switch cost ($F(1,22) < 1$, $p = .79$) and the preparation effect ($F(1,22) < 1$, $p = .35$), it had a main effect on error rate ($F(1,22) = 5.16$, $p = .03$), indicating an overall decrease in accuracy in the tVNS condition (94.1%) compared to the sham condition (95.1%). For completeness we report a significant two-way interaction between tVNS intensity and CSI, ($F(1,22) = 5.56$, $p = .03$), but that interaction was largely driven by a spurious difference in the effect of CSI between low and high sham stimulation intensity. Together, these findings suggest that tVNS at 1.0 mA caused a change in the participants' speed-accuracy tradeoff.

STUDY 2

Effects of atomoxetine

Methods

Participants
Twenty-four young volunteers (six male; 18-25 years old; mean age: 21.7) participated as part of a larger pharmacological neuroimaging study (van den Brink et al., 2016). Participants were screened by a physician for the following exclusion criteria: standard contraindications for MRI; current use of psychoactive or cardiovascular medication; a history of psychiatric illness or head trauma; cardiovascular disease; renal failure; hepatic insufficiency; glaucoma; hypertension; drug or alcohol abuse; learning disabilities; poor eyesight (myopia ≤ -6 diopters); smoking > 5 cigarettes a day; and pregnancy. All participants gave written informed consent prior to their participation, and were compensated with €135. The study was approved by the Leiden University medical ethics committee.

Design and Procedure
The task performed by participants was identical to that used in Study 1. As in Study 1, blocks with long and short CSI alternated in an ABABAB or BABABA fashion, and this between-subject factor was stratified with treatment order. The study was conducted according to a double-blind placebo-controlled cross-over design. In each of the two sessions, scheduled one week apart at the same time of the day, participants received either a single oral dose of atomoxetine (40 mg) or placebo (125 mg of lactose monohydrate with 1% magnesium stearate, visually identical to the drug), in counterbalanced order. Data reported elsewhere show that for these participants the atomoxetine treatment significantly increased salivary levels of cortisol and α-amylase, reliable markers of sympathetic nervous system and hypothalamus-pituitary-adrenal axis activation, respectively (Warren, van den Brink, Nieuwenhuis, & Bosch, 2017).

The procedure of Study 2 is illustrated in Figure 4. The practice of the task switching paradigm started immediately after the pill ingestion, when the drug did not have any effects yet ($t = 0$ min; see Warren et al., 2016). The actual experiment was conducted

when atomoxetine plasma levels were at their peak (t = 140-170 min; Sauer, Ring, & Witcher, 2005). Between the practice phase and the experimental phase, participants underwent resting-state functional neuroimaging.
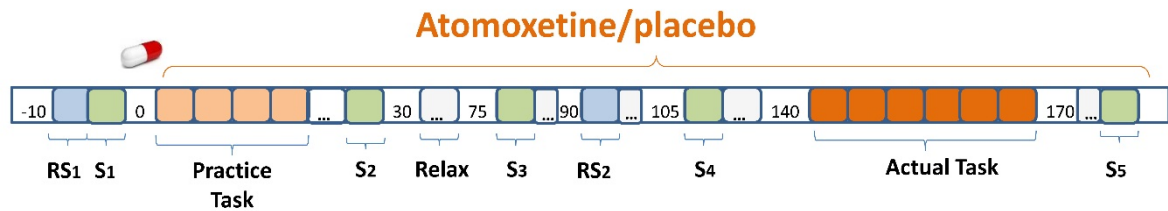

Figure 4. Timeline of procedures in Study 2. $RS_{(1,2)}$ = resting-state MRI, $S_{(1,2,3,4,5)}$ = saliva measurement

Analysis

We performed repeated-measures ANOVAs with correct RT and accuracy (% errors) as dependent variables, treatment (atomoxetine/placebo), CSI (150 ms/800 ms), trial type (switch/repeat) and task (odd/even or high/low) as within-subject independent variables and treatment order as a between-subject variable. As in Study 1, treatment order and task were factors of no interest, so we do not report any statistical terms involving these factors. Trial exclusion criteria were the same as in Study 1.

Results

Reaction times

Mean RTs are presented in Figure 5. The pattern of findings was similar to that in Study 1. Participants showed a typical switch cost of 85 ms ($F(1,22)$ = 47.21, $p < .001$; switch 733 ms, repeat 818 ms), which decreased with the time available for preparation ($F(1,22)$ = 24.68, $p < .001$). The switch cost was smaller in long CSI blocks (44 ms) than in short CSI blocks (127 ms). There was also a main effect of CSI ($F(1,22)$ = 128.63, $p < .001$).

Treatment ($F(1,22)$ = 1.24, $p = .27$) did not have a main effect on RT, but the means indicated somewhat slower responses in the atomoxetine condition (785 ms) than in the placebo condition (765 ms). Importantly, the switch costs ($F(1,22)$ = 2.88, $p = .10$) and the preparation effect ($F(1, 22) < 1$, $p = .62$) were not modulated by atomoxetine and there were no interactions between treatment and other factors of interest.

Accuracy

As shown in Figure 5, the pattern of mean accuracy scores was also similar to that in Study 1. Participants showed a typical switch cost of 3.8% ($F(1,22)$ = 49.8, $p < .001$; switch 91.2%, repeat 95.0%), which decreased with the time available for preparation ($F(1,22)$ = 9.8, $p = .005$). The switch cost was smaller in long CSI blocks (2.1%) than in short CSI blocks (5.4%). There was also a main effect of CSI ($F(1,22)$ = 17.9, $p < .001$).

Treatment ($F(1,22) < 1$, $p = .78$) did not have a main effect on accuracy (atomoxetine 93.2%, placebo 93.0%). Importantly, the switch costs ($F(1,22) < 1$, $p = .92$) and the

preparation effect ($F(1,22) < 1$, $p = .43$) were not modulated by atomoxetine and there were no interactions between treatment and other factors of interest.
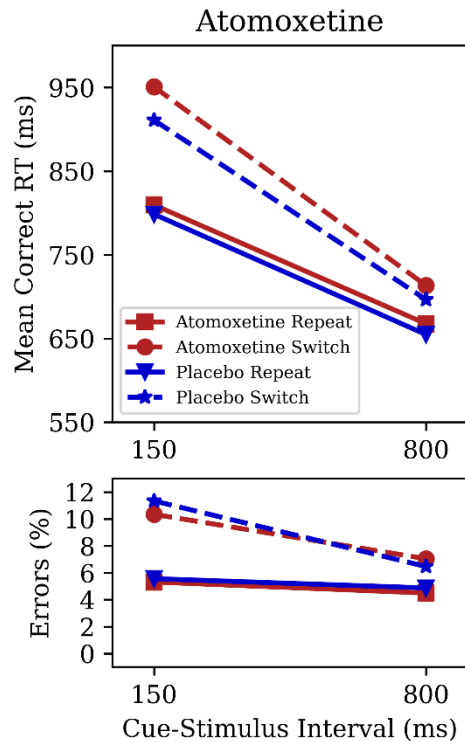

Figure 5. Effects of atomoxetine. Mean correct RT (top) and error rate (bottom) as a function of treatment, CSI and trial type.

General Discussion

Previous work has suggested an important role for the LC-NE system in modulating several forms of cognitive flexibility, possibly by global modulation of gain and corresponding levels of decision noise (Aston-Jones & Cohen, 2005; Kane et al., 2017; Warren, van den Brink, et al., 2017). However, it is still unknown whether NE levels are also critical for task switching (Kehagia et al., 2009; Kehagia et al., 2010), which requires the dynamic transformation of task-set representations from trial to trial. We addressed this question by examining cued task-switching performance after manipulating activity of the LC-NE system using stress induction, tVNS, and administration of atomoxetine. Our findings were highly consistent: none of these manipulations affected measures of task-switching performance, suggesting that NE is not involved in the cognitive flexibility required to switch between relatively abstract rules and sets of stimulus-response mappings.

A potential explanation for these findings is that our manipulations of the noradrenergic system were unsuccessful. However, this explanation is implausible. First, we used an effective, standardized protocol for experimental stress induction (Schwabe & Schachinger, 2018), which led to increased pain perception and elevated salivary cortisol levels in our participants. And as argued above, the relationship between stress and noradrenergic activity has been well-documented. The success of our tVNS manipulation

is less clear. Invasive VNS in rodents results in increased NE levels. Elsewhere we report that our 0.5-mA tVNS manipulation significantly increased salivary cortisol and α-amylase, two indirect hormonal markers of noradrenergic function, in a partially overlapping group of participants (Warren et al., 2018). However, reported effects of tVNS on psychophysiological markers of noradrenergic function in humans (pupil size and P3 amplitude) are mixed (Fischer et al., 2018; Warren et al., 2019), perhaps due to differences in stimulation intensity or choice of sham stimulation location. It is also worth noting that our tVNS manipulation did not affect subjective (psychological, declarative) arousal levels as assed with affect grid questionnaire; this dissociation between subjective (psychological) and physiological arousal/ stress levels has been reported in prior literature (Kindt, Soeter, & Vervliet, 2009). Finally, there is a wealth of evidence that atomoxetine has dose-dependent effects on LC firing rate and synaptic NE levels (Bari & Aston-Jones, 2013), and we previously reported data obtained in the same study and participants, showing that our manipulation increased salivary cortisol and α-amylase (Warren, van den Brink, et al., 2017). Taken together, there is little doubt that at least two of our manipulations were successful at manipulating noradrenergic activity.

What do our null findings mean? The two tasks our participants had to perform involved the same inputs (digits 1-4, 6-9) and outputs (keys Q and P) but differed in the mappings from input to output. The difficulty of combining these tasks lies in the brain's propensity to use the same representations for different purposes. That is, although in general this "multiplexing" offers an efficient way of encoding information, mutual interference, or cross-talk, arises when two tasks make simultaneous demands on the same representations (Feng, Schwemmer, Gershman, & Cohen, 2014). In principle, task switching is different from multitasking, in that the two sets of stimulus-response rules need not be active at the same time. However, it takes long for an irrelevant task-set representation to dissipate. In a typical, fast-paced task-switching experiment, this task-set inertia causes interference between the relevant and irrelevant task sets (Monsell et al., 2003; Yeung et al., 2006), which is reflected in the switch cost (i.e., a main effect of trial type). In such circumstances cognitive control can be used to reduce cross-talk in the service of overall task performance (Feng et al., 2014). In a task-switching experiment, participants can -to some extent- actively prepare for the upcoming switch trial by proactively reconfiguring the task set. This process is reflected in the preparation effect— the reduction of switch costs with growing CSI (i.e., an interaction between CSI and trial type). Importantly, the present findings suggest that our manipulations affected neither task-set inertia nor proactive task-set reconfiguration.

We studied higher-order cognitive flexibility using a task switching paradigm (Monsell et al., 2003) in which the task to be performed on each trial was indicated by a cue presented at the start of the trial. This paradigm distinguishes between trials on which the task changes (" switch trials") and trials on which the task stays the same ("repeat trials"). The finding of interest is that reaction time is longer, and error rate greater, on switch trials (the "switch cost"); and as the cue–stimulus interval is prolonged—allowing more opportunity for advance preparation—the switch cost is reduced (the "preparation

effect"). Switch costs are attributable to a combination of the time required for resolving interference from residual activation of the previous, no-longer relevant task set ("task-set inertia") and of the time required for retrieving the newly cued task set ("task-set reconfiguration"; Monsell et al., 2003). The cognitive flexibility required to switch between tasks depends on the dynamic transformation of neural task-set representations from trial to trial (Qiao et al., 2017; Yeung et al., 2006). In order to ensure that the observed switch costs and preparation effect would accurately reflect this type of cognitive flexibility instead of a cue-repetition effect, we used two cues per task, which allowed us to avoid direct cue repetitions between trials (Logan & Bundesen, 2003; Monsell & Mizon, 2006).

We are aware of two published studies on the effect of acute stress on task switching, which produced mixed results. Steinhauser and colleagues found that stress modulated the preparation effect (for response times, not error rates), but not the switch costs itself (Steinhauser, Maier, & Hübner, 2007). Their control group showed the typical reduction in switch costs at a long CSI, their stress group did not, which led the authors to conclude that stress induces a change in task-set reconfiguration strategy. Plessow and colleagues, using the same tasks as our study, found no effect of stress on the preparation effect and no effect on the RT switch cost; stress only led to a significant but small increase in the accuracy switch cost (stress group: 2.9%, control group: 1.4%; Plessow, Kiesel, & Kirschbaum, 2012). Both studies used a stress-induction procedure that involved a psychological component (social evaluation and increased cognitive load), but not a physiological component such as our cold pressor test. Plessow and colleagues confirmed the success of their stress manipulation by examining salivary cortisol levels; Steinhauser and colleagues report no cortisol data. Neither study controlled for cue-repetition effects and hence examined a confounded measure of task switching ability. More research is needed to fully understand the effect of acute stress on task switching.

We found no effects of tVNS (versus sham) and tVNS intensity on measures of task switching. A previous study found that invasive VNS (versus sham) impaired cognitive flexibility of epilepsy patients on an anagram task (Ghacibeh et al., 2006), so more research is needed in this area. In a post hoc exploratory analysis we found that tVNS at a higher intensity was associated with a speed-accuracy tradeoff: Stimulation at 1.0 mA resulted in faster (Δ 35 ms) but less accurate responses (Δ 1.0%). This finding seems at odds with findings that invasive VNS *decreases* excitability of the motor cortex in active rats (Mollet et al., 2013), even at mild stimulation intensities, which would predict slower and more accurate responses. One study examined effects of tVNS on excitability of the motor cortex in healthy human volunteers (Capone et al., 2015). However, this study was underpowered (N=10), used an unusually high stimulation intensity (8.0 mA), and did not find significant effects after correction for multiple statistical comparisons. Thus, our finding that 1.0 mA changes the speed-accuracy tradeoff requires replication, preferably augmented with measurements of motor cortex excitability.

Finally, although we used a drug (atomoxetine) and dose (40 mg) known to influence various aspects of cognitive task performance and cortical state (Jepma et al., 2016;

Pfeffer et al., 2018; van den Brink, Nieuwenhuis, & Donner, 2018; Warren, van den Brink, et al., 2017), even our pharmacological manipulation failed to modulate task switching performance. Two other pharmacological studies have yielded consistent results. One study found no effect of the beta-adrenergic antagonist propranolol (80 mg) on switch costs (Steenbergen, Sellaro, de Rover, et al., 2015). However, there is some evidence that higher-order cognitive flexibility is mediated by alpha receptors, not beta receptors (Lapiz & Morilak, 2006). Another study found no effect on switch costs of the dopamine and norepinephrine transporter blocker methylphenidate (20 mg (Frobose et al., 2017). Neither study was designed to examine the preparation effect.

While the cortical areas involved in task switching are rather well-known (Worringer et al., 2019), very little is known about the neurochemical basis supporting efficient task switching. In a much-cited review, Kehagia and colleagues (Kehagia et al., 2010) hypothesized that task switching may have a noradrenergic substrate, similar to extra-dimensional set-shifting, another example of higher-order cognitive flexibility. However, the present findings suggest that norepinephrine does not play an important role in task switching. This has applications for everyday life activities given that cognitive flexibility allows us to quickly adapt ongoing behavior to salient changes in the environment and is important for human success (e.g. at work) and survival (e.g. threatening conditions). It also has clinical applications related to disorders such as ADHD, anxiety disorders, epilepsy and Parkinson's disease where pharmacological administration of noradrenaline is the most common treatment or excessive flexibility/rigidity is one of the main symptom.