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Bursts of transcranial electrical stimulation increase arousal in a continuous performance test

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ABSTRACT

Arousal reflects a state of generalised physiological activation, and its key role in cognition and behaviour has been extensively described. The regulation of arousal is controlled by specific nuclei located in the brainstem that contain widely distributed projections to the cortex and form the arousal systems. In humans, arousal has been commonly studied and modulated through behavioural paradigms, whereas in animals, direct electrical stimulation has been used to confirm the important role of these widely distributed structures. Recent evidence suggests that it might be possible to use transcranial electrical stimulation (tES) to non-invasively induce currents in the brainstem regions of the brain. Therefore, we hypothesise that, using a specific electrode arrangement, it might be possible to employ tES to stimulate subcortical–cortical neuromodulatory networks, inducing modulation of general arousal. The aim of the present study was to determine if it is possible to increase arousal during a discriminative reaction times (RTs) task, through the application of tES, to improve the subjects' performance.

We developed 3 experiments: Experiment 1 validated the behavioural task, which was an adapted version of the continuous performance test. Experiment 2 aimed to show the task sensitivity to the level of activation. The results confirmed that the task was sensitive enough to reveal modulations of arousal. In Experiment 3, we applied bursts of tES concurrent with the onset of the relevant stimuli of the task to increase the physiological phasic activation of arousal. The skin conductance response was recorded during the experiment in addition to the RTs. The results showed a reduction of RTs and a concurrent increase in skin conductance during the real stimulation condition, which is consistent with a general increase in arousal. In all, these data support the effectiveness of bursts of tES in modulating arousal.

1. Introduction

Arousal reflects a state of generalised physiological and psychological activation that is closely related to a variety of phenomena such as attention, motivation, anxiety and sleep (Robbins and Everitt, 1995a; Sara, 2009). Regulation of arousal and sleep–wake transitions is achieved by several nuclei that are widely distributed in the brainstem. These nuclei have neuromodulatory functions and various projections to the cortical and subcortical structures. Instead of carrying detailed sensory information, they modulate large groups of post-synaptic neurons (e.g., neurons of the cerebral cortex, thalamus, and spinal cord) by increasing or decreasing their excitability. One of these neuromodulatory nuclei is the locus coeruleus (LC), which has historically been related to the regulation of arousal (Aston-Jones and Cohen, 2005; Aston-Jones et al., 1999; Sara and Bouret, 2012). Recordings from primate LC neurons during several tasks have shown two distinguishable functioning modes (Clayton et al., 2004; Usher et al., 1999). In the phasic mode, bursts of LC activity are observed during the processing of motivationally relevant stimuli, leading to the release of noradrenaline (NA) in the hippocampus, neocortex, and many other projection areas. This state of activation has been proposed to facilitate reward-seeking behaviours and help optimise task performance (exploitative behaviour). Conversely, in the tonic mode, the basic activity of the LC is increased, while the bursts of phasic activity are absent. During the tonic mode, subjects tend to explore the context, searching for other motivationally relevant stimuli, resulting in a more distractible behaviour (explorative behaviour) that might decrease task performance (Aston-Jones and Cohen, 2005).

Several studies on humans and animals have reported the key role of arousal in cognition (Berridge and Waterhouse, 2003; Brown et al., 2014). In humans, arousal is commonly modulated through emotional stimuli (Sutherland and Mather, 2012; Dew

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et al., 2014), warning cues (Hackley, 2009) and conflict paradigms (Brown et al., 2014), inducing an improvement of the behavioural performance as arousal increased. It has been demonstrated that an arousing stimulus can amplify the effect of saliency in short-term memory (Sutherland and Mather, 2012) and even enhance visual perception (Zeelenberg and Bocanegra, 2010) and memory retrieval (Mather and Sutherland, 2011). The importance of arousal in cognition has also received empirical support from studies regarding rehabilitative interventions in brain injured patients (Levine et al., 2011; Manly et al., 2002) and children with attention-deficit hyperactivity disorder (O'Connell et al., 2006). These studies showed that alerting cues improve executive functions.

In animals, the role of arousal has been widely studied using electrical stimulation applied directly to the LC. Activation of the LC during a demanding task, with the consequent release of NA to the cortex, has been demonstrated to have an impact on the focus of attention and processing of the stimuli (Clayton et al., 2004; Sara, 2009). In a recent study, Lim et al. (2010) showed that electrical stimulation of the LC in rats can promote long-term potentiation of hippocampal-frontal synapses, which are involved in long-term offline memory consolidation. Further evidence has shown that the release of NA by electrical stimulation of the LC in rats facilitates retrieval of the correct directions in a maze (Sara and Devauges, 1988).

In recent years, electrical stimulation has been widely applied to humans due to its ability to modulate cortical excitability in a non-invasive manner (Nitsche et al., 2008; Priori, 2003). Transcranial electrical stimulation (tES) involves the application of weak electrical currents by a pair of electrodes applied directly to the head, and has been used both to modify behavioural performance in a wide range of cognitive tasks (e.g., Jacobson et al., 2012; Vallar and Bolognini, 2011; Brignani et al., 2013; Pellicciari et al., 2013) and in the treatment of neurological disorders such as chronic pain, Parkinson's disease and Alzheimer's disease (e.g., Boggio et al., 200·9, 2012; Ferrucci et al., 2008). tES generates an electrical field that modulates neuronal activity according to the modality of the application, which can be direct (transcranial direct current stimulation), alternating (transcranial alternating current stimulation) or random noise (transcranial random noise stimulation). Another type of non-invasive electrical stimulation consists of cranial electrotherapy stimulation (CES). This technique has been in clinical use for the last fifty years for the treatment of many emotional and physical disorders such as depression, anxiety and insomnia. CES provides small pulses of electric current across the head of the patients at different frequencies, usually between 0.5 Hz and 100 Hz (Kirsch and Nichols, 2013; Smith, 2006), with specific electrode arrangements. Although CES has been regulated by the U.S. Food and Drug Administration since 1977, its mechanism of action is not clear, but it may affect the release of neurotransmitters across the cortex. This explanation is consistent with evidence suggesting a broad diffusion of the current under the stimulation sites, which could affect several distributed neuromodulatory systems. Evidence for a generalised effect of CES was reported in studies during the first half of the last century (Hayes, 1950; Smitt and Wegener, 1944), and has received further confirmation from more recent investigations. Modelling studies, based on computational simulations of the brain (Bikson et al., 2012; Laakso and Hirata, 2013; Sadleir et al., 2010; Wagner et al., 2014), as well as studies using neuroimaging techniques (Alon et al., 2011; Antal et al., 2011; Lang et al., 2005; Lindenberg et al., 2013), have reported that, according to the arrangement used, there is extensive diffusion of the current that is not limited to the areas under the stimulation electrodes; CES also influences other structures (e.g., the brainstem).

Therefore, we hypothesise that the application of tES with a certain electrode arrangement should stimulate neuromodulatory cortical and subcortical networks, inducing an exogenous modulation of arousal. The aim of this study was to use tES to increase the arousal of participants during a discriminative reaction time task to improve performance. We predict that the application of bursts of tES concurrent with the presentation of behaviourally relevant stimuli could increase the phasic arousal response.

2. Experiments 1 and 2: behavioural task validation

The first step of our research was to verify if the paradigm we chose was sensitive enough to reveal arousal modulations. Therefore, we developed two experiments using an adapted version of the continuous performance test (CPT), which has been widely used in many studies to measure sustained and selective attention (Conners et al., 2003; Riccio et al., 2002; van den Bosch et al., 1996). During the task, participants had to press response buttons for target digits that appeared after a warning digit. Because the warning digit forces participants to prepare for the response, an endogenous increase of arousal during the warning-target interval is expected. To further increase this level of arousal, we used bursts of white noise presented to the participants through headphones at a volume of 90 db. We chose to use this type of arousing auditory stimulus because we wanted to induce a nonspecific activation concurrent to the preparation of the response, and we were not interested in the processing of the emotional connotation of the stimulus.

We evaluated the response speed as a measure of the behavioural performance because a reduction in reaction time (RT) has been demonstrated in conditions of increased arousal, indicating a performance improvement (Bagherli et al., 2011; VaezMousavi et al., 2009; VaezMousavi et al., 2007a). In order to get an indication about the level of activation experienced by the participants, we recorded a subjective report before the experiment. Given (i) the lack of a specific questionnaire for the evaluation of the arousal state and (ii) the strict relation reported in literature between anxiety sensitivity and somatic arousal sensations (Pané-Farré et al., 2014; VaezMousavi and Osanlu, 2011), we administered the State-Trait Anxiety Inventory (STAI-Y) (Spielberger et al., 1983) to all the participants. As reported by Eysenck (1967), subjects with high levels of anxiety are more aroused than those with low levels of anxiety. The activity of NA neurons in the LC is facilitated during stressful conditions, with a strict relation to increased anxiety (Mizuki et al.,1997; Robbins and Everitt, 1995b). Several studies, in addition, reported anxiety disorders as characterized by elevated autonomic arousal driven by NA activity. This system itself has been shown to mediate both anxiety, vigilance and attention (Aston-Jones et al., 1991, 1994; Berridge and Waterhouse, 2003; Grisham et al., 2015).

2.1. Experiment 1

In the Experiment 1 we tested whether bursts of white noise combined with a warning stimulus could increase the arousal of the participants, resulting in an improvement in performance compared to a condition in which the warning stimulus was presented without any white noise.

2.1.1. Materials and methods

2.1.1.1. Participants. Twenty-three healthy volunteers participated in Experiment 1 (18 females, mean age = 27.5 years; SD = 2.9). Two of them were excluded from the analyses due to a lower accuracy or to a higher slowness compared to the overall mean of the participants (see Section 2.1.1.3). All participants had normal or corrected-to-normal visual acuity and were right handed according to the Edinburgh handedness inventory test (Oldfield, 1971).

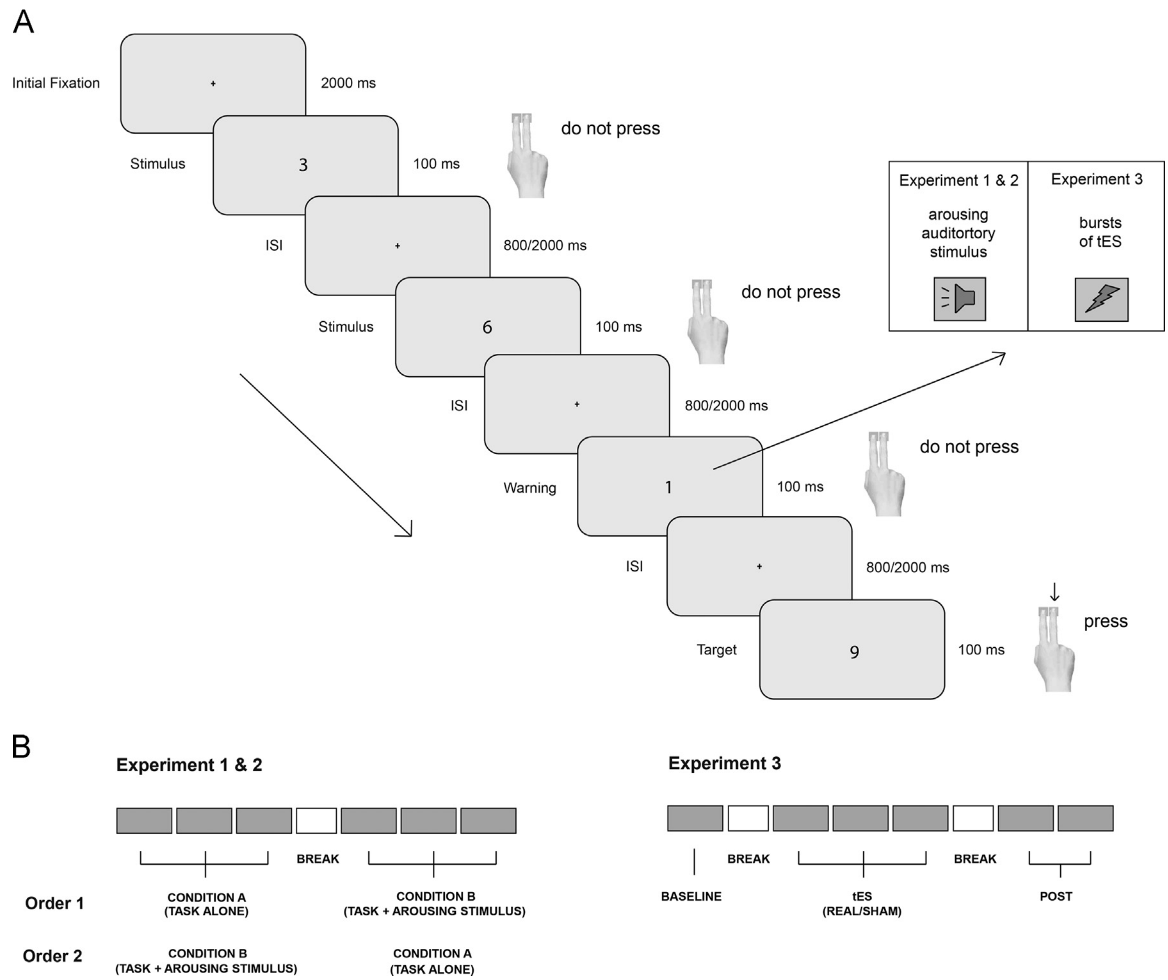

Fig. 1. A schematic illustration of the paradigm (panel A) and the designs of the experiments 1, 2 and 3 (panel B). In Experiments 1 and 2, the warning digit (1) was presented alone (condition A) or concurrently with an arousing auditory stimulus (condition B). In Experiment 3, bursts of tES were applied during the warning digit presentation.

The STAI-Y questionnaire was administered to each participant to measure the level of state and trait anxiety before the experiment (STAI-Y state, mean score=29.8, SD=4.7; STAI-Y trait, mean score=33.4, SD=5.4). The experimental methods were approved by the Ethics Committee of the IRCCS Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy. Informed consent was obtained from all participants.

2.1.1.2. Behavioural task and procedure. The task, which is schematically displayed in Fig. 1, was a variant of the CPT. Participants were instructed to maintain fixation on the centre of the screen where a series of digits, from 1 to 9, was presented in a quasi-random order. Each digit was presented for 100 ms with a variable inter-stimulus interval (ISI) of 800–2000 ms. The target digits (8–9) were presented after a warning digit (1) with the constraint that two consecutive pairs of warning-target digits were separated by at least 2 neutral digits. Participants were instructed to respond to the target digits (8–9) using one of the two response buttons on a serial response box (Science Plus Group, Groningen, NLD). Speed of response and accuracy were both emphasised. The subjects had to respond with the index or middle finger of their dominant hand focusing both on the accuracy and speed of the response. The response buttons were balanced across participants.

The black digits of ~$0.5^\circ \times 0.87^\circ$ in dimension were displayed on a light grey background using a Dell LED monitor with a screen resolution of 1920×1080 pixels. The presentation was controlled by E-Prime software (Psychology Software Tools Inc., Sharpsburg, PA). The screen was located at a distance of 70 cm from the participants.

Two experimental conditions were presented. In condition A, target digits (8–9) were presented after the warning digit (1) alone, while in condition B, an arousing auditory stimulus was presented through headphones concurrent with the presentation of the warning digit. The arousing stimulus was a brief burst (100 ms) of white noise presented at a volume of 90 db, which was able to induce a startle reflex. Participants were randomly assigned to two groups according to the presentation order of conditions A and B (order 1: conditions A–B; order 2: conditions B–A).

All participants performed a short training session to become familiar with the task (25 stimuli), and then 3 blocks for each condition with a central break of 10 min were administered. Breaks of 1 min were observed between blocks. Each block lasted 5 min and 30 s and consisted of 225 visual stimuli including 24 target digits (digit 8 was presented 12 times and digit 9 was presented 12 times) and 3 catch trials (digit 1 not followed by a target digit). The total experiment lasted for approximately 50 min.

2.1.1.3. Behavioural analyses. As a first step, wrong and missed responses were considered as errors and excluded from the total number of responses. If the percentage of a participant's errors exceeded 1.96 standard deviations from the mean of the group, the data of that subject were rejected from the final analyses. As a second step, we measured the mean and the standard deviation of all the valid RTs not including responses that were over 1.96 standard deviations from the mean. As a result, two subjects were excluded from the final analyses due to a lower accuracy or to a higher slowness compared to the overall mean. The RTs of all correct responses were transformed by their natural logarithm to obtain a normal distribution of the data for the analyses. The mean of the RTs was analysed using a generalised estimating equation model with the factors *condition* (2 levels, A and B), *order* (2 levels, 1 and 2) and *block* (3 levels, block 1–2–3). This type of statistical model allows for a more robust estimate procedure in the case of repeated measures and avoids common assumptions for general linear models. Fisher's least significant difference (LSD) test was performed for multiple comparisons (Perneger, 1998).

2.1.2. Results and discussion

The *t*-test comparing the STAI-Y scores did not show any significant difference in the level of activation between participants performing the task in order 1 or order 2, neither in the level of state nor in the level of trait anxiety.

The analysis performed on RTs revealed no main effect of the factor condition [Wald Chi-Square $\chi^2=0.433$, df=1, p=n.s.], but significant interactions between condition and order [$\chi^2=8.227$, df=1, $p=0.004$] and between condition, order and block [$\chi^2=6.563$, df=2 $p=0.038$]. In order to better investigate these interactions, we applied two different generalised estimating equation models to each order, including *condition* (2 levels, A and B) and *block* (3 levels, block 1–2–3) as factors.

The analysis on order 1 showed a significant difference between condition A and condition B (Fig. 2), with reduced RTs when the arousing auditory stimulus was presented concurrent with the presentation of the warning digit [condition: $\chi^2=5.062$, df=1, $p=0.024$]. Moreover, the significant main effect block [$\chi^2=7.429$, df=2, $p=0.024$] indicated that in both condition A and B, participants performed blocks 2 and 3 faster than they performed the respective block 1. The interaction between condition and block did not reach the significance [$\chi^2=3.988$, df=2, p=n.s.]. These results suggest an improvement of the performance during condition A (i.e., when the warning digit was presented alone) as consequence of a learning effect, and a further improvement in condition B (i.e., when the arousing auditory stimulus was presented together with the warning digit), possibly due to an increase of the level of arousal.

The analysis applied to order 2 revealed no significant effect for the factor condition [$\chi^2=3.165$, df=1, p=n.s.], but a significant effect for the factor block [$\chi^2=13.947$, df=2, $p=0.001$], which also interacted with condition [$\chi^2=9.798$, df=2, $p=0.007$] (Fig. 2). These results show that participants reached their best performance at the end of the arousing condition (i.e., condition B, block 3) and they did not improve it any more. Interestingly, this improvement was maintained in the second part of the task (i.e., condition A) as a sort of after-effect induced by the arousing auditory stimulus or as consequence of a conditioning effect between the stimulus and the response. We suppose that the optimal level of performance achieved by participants in order 2 during the first part of the task (i.e., condition B) resulted by the summation between the normal learning effect and the increase of the level of arousal.

On the whole, these results are in line with the possibility to modulate arousal during an RT task using an auditory stimulus without any emotional connotation. Curiously, the performance of the participants suggests that an optimal level of arousal was not achieved immediately at the presentation of the arousing

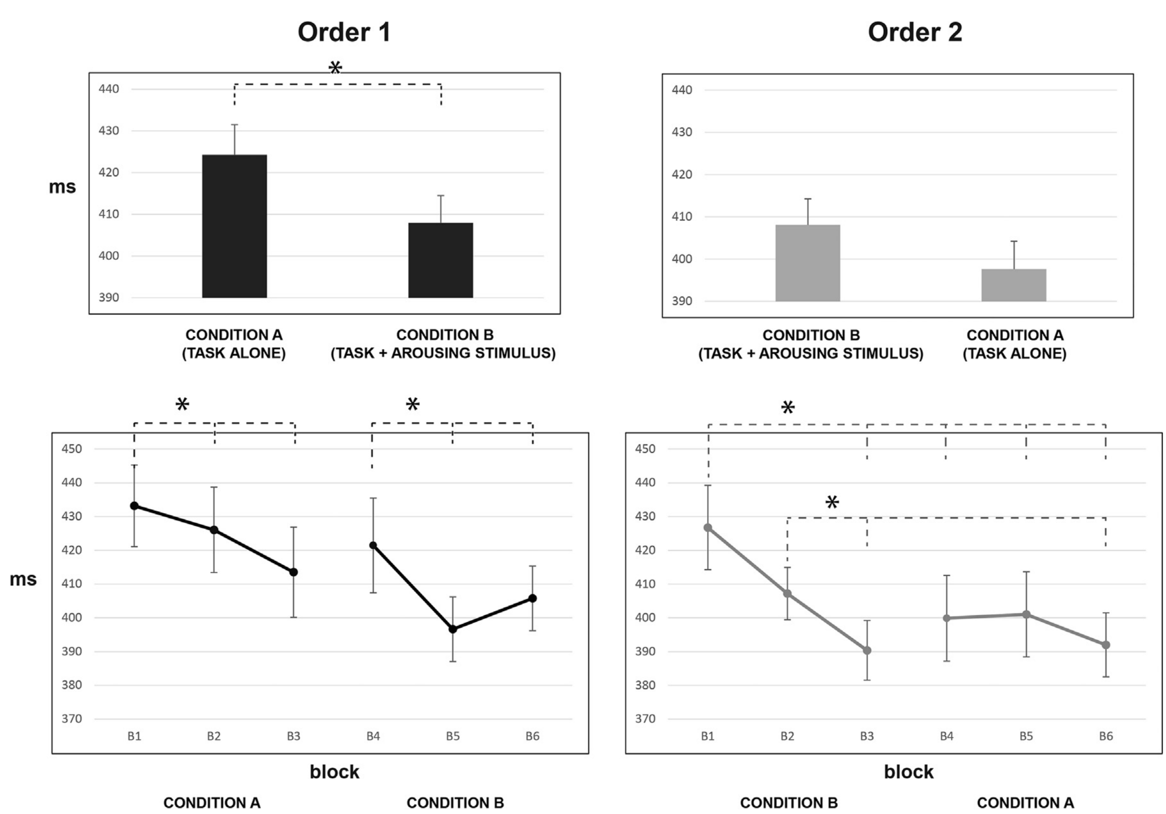

Fig. 2. Results of Experiment 1, reported according to the order of execution of the conditions (order 1 on the left; order 2 on the right). On the top of the figure, the histograms refer to the two conditions of the task (condition A: task alone; condition B: task+arousing stimulus) with the mean RTs of the three blocks of each condition expressed in milliseconds. In the graphs below, the horizontal axes represent the 6 blocks of the task, while the vertical axes represent RTs expressed in milliseconds. Significant effects are marked with ∗.

stimulus, but in the course of the arousing condition. Participants most likely had to become familiar with the high volume and the unexpected presentation of the arousing stimulus before they could show a facilitated performance.

2.2. Experiment 2

Once we verified the sensitivity of the paradigm in revealing arousal modulations during the task, we determined if it was also sensitive to the level of subjective activation reported by the participants at baseline. Therefore, we recruited a group of participants with a higher anxiety level at baseline. A group of students participated to this experiment for a credit reward for a university course, and we assumed this condition to be arousing, as reported in literature (Bagherli et al., 2011). The task performance of these participants (referred to as Group 2) was compared with that of the participants in Experiment 1 (referred to as Group 1) that were subjected to order 2.

2.2.1. Materials and methods

2.2.1.1. Participants. Group 1 was composed of ten participants (7 females, mean age=27.1; SD=2.9. STAI-Y state, mean score=29.4, SD=4.7; STAI-Y trait, mean score=34.2, SD=6.8). Thirteen additional students were recruited for Group 2 (11 females, mean age=20.9; SD=2.8. STAI-Y state, mean score=35, SD=4.2; STAI-Y trait, mean score=39.4, SD=7). Three subjects were excluded from the analyses because their accuracy or RTs were outliers compared to the overall mean, using the same statistical criteria applied in Experiment 1 (see Section 2.1.1.3). All participants had normal or corrected-to-normal visual acuity and all, except one, were right handed according to the Edinburgh handedness inventory test. Informed consent was obtained from all participants.

2.2.1.2. Behavioural task and procedure. The task and the stimuli used in Experiment 2 were the same as those used in Experiment 1. All of the participants performed the task using order 2 [condition B (task+auditory stimulus) followed by condition A (task alone)].

2.2.1.3. Behavioural analyses. We compared the level of activation at baseline using the scores of the STAI-Y questionnaire between the groups with a *t*-test for independent groups.

A generalised estimating equation model was applied to the RTs transformed by their natural logarithm, with the factors *group* (2 levels, Group 1 and Group 2), *condition* (2 levels, A and B), and *block* (3 levels, block 1–2–3). Fisher's LSD test was performed for multiple comparisons.

2.2.2. Results and discussion

The *t*-test on scores of the STAI-Y questionnaire showed a significant difference between the two groups regarding the state scale, with a higher level of activation for participants of group 2 [$p=0.012$], while the trait scale did not show any significant difference [$p=$n.s.]. This result indicates that participants were activated by the situation and not by a generalised higher level of anxiety.

The RT results showed no significant effect for the factors group [$\chi^2=0.090$, df=1, $p=$n.s.] or condition [$\chi^2=1.110$, df=1, $p=$n.s.], but significant interactions between group and block [$\chi^2=14.859$, df=2, $p=0.001$] and between group, condition and block [$\chi^2=6.286$, df=2, $p=0.043$]. To better investigate these interactions, we applied different generalised estimating equation models to each group, including *condition* (2 levels, A and B) and *block* (3 levels, block 1–2–3) as factors, and to each condition, including *group* (2 levels, Group 1 and Group 2) and *block* (3 levels, block 1–2–3) as factors. As already described in Experiment 1, participants

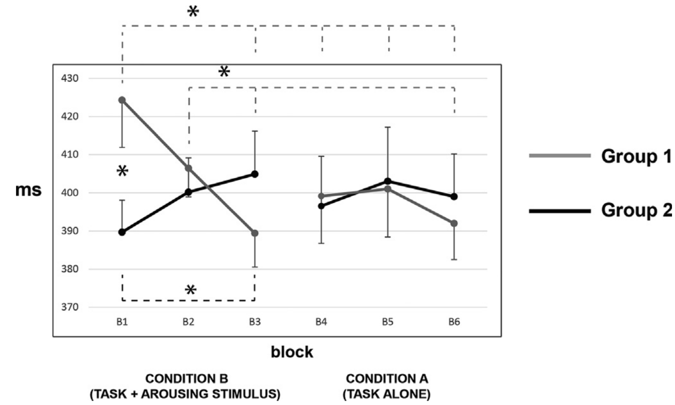

Fig. 3. Results of Experiment 2, showing RTs of Group 1 compared with those of Group 2. The horizontal axis represents the 6 blocks of the task, while the vertical axis represents RTs expressed in milliseconds. Significant effects are marked with ⁎, the grey dotted lines show the significant effects between the blocks of the Group 1, while the black dotted line shows the significant effect for the Group 2.

of Group 1 showed an improvement in performance during the presentation of the arousing stimulus (i.e., condition B), consisting of a reduction in RTs (i.e., block 1 vs. block 3, $p<0.001$; block 2 vs. block 3, $p=0.013$). In the subsequent condition A, they did not improve their performance any more.

In contrast, participants of Group 2 showed a worsening of the performance during the presentation of the arousing stimulus, with a significant increase in RTs between the first and the third block of condition B [$p=0.036$]. In addition, a difference between groups emerged when the arousing auditory stimulus was presented concurrent with the presentation of the warning digit (i.e., condition B), but not when the warning digit was presented alone (i.e., condition A). Specifically, participants of Group 2 were faster than participants of Group 1 in the first block of the task (i.e., condition B) [$p=0.013$] (Fig. 3), while their RTs increased in the following blocks, exactly in the opposite direction to the trend showed by Group 1, cancelling out differences between groups.

These results are consistent with the effects expected by a group with higher levels of arousal, that would explain a better performance in the starting block and a worse performance in the following blocks, when the arousing auditory stimulus would led to a non-optimal too high arousal level. This trend, resembles the inverted U-shape curve relationship between arousal and task performance expressed in the classical study of Yerkes–Dodson (Aston-Jones et al., 1999; Yerkes and Dodson, 1908). According to this relationship, an optimal performance is obtained with a moderate level of arousal, while excessively high or low levels lead to a decline in task performance. In a work similar to the present one, VaezMousavi et al. (2007b) reported data consistent with this model. In their study, 22 university students performed a continuous performance test. RTs and skin conductance were recorded as performance and physiological indexes, respectively. The results showed that RTs decreased significantly with high levels of skin conductance during the task, except for 5 subjects who showed higher levels of skin conductance response during the baseline. The authors suggested this result to be a consequence of a high anxiety level at the beginning of the experimental session. A similar finding was reported by Barry et al. (2005) in a study with children and also in a balance task (Vaezmousavi and Osanlu, 2011). In the lights of these results, the higher score to the STAI-Y questionnaire of the Group 2 could be indicative of higher levels of arousal, supporting the worst performance during the task. However, because we do not have a direct physiological measure of arousal in a baseline condition, but only a subjective report of the level of activation experienced by the participants, this interpretation is only speculative and further evidence should be collected to clarify the relation between anxiety and arousal levels.

3. Experiment 3: arousal modulation by tES

The aim of Experiment 3 was to exogenously increase the participants' level of arousal with tES to improve the behavioural performance. To further increase the phasic endogenous activation related to the warning stimuli, we replaced the arousing auditory stimuli with bursts of tES. The rationale for this is based on the assumption that CES has principally been used to reduce the level of arousal in patients suffering from insomnia, chronic pain and anxiety. In these studies, the current was administered at frequencies between 0.5 and 100 Hz with a monophasic or biphasic quadratic waveform shape. Considering these results, we chose an alternating current with higher frequencies (100–640 Hz) to obtain an opposite effect on arousal. To obtain a physiological measure of arousal, we recorded the skin conductance response (SCR) during the task. The SCR is considered a useful index of cognitive engagement during a task and has been recorded in various studies as a reliable measure of arousal (Dawson et al., 2000; Bagherli et al., 2011).

3.1. Materials and methods

3.1.1. Participants

Sixteen healthy participants were recruited (8 female, mean age=24.5; SD=3.8; STAI-Y state, mean score=31.4, SD=7; STAI-Y trait, mean score=36.8, SD=6.9). All participants were right handed according to the Edinburgh handedness inventory test, had normal or corrected-to-normal visual acuity and showed no risk factors for tES application, as assessed through safety questionnaires. The data from two participants were discarded from the final analyses due to a clear perception of the bursts of stimulation during the experiment (see Section 3.1.4).

The experimental methods were approved by the Ethics Committee of the IRCCS Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy. Informed consent was obtained from all participants. Participants received a financial reward at the end of the experiment.

3.1.2. Behavioural task and procedure

The task and the stimuli were the same as those used in the previous experiments, but the arousing auditory stimulus was replaced by bursts of tES administered to the participants concurrent with the presentation of the warning digit. To verify the effect of the stimulation on arousal, all participants took part in two experimental sessions, during which they received real or sham tES during the execution of the task. The order of the tES condition (real vs. sham) was balanced across participants in a within subjects design, half of the participants received real stimulation during the first day and sham stimulation during the second day, whereas the opposite order of stimulation was followed for the other half of the participants. The temporal interval between the two experimental sessions was between 1 and 7 days. All participants performed a short training session to become familiar with the task (25 stimuli) followed by six blocks of the task: the first block without tES (baseline), three blocks with concurrent tES application (tES) and two final blocks without tES (post). The duration and the number of visual stimuli of each block were the same as in previous experiments. The experiment lasted approximately 60 min including application of the tES electrodes.

3.1.3. tES

Brief bursts of high frequency random noise stimulation (100–640 Hz) were delivered concurrent with the presentation of the warning digit using a battery-driven current stimulator (BrainSTIM, EMS, Bologna, Italy) through a pair of rounded conductive-rubber electrodes (22.8 cm^2) prepared with a conductive gel solution. The electrodes were placed over FPz and Oz as determined by the International 10–20 EEG system. The stimulation was triggered with E-Prime software and was applied during the 3 central blocks of the task (tES) for a total of 81 bursts of stimulation. Each burst of stimulation had a fixed duration of 900 ms so that the same amount of current would be delivered to each participant. The stimulation parameters (current intensity=2 mA; max current density=0.087 mA/cm^2) were maintained below the safety limits (Nitsche et al., 2008). The current was not delivered after the initial impedance check in the sham condition.

3.1.4. tES sensations

At the end of each experimental session, all participants completed a questionnaire to evaluate possible discomfort and perceived influences on the performance induced by tES (Fertonani et al., 2010, 2015). In the present experiment, it was absolutely necessary that participants did not perceive any difference between real and sham stimulation, because the mere sensory stimulation could mimic the expected arousal effects. For this reason, we paid considerable attention to this matter during the experimental set-up phase. Different studies investigating the sensations induced by tES had already reported that random noise is the “less perceived” stimulation compared to the other types (Ambrus et al., 2011, 2010; Fertonani et al., 2011; Pirulli et al., 2013). Here, however, we applied bursts of stimulation instead of the classical stimulation for several minutes, which could increase the degree of perceived sensations. To avoid this inconvenient, we ran a pilot experiment to individualise the appropriate current intensity, which was fixed at 2 mA.

In addition, at the end of Experiment 3 all participants were explicitly asked if they had perceived different sensations between the two experimental sessions. Only two participants clearly perceived the bursts of tES during the real stimulation, differentiating real from sham session. Their oral report was consistent with their responses to the questionnaire. In order to avoid any possible confounding effect, the data of these two participants were not considered in the analyses. The responses to the questionnaire of the remaining participants were submitted to a Wilcoxon matched pair test. No significant difference occurred between the sensations reported after real and sham stimulation, supporting that participants were completely unaware of the type of stimulation they received. Therefore we did not perform a control experiment applying this type of tES at peripheral level, to evaluate if the sensations induced by the short stimulation protocol could modify subjects’ performance. This can be consider a limitation of the study.

To our knowledge, this is the first study to evaluate the sensations of tES induced by bursts of stimulation. The absence of any clear perception reported by almost all the participants supports the reduced sensations related to random noise waveform already reported in previous studies.

3.1.5. Skin conductance recording

Skin conductance was recorded from 1.3 cm of diameter Ag/AgCl electrodes placed on the distal phalanges of the second and third finger of the participant's non-dominant hand. The electrodes were prepared with an isotonic paste (Discount Disposables, St. Albans, Vermont), and the activity was recorded using a galvanic skin response (GSR) module (BrainProducts GmbH, Munich, Germany) with a constant voltage applied across the electrodes. Electrodermal data were DC-recorded continuously with a resolution of 0.1 and digitised at a sampling rate of 5000 Hz (BrainAmp ExG MR 16 channels, BrainProducts GmbH, Munich, Germany).

3.1.6. Behavioural analyses

We analysed response speed as a measure of behavioural accuracy as in Experiments 1 and 2. Due to the within subjects design, the data from all blocks of every tES condition (real stimulation vs. sham) were normalised to the respective baseline block to produce a common starting point for both days of the experiment. RTs were transformed by their natural logarithm and analysed using a generalised estimating equation model with the factors *tES condition* (2 levels, real and sham) and *block* (5 levels, block 2–3–4–5–6). Fisher's LSD test was performed for multiple comparisons.

3.1.7. Skin conductance analyses

The measure of interest was the phasic activation of the skin conductance response in relation to the presentation of the warning digit. The periods of interest were extracted from the raw data using BrainVision Analyzer v2 (BrainProducts GmbH, Munich, Germany) and then analysed using Ledalab software (Benedek and Kaernbach, 2010). Similar to the work of Benedek and Kaernbach (2010), we focused on the integrated skin conductance response values (ISCR) to obtain an indicator of response magnitude using an event-related paradigm. The window of interest was 1–4 s after the onset of the warning digit, and the minimum amplitude criterion was set to 0.05 microSiemens (μS). All values were standardised with the formula $y=\log(1+x)$ due to the positively skewed distributions of the skin conductance responses (Martin and Venables, 1980). For both the real and sham conditions, the values from all blocks were normalised to the respective baseline block, as in the behavioural analyses.

The data from 3 participants were excluded from the analyses due to technical problems during the recording (1 participant) or because their responses were outliers compared to the overall mean of the participants (2 participants). Skin conductance responses (SCR) were then analysed using a generalised estimating equation model with the factors *tES condition* (2 levels, real and sham) and *block* (5 levels, block 2–3–4–5–6). Fisher's LSD test was performed for multiple comparisons.

3.2. Results and discussion

3.2.1. Behavioural data

The *t*-test on the scores of the STAI-Y did not show any significant difference between this group of participants and the participants from Experiment 1.

The analyses of response speed (Fig. 4) showed a main effect of the factor block [$\chi^2=12.121$, df=4, $p=0.016$] and a significant interaction between tES condition and block [$\chi^2=24.766$, df=4, $p<0.001$]. Post-hoc analyses showed a significant difference between real and sham conditions in the second block of tES application (i.e., block 3) [$p=0.037$] with reduced RTs in the real compared to the sham tES condition, denoting a performance improvement. Another significant difference emerged between block 2 and block 3 in the sham condition [$p=0.008$], indicating a decline in performance during the session without real stimulation. No difference was present between the real and sham conditions in the last two blocks post stimulation (i.e., blocks 5 and 6), indicating the absence of after-effects. The effect on the RTs was present only in the second block of stimulation, similarly to the delayed effects observed in Experiments 1 and 2 with the arousing auditory stimulus. Nevertheless such effect was not present in the last block

The effect of the real stimulation on RTs showed a trend toward a significant linear correlation with the STAI-Y state questionnaire scores [$p=0.055$, $r=0.522$]. This result suggests a possible role of the initial level of activation, here measured as a subjective report with the STA-Y questionnaire, in modulating the effects of the

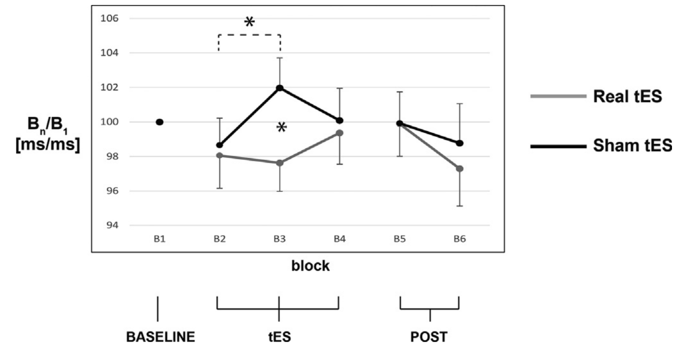

Fig. 4. Behavioural results of Experiment 3, showing RTs in the real tES condition (in grey) and in the sham tES condition (in black). The horizontal axis represents the 6 blocks of the task, while the vertical axis represents RTs normalised to block 1 in order to have the same baseline across conditions. Significant effects are marked with ⁎.

current stimulation on the behavioural performance. This correlation showed that a high level of activation at baseline could impair performance, resulting in smaller effects of real stimulation on RTs.

3.2.2. Physiological data

Skin conductance analyses showed a main effect of tES condition [$\chi^2=15.301$, df=1, $p=<0.001$] with a higher phasic activation during the real condition compared to the sham condition (Fig. 5), which was maintained in blocks 5 and 6 following tES application. Another significant effect was present for the factor block [$\chi^2=16.878$, df=4, $p=0.002$], and post-hoc comparisons between blocks showed a general decrease in phasic activation throughout the experiment in response to a physiological habituation to the repetitive stimuli with a subsequent reduction of the response amplitude over time. The interaction between tES condition and block did not reach significance [$\chi^2=2.568$, df=4, p=n.s.].

These results are consistent with an increase of arousal induced by tES and suggest that skin conductance is a physiological index steadier and sensitive to tES induced arousal modulations than the behavioural measure here used. Interestingly, the higher phasic activation observed during the real stimulation in comparison to the sham condition was maintained also in the two final blocks without tES, suggesting an after-effect of the stimulation on the skin conductance. The maintenance of tES effects over time is extremely relevant in a rehabilitative prospective. However, here we must be cautious about this interpretation. A decrease of the skin conductance response was present after both real and sham

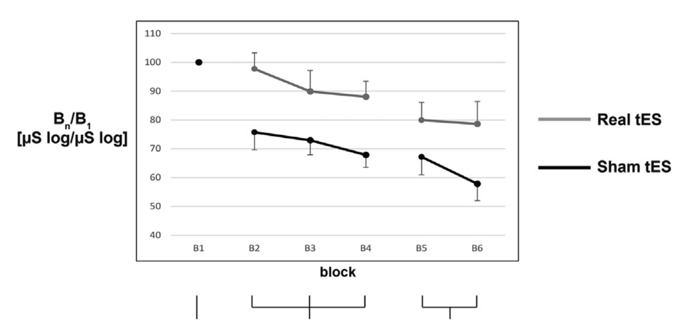

Fig. 5. Physiological results of Experiment 3, showing skin conductance response in the real tES condition (in grey) and in the sham tES condition (in black). The horizontal axis represents the 6 blocks of the task, while the vertical axis represents skin conductance response in microSiemens (μS) normalised to block 1 in order to have the same baseline across conditions. A significant difference between the two conditions was present.

stimulation and the difficulty of returning to the same levels could be simply due to the high difference developed during the stimulation. Possible after-effects induced by tES on skin conductance, thus, need to be further explored.

According to our expectations, bursts of high frequency alternating current administered concurrent to the presentation of a warning digit induced behavioural and physiological effects consistent with increased arousal. During the real tES condition, participants showed reduced RTs, denoting a performance improvement, and higher phasic activation of the skin conductance response compared to the sham condition.

4. General discussion

The aim of the present study was to determine whether tES can modulate arousal by improving performance during a behavioural task. Indeed, a state of increased arousal has been related to better performance in different behavioural tasks (Bagherli et al., 2011; Sutherland and Mather, 2012; VaezMousavi et al., 2007a). Arousal plays a key role in cognition and different neurological and psychiatric pathologies, in which its dysregulation is strictly related to cognitive deficits (Berridge and Waterhouse, 2003), depression, insomnia or anxiety (Cortoos et al., 2010; Schock et al., 2011). Support for this view also comes from several neurorehabilitation studies aimed at modulating attentional network activity in patients with brain injuries by using external auditory stimuli. The results showed that it was possible with such an approach to induce performance improvements in these patients (Manly et al., 2002). The physiological effect induced by this exogenous stimulation was identified as a general increase in arousal (Manly et al., 2002). The importance of arousal has been proposed also for the disengagement of the right-sided spatial attentional bias in patients with unilateral neglect (Robertson et al., 1998). Overall, these studies suggest that arousal might play a crucial role in patient rehabilitation. In this context, the use of a non-invasive technique such as tES, which is associated with the absence of any clear perception of an external stimulation by the participants, to modulate arousal could lead to several benefits, especially for patients. Moreover, tES might provide the possibility to induce long-term effects (Nitsche and Paulus, 2001) based on neuroplasticity mechanisms (Cooke and Bliss, 2006), which is a desirable effect in every rehabilitative intervention.

In the present study, first we proved that the paradigm used was sensitive enough to disclose modulations of arousal. The reduced RTs observed during a task requiring sustained and focused attention could be ascribed to the increase in arousal exogenously induced by an auditory stimulus (Experiment 1). In addition, this improvement in performance was modulated according to the level of activation experienced by the participants at baseline (Experiment 2).

In Experiment 3, we applied bursts of tES with the goal of inducing an exogenous increase in arousal. We used bursts of stimulation because our aim was to mimic the physiological phasic activation of the LC induced by the relevant stimuli of the task. The timing of the stimulation was a crucial factor. A generalised activation of the system involved in an attention task generally occurs during the interval between the warning and the response digits (Bouret and Sara, 2004; Sara, 2009), and results in an increased release of neurotransmitters throughout the cortex due to the distributed projections of the LC-NA system. To increase this phasic activation, we applied bursts of tES concurrent with the onset of the warning digit. In this manner, the bursts of tES would have to increase the release of neurotransmitters through a direct or indirect stimulation of the LC-NA system. Because the target of the stimulation was a widespread neuromodulatory system, we placed the stimulating electrodes over Fpz and Oz, an arrangement that should allow a larger area of stimulation of the brain, including the brainstem (Bikson et al., 2012; Laakso and Hirata, 2013; Sadleir et al., 2010; Wagner et al., 2014). Several lines of evidence suggest that a frontal electrode is important for the induction of a generalised diffusion of the current. During the last century, Hayes (1950) ascribed the increase in current in the frontal regions to its diffusion through the orbits while measuring the distribution of the current in a monkey. Some years later, Lippold and Redfearn (1964) stated that to obtain “psychological effects at imperceptible current strengths”, the current flow needs to enter the orbital fissure. Additionally, recent modelling studies support the diffusion of the current through different regions of the brain. Laakso and Hirata (2013) used a simulation model to replicate different arrangements of current stimulation used in the literature, and they showed that, despite the placement of the electrodes, at least a small portion of the current travels through the eye orbits. The current travels along the pathway of least resistance during the stimulation of different cortical and subcortical regions; it is not limited to the areas under the electrodes. Sadleir et al. (2010), using a finite-element model, showed that high current densities reached structures outside of the regions under the stimulating electrodes. Values of the same order of magnitude as those applied to the cortex (inferior frontal gyrus in their simulation) were observed in sub-cortical regions such as the putamen, amygdala, hippocampus and caudate nucleus. Other recent studies have used neuroimaging techniques to evaluate the effect and diffusion of the current. Lang et al. (2005) used positron emission tomography in a group of healthy volunteers and showed that tES stimulation of the primary motor cortex, compared to sham conditions, induced a widespread modulation of the regional cerebral blood flow in cortical and subcortical areas. Another study that acquired fMRI sessions during tES stimulation (Alon et al., 2011) showed a reduction of the resting-state connectivity as a consequence of the generalised effect of the current on the brain. Considering this evidence, the assumption of the direct or indirect stimulation of the distributed pathway of the LC-NA system seems possible.

Finally, we choose to use a high band random frequency alternating current, as opposed to CES studies, in which low frequencies were used, because we aimed to obtain an increase in arousal. Random noise stimulation, compared to other types of alternating current, has the advantage that almost no sensations are induced by the current (Ambrus et al., 2010; Fertonani et al., 2011; Pirulli, et al., 2013).

The results of Experiment 3 showed an improvement of the performance and a concurrent increase in skin conductance during the real stimulation condition. tES, however, induced a stronger modulation of the physiological response respect to the behavioural ones (i.e., RTs), that was delayed and short-lived. It is known that RTs are a low sensitivity measure, usually affected by many factors, such as fatigue and general activation of the arousal system (Davranche et al., 2006; van den Berg, 2006; Welford, 1980), but this inconsistency between physiological and behavioural responses remains an unexpected result, although it has already been reported in other studies. VaezMousavi et al., (2009) found that the physiological effect (measured through skin conductance response) did not always predict the behavioural effect (measured through RTs), showing also a large inter-individual variability for this relation. The authors reported that a high level of arousal increased the skin conductance response but it was not always related to a speeded behavioural performance. They ascribed this variability to a possible fluctuation in the task involvement throughout the experiment, due to individual-difference variables related to physiological (i.e., cardiovascular fitness) and psychological aspects (coping styles or task/outcome orientation). Therefore the differential susceptibility of RT and skin conductance measures to tES modulations remains the most likely explanation.

Studies with random noise stimulation are still very few, and it is difficult to provide a clear definition of its mechanism of action. Moreover most of these studies investigated tES effects only at behavioural level and reported either delayed or immediate effects. For example Terney et al. (2008) studied the effects of stimulation on implicit motor learning. Random noise stimulation was applied during a serial RT task and the results showed a reduction of RTs only during the final part of stimulation. In contrast Fertonani et al. (2011), found that random noise stimulation improved the behavioural performance in a visual perceptual learning task from the beginning of the stimulation.

In our experiment we applied bursts of stimulation while tES has usually been applied in a continuative way for several minutes. These two stimulation approaches, continuous vs. burst of stimulation, can differently impinge in to physiological brain's homoeostatic mechanisms via distinctive dynamics of response (Bienenstock et al., 1982) reflected in overt responses.

To our knowledge, this is the first study that used bursts of random noise stimulation with the aim of increasing arousal and measuring skin conductance, thus further studies are needed to better understand the characteristics of this stimulation and its mechanisms of action.

In conclusion, the data from our study support the possibility of using tES to modulate arousal. Moreover, the possibility of using this protocol in patients to increase arousal and facilitate neurorehabilitation is an additional important prospective that should indeed be evaluated. Further investigations exploring the effects of tES on other behavioural tasks, possibly using additional physiological measures of arousal (e.g., pupil dilation, EEG; see Nieuwenhuis et al., 2011), are needed to support this research line.

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