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Effects of Concomitant Stimulation of the GABAergic and Norepinephrine System on Inhibitory Control – A Study Using Transcutaneous Vagus Nerve Stimulation

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ABSTRACT

Background: Inhibitory control processes are a central executive function. Several lines of evidence suggest that the GABAergic and the norepinephrine (NE) system modulate inhibitory control processes. Yet, the effects of conjoint increases in the GABAergic and NE system activity on inhibitory control have not been examined.
Objective/hypothesis: We examine the conjoint effects of the GABA and NE system for inhibitory control.
Methods: We used transcutaneous vagus nerve stimulation (tVNS), which has been shown to modulate both the GABAergic and NE system. We examine the effects of tVNS in two experimental paradigms examining different aspect of inhibitory control; i.e. a backward inhibition paradigm and a response inhibition paradigm modulating working memory load.
Results: There were no effects of tVNS on backward inhibition processes, but on response inhibition processes. Yet, these only emerged when working memory processes were needed to control response inhibition. Compared to a sham stimulation, tVNS induced better response inhibition performance (i.e. fewer false alarms).
Conclusions: A concomitant modulation of the GABAergic and NE system, as induced by tVNS, affects inhibitory control processes, but only when working memory processes play an important role for inhibitory control. Even though both the GABAergic and the NE system are modulated by tVNS, the results suggest that the modulation of the NE system is most important for the emerging effects.

Introduction

Inhibitory control processes are a central executive function allowing to control one's attention, behavior, and thoughts by overriding a strong internal predisposition or external lure [1,2]. Yet, other executive control subprocesses may interfere with the processes in most daily life situations. Working memory processes have been shown to modulate response inhibition processes, with high working memory load impairing response inhibition [3–5]. However, response inhibition functions can also work as subprocesses supporting executive control functions. One example for this is cognitive flexibility, which is often examined using task switching paradigms. Switching between tasks is effortful and time consuming. Importantly, it has been proposed that for flexible task switching, the efficient activation of a new task goes along with the inhibition of the previous, no longer relevant task [6]. The process that inhibits the most recently performed task when a new task is to be performed is referred to as 'backward inhibition' (BI) [7]. Stronger backward inhibition processes are related to better task switching.

While there has been considerable advance in the understanding of the neurobiological processes subserving inhibitory control [1], the neurobiological mechanisms underlying backward inhibition processes as well as the impact of working memory load on inhibitory control are not yet fully understood. For example, it has been shown that increasing norepinephrine (NE) concentrations in the prefrontal cortex improve response inhibition performance in rodents and humans [8–10] as well as in patients suffering from attention deficit hyperactivity disorder [9] when examining response inhibition processes via stop signal and Go/Nogo tasks. In these

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studies (i.e. the stop-signal task), the stop signal reaction time (SSRT) was shorter increasing NE system turnover suggesting for better response inhibition performance. The NE system may therefore also modulate backward inhibition processes and the effect of working memory load on response inhibition processes. This is especially likely for the latter, as working memory processes are also modulated by the NE system [11]. Aside from the NE system, the GABAergic system has recently been found to be also important for response inhibition processes [12,13], with increasing (striatal) GABA levels being associated with better response inhibition performance. Similar results have been obtained for GABA concentrations in the anterior cingulate cortex (ACC) [14]. Since prefrontal-striatal circuits are important for backward inhibition processes [15–17] and the impact of working memory load on response inhibition processes [3,4], the GABAergic system is a potential second candidate modulator of backward inhibition processes and the impact of working memory load on response inhibition processes.

Due to all of these considerations, both the NE and the GABAergic system may therefore be important modulators of inhibitory control. This may especially be the case when both of these systems are conjointly modulated. One way to do so is to apply transcutaneous vagus nerve stimulation (tVNS) [18]. Stimulation of the vagus nerve in rats increases the NE concentration [19,20]. Moreover, two functional magnetic resonance imaging (MRI) studies in healthy humans have found that real tVNS compared to a sham stimulation increased activation in the brainstem region including the locus coeruleus [21]. These outcomes indicate that transcutaneous VNS also results in effective stimulation of the vagal afferents [22,23]. Moreover, it has been shown that vagus nerve stimulation increases the levels of free GABA in the cerebrospinal fluid [24]. Also, in epileptic patients receiving tVNS for one year, GABA-A receptor density was significantly increased as compared to untreated controls [25]. Lastly, a recent study also suggests that tVNS acts to increase the GABAergic cortical activity [26].

In the current study, we use tVNS to simultaneously increase NE and GABA concentrations. If there are additive effects of an increase of GABAergic and NE activity, this will foster inhibitory control processes. We hypothesize that there should hence be stronger backward inhibition effects leading to better task switching performance and better response inhibition under varying levels of working memory load. Due to the importance of the NE and also the GABAergic system for working memory processes it is also possible that modulatory effects of tVNS on inhibitory control processes are confined to conditions where working memory load is increased. However, the GABAergic and the NE system interact with each other [27] and the effects of NE depend on the receptor type stimulated and the cortical region [28]. Since tVNS effects are not selective for an NE receptor subtype and it is known that positive effects of NE on executive functions are mediated via α2 receptors, while α1 receptors and lower affinity β-receptors worsen executive control functions [29,30], it is also possible that there is little effect of tVNS stimulation on inhibitory control processes.

Materials and methods

Participants

Fifty-one Leiden University undergraduate students (37 females, 14 males, mean age = 23.62 years, range 18–29) took part in the study. Participants were recruited via an on-line recruiting system and were given course credit for taking part in the experiment. Participants were screened individually via a phone interview using the Mini International Neuropsychiatric Interview (M.I.N.I.), which is often used in pharmacological research [31–33]. A blind, sham-controlled design was used. Twenty-six participants were randomly assigned to the active tVNS group whereas 26 were assigned to the sham tVNS group; however, one subject in the tVNS group had to be excluded due to reasons of data quality. Following published protocols [34], participants fulfilled these inclusion 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; and (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. Adverse effects include: (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. 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 local ethics committee (Leiden University, Institute for Psychological Research). In each group of subjects (i.e. tVNS and sham tVNS group) the subjects were tested with both paradigms (detailed below). The paradigms were administered in a counterbalanced order.

Transcutaneous vagus nerve stimulation (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). 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 [22,35]. Stimulation was active for 30 seconds, followed by a break of 30 seconds. Following Kraus and colleagues [36], a sham condition using tVNS was created by attaching the stimulation electrodes to the center of the left ear lobe, which is known to be free of cutaneous vagal innervation [37]. This procedure is known to produce no activation in the cortex and brain stem [38]. tVNS is safe to be performed only in the left ear [39,40] as these do not affect vagal fibers to the heart which originate from the right site [41]. The placement of the electrode for tVNS and sham tVNS differs. During real tVNS the electrodes are placed in the inner ear (see above). During sham tVNS the electrodes are placed in the outer ear. Since the stimulation sites are therefore different people would notice that something is happening. That is why it is not possible to use a within-subject design, even though this would be desirable from the perspective of statistical power in the study design.

Backward inhibition paradigm

To assess the backward inhibition (BI) effect, we used a modified version of the backward inhibition paradigm proposed by Koch et al [42] (see also Ref. [16]).

Each trial consisted of a central cue which was complemented by a central target. A square cue was used to indicate task A (odd/even), a diamond was used to indicate task B (smaller/larger), and a triangle was used to indicate task D (double-press). Target stimuli consisted of digits 1–9 except for 5 and appeared within the cue frame with a stimulus onset asynchrony (SOA) of 100 ms (compare Fig. 1). Both cue and target stayed on the screen until the participants responded. In the odd/even task, participants should indicate whether the target digit was odd (left index finger press) or even (right index finger press). In the smaller/larger task, they should indicate whether the target was smaller (left index finger) or larger (right index finger) than five. In contrast to that, participants should

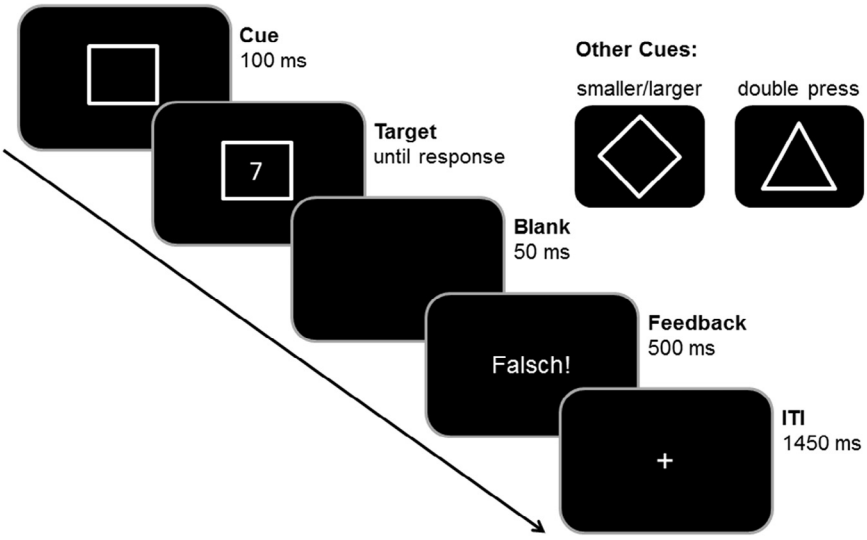

Figure 1. Experimental design of the backward inhibition paradigm. Each trial began with the presentation of a cue in the center of the screen. A square cue indicated the odd/even task (left button press for odd numbers, right button press for even numbers). A diamond cue (see bottom left) indicated the smaller/larger rule (left for smaller than five, right for larger than five). A triangle cue (see bottom left) indicated the double press rule (simultaneous button press within the first 1000 ms after target onset). After 100 ms, the target stimulus (any digit from 1 to 9, except 5) was presented within the target stimulus until a response was made. A speedup sign ("Faster!") appeared above the cue frame in case no response was given within the 1000 ms after target onset. During the inter-trial interval of 2000 ms, there was a 500 ms feedback for incorrect trials ("Wrong!"), but no feedback/only a fixation cross in correct trials.

press both buttons simultaneously (with an asynchrony of less than 50 ms) upon target presentation in the double-press task. Responses were given on the two Ctrl-buttons of a custom keyboard. If participants did not respond within 1000 ms after target onset, a speed-up sign **("Faster!")** appeared above the cue asking participants to respond more quickly. Between trials, there was a fixed 1500 ms response-stimulus interval (RSI), during which a fixation cross was centrally presented. In case of a slow responses (more than 1000 ms in the task D, 2500 ms in tasks A and B) and/or erroneous responses, the feedback "too late!" and/or "wrong!" was centrally presented during the first 500 ms of the RSI (as shown in Fig. 1). Incorrect key presses, too slow responses and non-simultaneous keypresses in the double-press task were counted as errors.

The experiment consisted of 768 trials divided into 8 equally sized blocks which were subject to a grammar proposed by Koch et al. [42]. Each cue and target as well as each possible combination of them were randomized and occurred with the same frequency. However, neither cues nor target could be the same in two consecutive trials. Furthermore, the target in the current trial was always different from the target used in the last trial with the same cue. Within each block, each trial (except for the first two trials, of course) built a triplet with the last two preceding trials. Hence, there was a total of 752 triplets and the experiment lasted approximately 30 minutes. All twelve possible triplet combinations (ABA; ADA; BAB; BDB; DAD; DBD; DBA; BDA; DAB; ADB; BAD; ABD) were equally frequent (±1 triplet for two of the triplet conditions in each block). Triplets where the last trial had the same cue as the n-2 trial were categorized as back-switching triplets while triplets without that n-2 cue repetition were categorized as baseline triplets. In accordance with previous results on this task [16], we however limited our analyses to the triplets which best depict the BI effect, i.e. ABA and BAB for the BI condition, and DAB and DBA for the baseline (BASE) condition.

All participants received both written and oral task instructions and were asked not to keep track of previous trials. To make sure that the participants understood the instructions and kept the rules in mind, they were asked to start with a practice block consisting of 12 trials. As behavioral measures, accuracy and RTs were separately collected for each condition.

Statistical analysis of the backward inhibition paradigm

For data analyses, we discarded all trials with RTs faster than 100 ms. Additionally, we excluded all trials from task D with RTs slower than 1000 ms and all trials from tasks A and B with RTs slower than 2500 ms (i.e. all trials in which a "Faster!" sign was shown). This cutoff decision was based on the paper by Koch et al. [41] and the fact that responses in tasks A and B are typically longer than those in task D. To form triplets of correctly responded trials for the analysis of the BI effect, we furthermore excluded the first two trials of each block, all erroneous trials and the two trials following an error or a too slow trial.

Next, we separately quantified the accuracy (percentage of correct triplets), mean RT (of the last trial of each triplet) and the standard deviation (SD) of the RTs for the ABA, BAB, DBA, and DAB triplets. Finally, we obtained measures for the BI condition by averaging the respective measures of the ABA and BAB triplets while the DAB and DBA were averaged to create values for the BASE condition. Additionally, we decided to investigate potential learning effects/effect of time by assessing these behavioral measures at four different stages (i.e. averaged over blocks 1&2/3&4/5&6/7&8).

Based on the fact that only triplets with three correct responses in a row were rated as correct, responses at chance level would yield an accuracy of approx. 12.5% (i.e. a probability of 0.5^3). To exclude subjects who responded at or close to chance level, we excluded all subjects who yielded an accuracy of less than 20% in any block and/or condition. After this procedure, 45 subjects (n = 22 active, n = 23 sham) remained within the sample and entered statistical analyses.

For the regular data analyses, the data were analyzed with IBM SPSS statistics version 23.0.0.0 using mixed effects ANOVAs comprising the within-subject factors "condition" (BI vs. BASE) and "block" (1&2 vs. 3&4 vs. 5&6 vs. 7&8) as well as the between-subject factor "stimulation" (active vs. sham). Separate ANOVAs were

calculated for each behavioral measure (accuracy, mean hit RTs, and RT SDs). Greenhouse–Geisser correction was applied whenever necessary. Post-hoc tests were Bonferroni-corrected whenever necessary.

Mental workload response inhibition paradigm

To examine response inhibition processes and its modulation by working memory load we used a task combining a classical Go/Nogo task with a mental rotation task as described in a previous study [4]. In this task the characters G and R and the digits 5 and 7 were employed as target stimuli due to their good visual discriminability and due to the fact that these stimuli are known to not evoke sex-dependent differences in the mental rotation process [43–45]. By means of target rotation, varying working memory load was induced and varied in this Go/NoGo paradigm. Equal proportions of each target stimulus were rotated by 30, 90, or 150°, thus evoking an increasing workload from the smallest to the largest rotation angle [46,47]. Targets were presented rotated clockwise and counter-clockwise in a normal (not mirrored) and a mirrored fashion [45,48–51]. To ensure the classic characteristics of a Go/NoGo task, 30% NoGo trials requiring no response and 70% Go trials that required a specific response by the participants were utilized.

The trials were presented in two blocks. In block A, the less demanding block, all letter targets required a response, and thus constituted the Go stimuli. Digits required no response and thus served as Nogo stimuli. It was therefore possible to discriminate between responding and inhibition on the basis of stimulus category (letter vs. number) and it was thus not necessary to rotate the different stimuli to know whether a response was required or not. On Go trials in block A, the presentation of a mirrored letter required a button press with the index finger of the left hand, while the presentation of a normal (not mirrored) letter required a right index finger response.

In contrast to block A, both numbers and letters were utilized as targets in block B. This block was hence more challenging, since the decision to respond or to refrain from responding could not be reached merely on the basis of perceptual category (letter vs. number). In block B, responses had to be carried out to un-mirrored targets, while mirrored numbers or letters required no response and thus served as NoGo trials. It was therefore necessary to perform mental rotation processes in order to decide whether or not to respond. These mental rotation processes increase working memory load, thus allowing to investigate the influence of mental workload on response inhibition processes. As both blocks A and B required a response to normal, un-mirrored letters, transfer effects between the two blocks needed to be ruled out. To do so, normal, un-mirrored letters required a left hand response in block B (as compared to a right-hand response in block A). Consequently, the presentation of normal, un-mirrored numbers required a right hand response in block B. In order to provoke inhibition errors and to further amplify the effect of workload manipulation through time pressure, participants were generally requested to respond as fast and accurately as possible in each trial during the whole task. An example of experimental conditions and stimulus combinations is given in Fig. 2.

Each trial began with an 800 ms presentation of a fixation cross, which was followed by a 1100 ms target presentation. Irrespective of correctness, target presentation was terminated when a response was executed. The two blocks were presented in a counterbalanced order and always preceded by a standardized instruction. Furthermore, an exercise with 60 trials was conducted before the experiment in order to familiarize subjects with the task. In the subsequent experimental blocks, 360 trials (252 Gos and 108 NoGos, equally distributed across the different rotation angles and mirror conditions) were presented in a randomized order, thus summing up to a total of 720 trials in the two blocks. The experiment lasted for about 30 minutes.

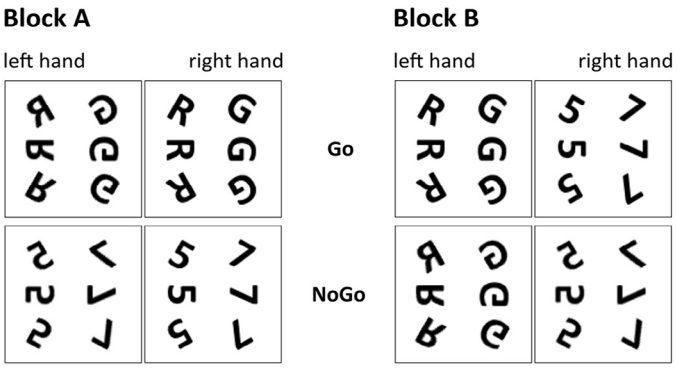

Figure 2. Overview of the experimental conditions in the mental workload inhibition paradigm. At the left of the figure, block A is shown, and the right shows block B. For the Go and Nogo trials, examples of the used stimuli are shown. For Go trials, it is indicated which stimulus required a left or a right-hand response.

Statistical analysis of the mental workload inhibition paradigm

For statistical analyses, we quantified the accuracy (in percent correct) and hit RTs for Go trials as well as the false alarm rate for Nogo trials (separately for each block and rotation). Individuals with less than 70% accuracy on Go trials were excluded from the sample. After this procedure, 46 subjects (n = 22 active, n = 24 sham) remained within the sample and entered statistical analyses. Our three measures were separately analyzed with IBM SPSS statistics version 23.0.0.0 in repeated-measures ANOVAs using the factors "block" (block A vs. block B) and "rotation" (0° vs 90° vs. 150°) as within-subject measures and the factor "stimulation" (active vs. sham) as a between-subjects factor. Greenhouse–Geisser correction was applied whenever necessary. Post-hoc tests were Bonferroni-corrected whenever necessary.

Results

Results of the backward inhibition paradigm

The descriptive results of performance in the backward inhibition paradigm are shown in Fig. 3.

The analyses of the accuracy data obtained from the backward inhibition paradigm revealed a main effect of block ($F(3129) = 15.017$; $p < .001$; $\eta_p^2 = .259$). Post-hoc t-tests showed that this was due to lower accuracy in the first two blocks as compared to all other blocks. Importantly, the remaining blocks did not differ from each other (all $p \geq .158$). All other main effects or interactions were non-significant (all $F \leq 2.664$; $p \geq .110$; see Table 1).

The analyses of the RT data obtained from the backward inhibition paradigm revealed a main effect of block ($F(3129) = 64.410$; $p < .001$; $\eta_p^2 = .600$). Post-hoc t-tests showed that all blocks significantly differed from each other (all $p \leq .011$) and participants responded faster as they progressed with the paradigm. There was furthermore a main effect of condition ($F(1,43) = 35.039$; $p < .001$; $\eta_p^2 = .449$), with faster RTs in the BASE condition than in the BI condition. All other main effects or interactions were non-significant (all $F \leq .616$; $p \geq .437$; see Table 1).

Lastly, the SD of hit RTs only showed a main effect of block ($F(3129) = 15.344$; $p < .001$; $\eta^2_p = .263$). Post-hoc t-tests revealed that all hit RT SDs except for the comparison of the last two blocks ($t = .956$; $p = .344$) significantly differed from each other (all $p \leq .011$)

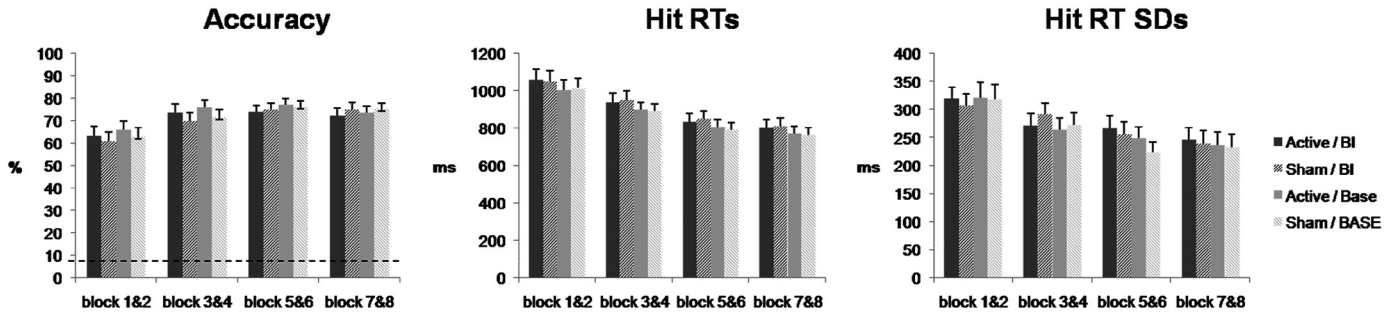

Figure 3. Descriptive data in the backward inhibition paradigm showing the accuracy (in percent), the reaction times (RTs) on correctly responded trials as well as SDs of hit RTs. These data are given for the tVNS and sham tVNS procedure in the backward inhibition (BI) and baseline (BASE) condition, as well as for the different blocks in the paradigm.

and decreased over the course of the experiment. All other main effects or interactions were non-significant (all $F \leq 2.042$; $p \geq .160$).

In sum, we found effects of initial learning as well as the effects typically observed in backward inhibition paradigms (compare Refs. [16,41]). Yet, stimulation did not make a difference in any of the observed effects and measures. We hence used Bayesian statistics to analyze this lack of group effects. Using the "Bayesian Repeated Measures ANOVA" function of the JASP 0.7.5.5 software (available on https://jasp-stats.org/), we estimated the Bayes factor ($BF_{INCLUSION}$) as well as p(incl|data), which is the posterior probability that the data favor the alternative hypothesis given the measured effects (i.e. $p(H_1|D)$). Of note, this yields individual values for all potential main effects and interactions investigated with the regular SPSS repeated-measures ANOVA. In this context, it should however be noted that the posteriors for the ANOVA factors are composed of several posteriors obtained from a model comparison taking all potential combinations of main and interaction effects into account (see supplementary tables). Due to the fact that some of the models are included in several factors, the sum of the posteriors of model comparisons is 1, but exceeds the value of 1 for the summed up posteriors obtained from the analysis of effects reported in Table 1. A more detailed description of the mathematical approach underlying this model can be found in Ref. [52]. The results of the Bayesian analyses are shown in Table 1.

Results of the mental workload inhibition paradigm

The descriptive results of performance in the mental workload inhibition paradigm are shown in Fig. 4.

The analyses of the accuracy data obtained from the mental workload inhibition paradigm revealed a main effect of block ($F(1,44) = 28.014$; $p < .001$; $\eta_p^2 = .389$) with higher accuracy in block B than in the block A. There was a main effect of rotation ($F(2,88) = 46.148$; $p < .001$; $\eta_p^2 = .512$) with a decrease in accuracy as rotation/workload increased. There was however also an interaction of block*rotation ($F(2,88) = 17.437$; $p < .001$; $\eta_p^2 = .284$). Post-hoc t-tests showed that block B always yielded higher accuracy than block A (all $p \leq .043$). However, this difference was larger in the 150° condition (all $p \leq .001$) while it did not differ between the 30° and 90° conditions ($t = .192$; $p = .849$). All other main effects or interactions were non-significant (all $F \leq 1.989$; $p \geq .154$; see Table 2).

The analyses of Go hit RTs revealed a main effect of block ($F(1,44) = 63.500$; $p < .001$; $\eta_p^2 = .591$) with slower responses in block A than block B. There was furthermore a main effect of rotation

Table 1
Results of the Bayesian analyses of the backward inhibition paradigm data.

ACCURACY	F-value	Sign./p(D|H0)	Partial η^2	p(incl|data)	$BF_{Inclusion}$
Group	.094	.761	.002	0.277	0.137
Blocks	15.017	.000	.259	1.000	2.280e +9
Condition	2.664	.110	.058	0.283	0.141
Group*Blocks	.951	.401	.022	0.036	0.081
Group*Condition	.199	.658	.005	0.013	0.029
Blocks*Condition	.133	.925	.003	0.009	0.020
Group*Blocks*Condition	.042	.983	.001	3.250e -6	5.850e -5
Hit RTs	**F-value**	**Sign./p(D|H0)**	**Partial η^2**	**p(incl|data)**	**$BF_{Inclusion}$**
Group	.000	.991	.000	0.371	0.211
Blocks	64.410	.000	.600	1.000	∞
Condition	35.039	.000	.449	0.999	326.132
Group*Blocks	.003	.996	.000	0.011	0.024
Group*Condition	.616	.437	.014	0.054	0.124
Blocks*Condition	.157	.898	.004	0.030	0.068
Group*Blocks*Condition	.445	.688	.010	3.278e -6	5.900e -5
Hit RT SDs	**F-value**	**Sign./p(D|H0)**	**Partial η^2**	**p(incl|data)**	**$BF_{Inclusion}$**
Group	.026	.872	.001	0.276	0.136
Blocks	15.344	.000	.263	1.000	1.624e +9
Condition	2.042	.160	.045	0.235	0.110
Group*Blocks	.581	.596	.013	0.018	0.040
Group*Condition	.067	.797	.002	0.023	0.051
Blocks*Condition	.960	.409	.022	0.014	0.030
Group*Blocks*Condition	.185	.894	.004	3.088e -6	5.558e -5

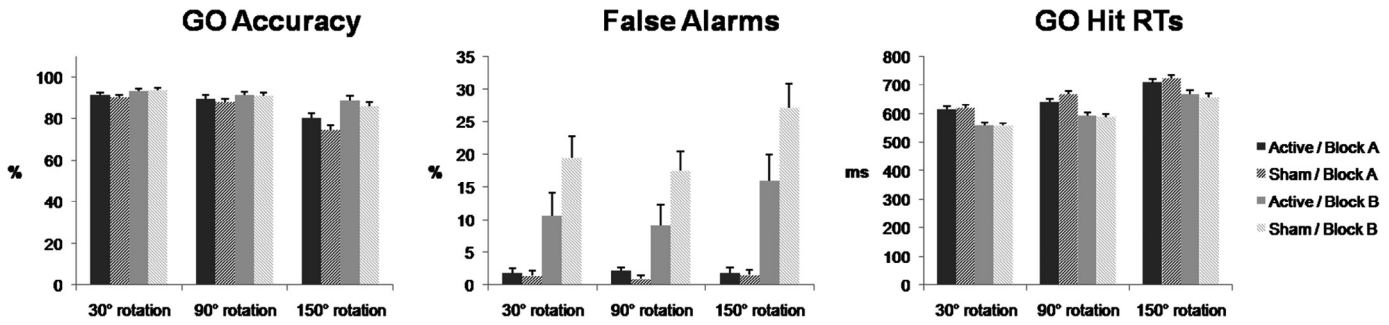

Figure 4. Descriptive data in the mental workload inhibition paradigm showing the accuracy (in percent) on Go trials, the absolute frequency of false alarms (i.e. responses on Nogo trials) and the reaction times (RTs) on Go trials. These data are given for the tVNS and sham tVNS procedure in the different experimental blocks (A and B).

($F(2,88) = 380.018$; $p < .001$; $\eta_p^2 = .896$). Post-hoc t-tests showed that all RTs significantly differed from each other (all $p \leq .001$) as they increased with rotation angle. All other main effects or interactions were non-significant (all $F \leq 1.897$; $p \geq .175$; see Table 2).

The analyses of the false alarms revealed a main effect of block ($F(1,44) = 51.803$; $p < .001$; $\eta_p^2 = .541$) with more false alarms in block B than in block A. There was a main effect of rotation ($F(2,88) = 11.397$; $p < .001$; $\eta_p^2 = .206$). There was however also an interaction of block*rotation ($F(2,88) = 11.129$; $p < .001$; $\eta_p^2 = .202$). Post-hoc repeated measures ANOVAs showed that there were no effects of rotation in block A ($F(2,90) = .076$; $p = .927$; $\eta_p^2 = .002$) but in block B ($F(2,90) = 12.401$; $p < .001$; $\eta_p^2 = .216$). Post-hoc t-tests revealed increased false alarm rates in the 150° condition as compared to the other two conditions (all $p \leq .002$) while false alarm rates did not differ between the 30° and 90° conditions ($t = 1.287$; $p = .205$). Most importantly, however, there was an interaction of block*group ($F(1,44) = 5.823$; $p = .020$; $\eta_p^2 = .117$). Post-hoc t-test revealed that the active group committed significantly less false alarms than the sham group in block B ($t = -2.144$; $p = .038$), but not in block A ($t = .804$; $p = .406$). All other main effects or interactions were non-significant (all $F \leq 3.483$; $p \geq .069$; see Table 2). In sum, we found that in the more challenging task B, subjects committed fewer false alarms when actively stimulated. We additionally used Bayesian statistics to analyze all effects in the same fashion as for the backward inhibition paradigm. The results of the Bayesian analyses are shown in Table 2, the model comparisons on which these values are based, are again provided in the supplementary data.

Discussion

In the current study, we examined the role of the GABAergic and NE system for inhibitory control processes. Both the GABAergic system and the NE system have previously been shown to modulate response inhibition performance [8–10,12,14]. Therefore, we examined the conjoint effect of increases in GABAergic and NE system on neural transmission by means of tVNS.

tVNS did not generally modulate inhibitory control processes. There were no effects of tVNS on backward inhibition processes, which was supported by a Bayesian analysis of the obtained data. There were also no effects of sham or active tVNS stimulation in block A, but in block B of the mental workload inhibition paradigm. In contrast to block A, both numbers and letters were utilized as targets in block B. This block was hence more challenging, as the decision to respond or to refrain from responding could not be reached on the basis of perceptual category (letter vs. number) alone. In block B, responses had to be carried out to un-mirrored targets (both letters

Table 2
Results of the Bayesian analyses of the mental workload inhibition paradigm data.

GO ACCURACY	F-value	Sign./p(D|H0)	Partial η^2	p(incl|data)	$BF_{Inclusion}$
Block	28.014	.000	.389	1.000	2.815e +8
Rotation	46.148	.000	.512	1.000	3.217e +15
Group	1.331	.255	.029	0.491	0.345
Block*Rotation	17.437	.000	.284	0.995	451.427
Block*Group	.993	.324	.022	0.135	0.338
Rotation*Group	1.989	.154	.043	0.187	0.499
Blocks*Rotation*Group	.281	.710	.006	0.006	0.108
GO RTs	**F-value**	**Sign./p(D|H0)**	**Partial η^2**	**p(incl|data)**	**$BF_{Inclusion}$**
Block	63.500	.000	.591	1.000	∞
Rotation	380.018	.000	.896	1.000	∞
Group	.135	.715	.003	0.590	0.514
Block*Rotation	.802	.419	.018	0.095	0.226
Block*Group	1.897	.175	.041	0.421	1.574
Rotation*Group	1.048	.342	.023	0.057	0.131
Blocks*Rotation*Group	1.789	.183	.039	9.767e -4	0.018
NOGO FAs	**F-value**	**Sign./p(D|H0)**	**Partial η^2**	**p(incl|data)**	**$BF_{Inclusion}$**
Block	51.803	.000	.541	1.000	3.217e +15
Rotation	11.397	.000	.206	0.952	7.008
Group	3.483	.069	.073	0.999	420.534
Block*Rotation	11.129	.000	.202	0.765	7.071
Block*Group	5.823	.020	.117	0.998	1.210.543
Rotation*Group	.456	.613	.010	0.083	0.195
Blocks*Rotation*Group	.195	.792	.004	0.007	0.128

and numbers), while mirrored numbers or letters required no response and thus served as NoGo trials. It was therefore necessary to perform mental rotation processes in order to decide whether or not to respond. These mental rotation processes increase working memory load and it therefore seems that working memory load is the important factor determining whether a concomitant modulation of the GABAergic and the NE system has an effect on inhibitory control processes. Since the rotation angle did not further modulate the pattern of results, additional (smaller) gradual variations in working memory load do not seem to be affected by the stimulation of the GABAergic system and NE system as induced by tVNS. Yet, the lack of effects observed in the backward inhibition paradigm underlines the interpretation that working memory load is the most important variable here. Backward inhibition processes have been suggested to take place at the response selection level [53] and have recently been shown to be modulated by flexible attention processes but not by working memory updating processes [16].

Even though tVNS leads to a conjoint modulation of the GABAergic and the NE system [18], it seems most likely that it is the NE system-related aspect of tVNS that underlies the obtained effects. Currently, there is no evidence that the effects of tVNS are specific for an NE receptor subsystem. However, the effects of NE differ depending on the receptor type affected in the prefrontal cortex [28] as well as the precise region with the prefrontal cortex [27]. It has further been suggested the beneficial effects of NE on executive control function are associated with pre- and post-synaptic α2 receptors, while α1 receptors and lower affinity β-receptors worsen executive control functions [29,30]. Concerning working memory processes, α2 receptors promote and α1 receptors impair performance in tests of working memory [28]. The results of the current study can therefore well be explained by the effects on NE α2 receptors. However, it may be argued that modulations of the NE α2 receptors, especially when increasing NE α2 receptor using agonists, have been shown to impair to inhibitory control functions [54]. Yet, this was only the case at high doses [54] and when directly infused in the dorsomedial prefrontal cortex [55], which was certainly not the case using tVNS stimulation used in this study.

While it has been shown that tVNS modulates GABA-A receptors [25], the effect of tVNS on the GABAergic system may be less important for the effects observed for inhibitory control modulated by working memory load. The reason for that is that upregulating GABAergic conductance impairs performs in working memory [56]. If the GABA system had played an important role for modulation of response inhibition processes by working memory, the opposite direction of effects should have been observed. The fact that this was not the case either suggests that the GABA-system is not important for response inhibition, or that the concomitant modulation of the NE system counteracts possible negative effects of the GABAergic system. Interestingly, GABA-A receptors possess a large variety of responses to NE including decreases, increases and also no responses depending on the cortical area [27]. It is therefore possible that the concomitant modulation of the NE system interferes with the modulation of the GABAergic system thereby eliminating negative effects of GABAergic stimulation. The fact that other studies showed that increases in GABAergic concentrations increase inhibitory control [12–14] is most likely due to the fact that these studies examined the effect of the GABAergic system in circumscribed brain regions, i.e. the anterior cingulate cortex or the striatum. tVNS does not specifically modulate the GABAergic system in the striatum and/or the anterior cingulate cortex, but exerts a systemic effect unspecific of any functional neuroanatomical region. The neuroanatomical specificity is therefore important to consider regarding the effects of the GABAergic system on inhibitory control. However, due to ethical reasons the stimulation intensity during the tVNS procedure was quite low. It is therefore reasonable to assume that effects of the NE and/or GABA system were only modest. When using higher stimulation intensities it is conceivable that effects (e.g. regarding working memory dependent modulations of response inhibition processes) can be seen in that tVNS modulates performance in conditions with highest working memory load (i.e. 150° degree condition) and less so in the other conditions with lower working memory load (i.e. 30° and 90° conditions). From a more clinical perspective it may be of interest to examine the effects of tVNS in patients with attention deficit hyperactivity disorder (ADHD). This is because pharmacological treatments in ADHD target the norepinephrine system [9] and these effects have been shown to modulate response inhibition processes in ADHD [9].

Conclusions

The study shows that tVNS and thus a concomitant modulation of the GABAergic and NE system affect inhibitory control processes, but only when working memory processes play an important role during inhibitory control. Even though both the GABAergic and the NE system are modulated by tVNS, the results suggest that it is the modulation of the NE system that is most important for the observed effects.

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Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.brs.2016.07.004.

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