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Effect of Serotonin on Paired Associative Stimulation-Induced Plasticity in the Human Motor Cortex

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Serotonin modulates diverse brain functions. Beyond its clinical antidepressant effects, it improves motor performance, learning and memory formation. These effects might at least be partially caused by the impact of serotonin on neuroplasticity, which is thought to be an important foundation of the respective functions. In principal accordance, selective serotonin reuptake inhibitors enhance long-term potentiation-like plasticity induced by transcranial direct current stimulation (tDCS) in humans. As other neuromodulators have discernable effects on different kinds of plasticity in humans, here we were interested to explore the impact of serotonin on paired associative stimulation (PAS)-induced plasticity, which induces a more focal kind of plasticity, as compared with tDCS, shares some features with spike timing-dependent plasticity, and is thought to be relative closely related to learning processes. In this single-blinded, placebo-controlled, randomized crossover study, we administered a single dose of 20 mg citalopram or placebo medication and applied facilitatory- and excitability-diminishing PAS to the left motor cortex of 14 healthy subjects. Cortico-spinal excitability was explored via single-pulse transcranial magnetic stimulation-elicited MEP amplitudes up to the next evening after plasticity induction. After citalopram administration, inhibitory PAS-induced after-effects were abolished and excitatory PAS-induced after-effects were enhanced trendwise, as compared with the respective placebo conditions. These results show that serotonin modulates PAS-induced neuroplasticity by shifting it into the direction of facilitation, which might help to explain mechanism of positive therapeutic effects of serotonin in learning and medical conditions characterized by enhanced inhibitory or reduced facilitatory plasticity, including depression and stroke.
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INTRODUCTION

Serotonin (5-HT) is a widely distributed neurotransmitter in the brains of animals and humans, affecting various physiological functions such as learning, memory formation, pain perception, mood and the sleep–wakefulness cycle (Bert *et al*, 2008; Geyer, 1996; Hasbroucq *et al*, 1997; Jacobs and Fornal, 1997; Meneses, 1999). One important foundation for these effects might be its impact on neuroplasticity. Activation of serotoninergic subreceptors is shown to affect long-term potentiation (LTP) or long-term depression (LTD) in animal slice preparations, depending on subreceptor type, location and frequency of application (Huang and Kandel, 2007; Kojic *et al*, 1997; Mori *et al*, 2001).

In the clinical domain, studies have suggested that depression might be a result of altered brain plasticity (Christoffel *et al*, 2011; Garcia, 2002; Henn and Vollmayr, 2004; Popoli *et al*, 2002), on which serotonin has a major impact. Distress has been proposed as one of the main factors preceding depression (Caspi *et al*, 2003), and in animals it inhibits LTP and facilitates LTD induction (Foy *et al*, 1987; Rocher *et al*, 2004; Shors *et al*, 1989; Xu *et al*, 1997). In accordance, LTD is facilitated in animal models of depression, which was prevented by chronic application of the selective serotonin reuptake inhibitor (SSRI) fluvoxamine (Holderbach *et al*, 2007). Besides depression, several studies have demonstrated that SSRIs improve motor functions in stroke patients (Chollet *et al*, 2011; Dam *et al*, 1996; Pariente *et al*, 2001) and in healthy individuals (Loubinoux *et al*, 1999; Loubinoux *et al*, 2002a; Loubinoux *et al*, 2002b; Loubinoux *et al*, 2005). Again, the physiological basis for this effect might be the impact of serotonin on plasticity.

Recently it was shown that motor cortex plasticity in healthy humans induced by transcranial direct current stimulation (tDCS) was affected by single-dose SSRI. Citalopram enhanced facilitatory plasticity induced by anodal tDCS and converted cathodal tDCS-induced inhibitory plasticity into facilitation (Nitsche *et al*, 2009). tDCS and paired associative stimulation (PAS) are non-invasive brain stimulation techniques inducing changes in cortical excitability that outlast the stimulation duration (Nitsche *et al*, 2003b; Nitsche and Paulus, 2000, 2001; Stefan *et al*, 2000; Wolters *et al*, 2003). These alterations in cortical

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excitability are NMDA- and Ca^{2+}-dependent (Nitsche *et al*, 2003a; Stefan *et al*, 2002; Wolters *et al*, 2003). tDCS induces non-focal plasticity, affecting relatively non-selectively neuronal populations beneath the large stimulation electrodes via subthreshold resting membrane potential modulation (Nitsche *et al*, 2008; Nitsche *et al*, 2007; Purpura and McMurtry, 1965). PAS induces focal and synapse-specific plasticity of the respective target neurons. In PAS, a repetitive electric pulse to a peripheral nerve at an intensity that activates somatosensory afferents is combined with transcranial magnetic stimulation (TMS) over the corresponding area of the primary motor cortex. Depending on the interstimulus interval (ISI), synchronous or asynchronous activation of the target group of neurons, which are motor cortex neurons connected with the respective somatosensory afferents, is accomplished, resulting in excitatory or inhibitory after-effects (Stefan *et al*, 2000). This mechanism of plasticity induction resembles some characteristics of spike timing-dependent plasticity (STDP), which is closely linked to learning and memory processes.

Interestingly, other neuromodulators have discernable effects of tDCS- and PAS-induced plasticity. Specifically, dopamine, acetylcholine, and nicotine have a focusing, or signal-enhancing effect on facilitatory plasticity (Kuo *et al*, 2007; Kuo *et al*, 2008; Monte-Silva *et al*, 2010; Thirugnanasambandam *et al*, 2012). These substances abolish tDCS-induced non-focal, but enhance PAS-generated focal facilitatory plasticity. This effect might explain the cognition- and behavior-enhancing impact of these substances.

After having explored the impact of serotonin on tDCS-induced plasticity, we were now interested to explore how this modulator affects PAS-generated neuroplastic cortical excitability alterations. We hypothesize that citalopram enhances PAS-induced focal excitatory plasticity and abolishes focal inhibitory plasticity or convert it into excitation, as it was shown for tDCS (Nitsche *et al*, 2009).

MATERIALS AND METHODS

Subjects

Fourteen healthy subjects aged 28.1 ± 4.7 years (7 males/7 females) were recruited. All subjects were right-handed according to the Edinburgh handedness inventory (Oldfield, 1971). None of them took any medication, had a history of a neuropsychiatric disease, present pregnancy, or metallic head implants. All volunteers gave written informed consent and were compensated for participation. The investigation was approved by the Ethics Committee of the University of Göttingen, and conforms to the principles laid down in the Declaration of Helsinki.

Paired Associative Stimulation

The peripheral electric pulse was delivered over the right ulnar nerve at the level of the wrist, followed by a TMS pulse over the M1 representation of the abductor digiti minimi muscle (ADM) at ISIs of 10 (PAS10) or 25 ms (PAS25). The peripheral pulse was delivered by a Digitimer D184 multipulse stimulator (Digitimer, Welwyn Garden City, UK) at an intensity of 300% of the sensory perceptual threshold. The TMS pulse was delivered by a Magstim 200 stimulator with an intensity to elicit single-pulse MEPs with peak-to-peak amplitudes of on average 1 mV. The participants were instructed to count silently the number of pulses they received at their wrist during the whole stimulation duration to guarantee sufficient attention to the procedure, which has been shown to be crucial to obtain the intended after-effects (Stefan *et al*, 2000; Stefan *et al*, 2004).

Pharmacological Interventions

Citalopram (20 mg) or equivalent placebo (PLC) drugs were administered 2 h before the start of the experimental session, allowing the verum drug to induce a stable plasma level and produce prominent effects in the central nervous system (Bezchlibnyk-Butler *et al*, 2000; Kragh-Sorensen *et al*, 1981; Robol *et al*, 2004).

Monitoring of Motor Cortical Excitability

MEPs were recorded from the right ADM by single-pulse TMS over the left primary motor cortex, conducted by a Magstim 200 magnetic stimulator (Magstim, Whiteland, Dyfed, UK) with a figure-of-eight magnetic coil (diameter of one winding—70 mm; peak magnetic field—2.2 T). The coil was held tangentially to the skull, with the handle pointing backwards and laterally at 45° from the midline. The optimal coil placement (hotspot) was defined as the site where TMS resulted consistently in the largest MEPs of the contralateral ADM. Surface MEPs were recorded from the right ADM with Ag–AgCl electrodes in a belly-tendon montage. The signals were amplified, and band-pass filtered (2 Hz to 2 kHz, sampling rate, 5 kHz). Signals were digitized with a micro 1401 AD converter (Cambridge Electronic Design, Cambridge, UK), controlled by Signal Software (Cambridge Electronic Design, v. 2.13) and stored for offline analysis.

Experimental Procedures

Each subject participated in four experimental sessions (PAS25 with citalopram or placebo, PAS10 with citalopram or placebo), which were carried out in randomized order and separated by minimum 1 week. A unique sequence of experimental sessions was randomly generated for each subject individually, which did not match any previously generated one for other subjects. The volunteers were seated in a comfortable chair with head and arm rests. First, the hotspot (the position of coil that produced the largest MEPs of the right ADM) was identified by TMS. Then the stimulation intensity was adjusted to elicit single-pulse MEPs with peak-to-peak amplitudes of on average 1 mV and 25 MEPs were recorded for the first baseline determination. After baseline recording, citalopram or placebo medication was administered. At 2 h after intake of medication, a second baseline was recorded to monitor for a possible influence of the drug on cortical excitability (baseline 2), and TMS intensity was adjusted, if necessary (baseline 3). After that procedure, PAS25 or PAS10 was administered and 25 MEPs were recorded immediately after stimulation and at time points of 5, 10, 15, 20, 25, 30, 60, 90 and 120 min after the stimulation PAS. Further TMS measurements were

conducted in the evening of the same day (SE), next morning, at ~0900 hours (NM), next noon, at ~1200 hours (NN), and next evening, at ~1800 hours (NE) (Figure 1). To keep the EMG electrodes and TMS coil at the same place for later measurements, their positions were marked with a waterproof pen. Subjects were blinded for both, stimulation and medication conditions; the experimenter was blinded only for the medication condition.

Analysis and Statistics

The experimenter was unblinded after finishing data collection and analysis. The individual means of 25 MEP amplitudes were calculated for all subjects and the after-stimulation mean MEP amplitudes were normalized to the respective mean baseline MEP amplitudes (quotient of post-PAS MEPs *vs* baseline values). Then the grand averages for each time point were calculated. A repeated measures ANOVA was performed on the above-mentioned data using MEP amplitude as the dependent variable and medication, stimulation type and time course as within-subject factors. The Mauchly test of sphericity was performed and the Greenhouse–Geisser correction applied when necessary. In case of significant results of the ANOVA, exploratory *post-hoc* comparisons were performed using Student's *t*-tests (paired samples, two-tailed, $p<0.05$, not corrected for multiple comparisons) between the MEP amplitudes before and after PAS administration within one experimental condition and between the single time points within the same stimulation condition.

To exclude differences between baseline values of different conditions, also between first and second baseline values, we compared the respective values by Student's *t*-tests (paired samples, two-tailed, $p<0.05$, not corrected for multiple comparisons).

RESULTS

All subjects tolerated the procedure well. None of them reported any side effect of either citalopram or the stimulation.

The average baseline MEP values did not significantly differ between groups as revealed by Student's *t*-tests (paired samples, two-tailed, $p>0.05$). Citalopram alone did not have any impact on cortical excitability, as revealed by Student's *t*-tests between first and second baseline values (paired samples, two-tailed, $p>0.05$; Table 1).

The ANOVA revealed significant main effects of medication ($F(1)=5.345$; $p=0.039$), stimulation ($F(1)=39.497$; $p<0.001$), stimulation × time ($F(14)=15.593$; $p<0.001$) and medication × time ($F(14)=2.456$; $p=0.004$) interactions (for details, see Table 2). The main effect of medication is caused by similarly directed effects of citalopram on MEP amplitudes for PAS10, and PAS25. As compared with placebo medication, citalopram enhanced motor cortical excitability. The main effect of stimulation is due to relatively larger MEP amplitudes in the PAS25 condition, as compared with PAS10, irrespective of medication condition or time point. The interaction of stimulation x time refers to different time courses of MEP alterations generated by PAS10, and PAS25. MEP reductions induced by PAS10 lasted longer than those accomplished by PAS25, and were antagonistically directed for up to 90 min after stimulation, but not with regard to the later time points. Finally, the interaction of medication and time course is caused by the MEP-enhancing effect of citalopram on MEP amplitudes, as compared with placebo medication, during the first 30 (PAS25) or 90 (PAS10) min after PAS, but not for later time points.

Post-hoc Student's *t*-tests show that in the placebo medication conditions, MEPs were significantly enhanced for 30 min after PAS-25 stimulation and diminished for 90 min after PAS10 stimulation. Citalopram abolished PAS10-induced LTD-like plasticity and enhanced PAS25-induced LTP-like plasticity, as compared with the respective placebo medication conditions. Student's *t*-tests show significant differences between drug and placebo conditions at all time points between 0 and 25 min after PAS10 administration and only at the single time point of 30 min after PAS25 (Figure 2).

For the effects of citalopram on PAS-induced plasticity with regard to the grand average calculated for the first 30 min after PAS, citalopram had a significant effect on focal

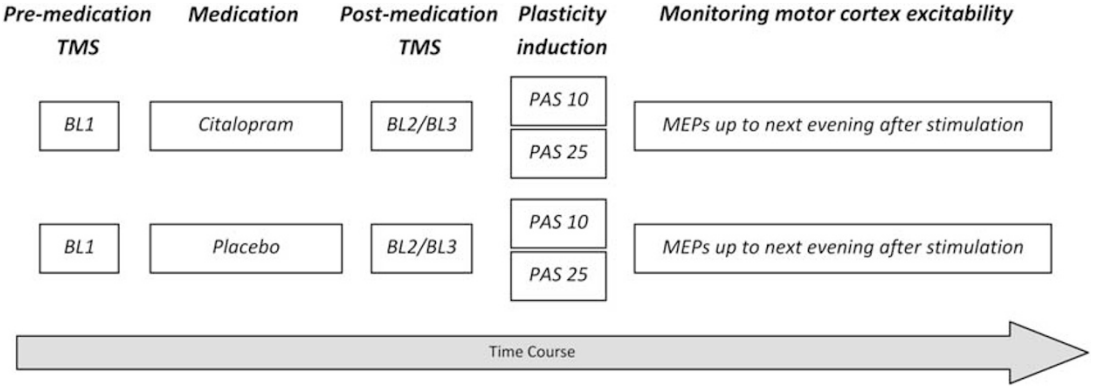

Figure 1 Course of the study. In the beginning of each session, before administration of citalopram or placebo medication, 25 baseline single-pulse MEPs were recorded at an intensity to elicit MEPs with peak-to-peak amplitudes of on average 1 mV. After 2 h, the second baseline was recorded to explore the effect of medication on cortical plasticity, and adjusted, if necessary. After obtaining the second (or third) baseline, PAS was administered and 25 MEPs were recorded immediately after stimulation and at time points of 5, 10, 15, 20, 25, 30, 60, 90, and 120 min after plasticity induction. Further transcranial magnetic stimulation (TMS) measurements were conducted in the evening of the same day (SE), next morning, at ~0900 hours (NM), next noon, at ~1200 hours (NN), and next evening, at ~1800 hours (NE).

Table 1 MEP Amplitudes and Stimulation Intensity Before and After Citalopram Administration

Stimulation	TMS parameter	Medication condition	Baseline 1	Baseline 2	Baseline 3
PAS25	MEP	Citalopram	1.04 ± 0.07	0.99 ± 0.17	0.99 ± 0.14
		Placebo	1.03 ± 0.11	0.93 ± 0.23	0.96 ± 0.14
	%MSO	Citalopram	49.3 ± 9.81	49.1 ± 9.85	49.3 ± 9.98
		Placebo	48.9 ± 9.42	48.9 ± 9.42	49.3 ± 9.46
PAS10	MEP	Citalopram	1.04 ± 0.12	1.00 ± 0.12	1.00 ± 0.10
		Placebo	1.04 ± 0.09	0.95 ± 0.11	1.03 ± 0.10
	%MSO	Citalopram	49.4 ± 9.48	49.4 ± 9.48	49.4 ± 9.53
		Placebo	49.1 ± 9.61	49.1 ± 9.61	49.6 ± 9.80

Shown are the mean MEP amplitudes ± SD and stimulation intensity (percentage of maximum stimulator output, %MSO) mean ± SD of baselines 1, 2 and 3. The intensity of TMS was adjusted to elicit MEPs with peak-to-peak amplitude of ~1 mV (baseline 1). A second baseline (baseline 2) was recorded 2 h after citalopram or placebo intake to determine the impact of the drug on cortical excitability and adjusted if necessary (baseline 3). Student's *t*-tests revealed no significant differences between conditions ($p > 0.05$).

Table 2 Results of the Repeated Measures ANOVA

Factor	Df	*F*	*p*
Medication	1	5.345	0.039*
Stimulation	1	39.497	<0.001*
Time	14	0.723	0.749
Medication × stimulation	1	0.543	0.476
Medication × time	14	2.456	0.004*
Stimulation × time	14	15.593	<0.001*
Medication × stimulation × time	14	0.622	0.845

*Significant results at $p < 0.05$.

excitability-diminishing plasticity, as revealed by respective *post-hoc* Student's *t*-tests (Student's *t*-test, paired samples, two-tailed, $p = 0.009$), whereas only a non-significant tendency towards excitability enhancement after PAS25 stimulation was detected (Student's *t*-test, paired samples, two-tailed, $p = 0.126$) (Figure 3).

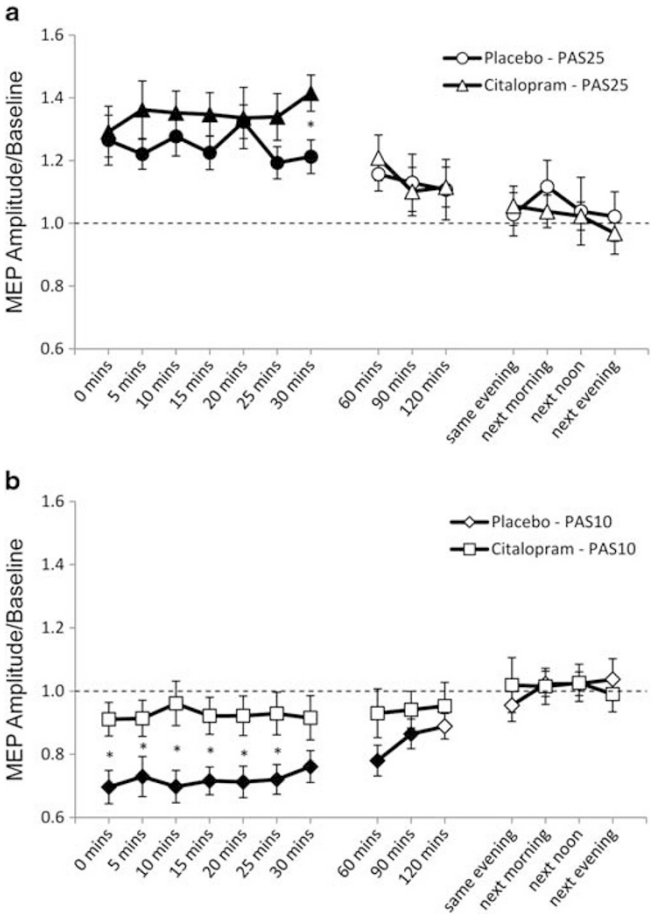

Figure 2 Impact of citalopram on paired associative stimulation (PAS)-induced neuroplasticity. Shown are baseline-normalized MEP amplitudes after plasticity induction by PAS25 (a) and PAS10 (b) under placebo or citalopram medication conditions up to the evening of the post-stimulation day. (a) In the placebo medication condition, PAS25 induced a significant excitability elevation up to 60 min after stimulation, which was enhanced, but not prolonged, by citalopram. (b). In the placebo medication condition, cortical excitability was significantly reduced after PAS10, this effect was abolished by citalopram. Error bars indicate SEM. Filled symbols indicate significant differences of post-stimulation MEP amplitudes from respective baseline values; asterisks indicate significant differences between the drug and placebo medication conditions at the same time points (Student's *t*-test, two-tailed, paired samples, $p < 0.05$).

DISCUSSION

The results of this study show that serotonin has specific effects on PAS-induced motor cortex plasticity in healthy humans. It abolishes focal LTD-like and trendwise enhances focal LTP-like plasticity induced by PAS10 and PAS25, respectively.

These results go in line with previous studies (Nitsche *et al*, 2009; Normann *et al*, 2007). Chronic application of SSRI enhanced facilitatory plasticity and resulted in a shift of inhibitory plasticity of early visual-evoked potentials (VEPs) toward excitation (Normann *et al*, 2007) or restored LTP induction and suppressed LTD facilitation in distressed animals (Von Frijtag *et al*, 2001). For the human motor cortex, another study has demonstrated that a single dose of the SSRI citalopram results in enhancement and prolongation of anodal tDCS-induced LTP-like facilitation and conversion of cathodal tDCS-induced LTD-like plasticity into facilitation (Nitsche *et al*, 2009). In further accordance,

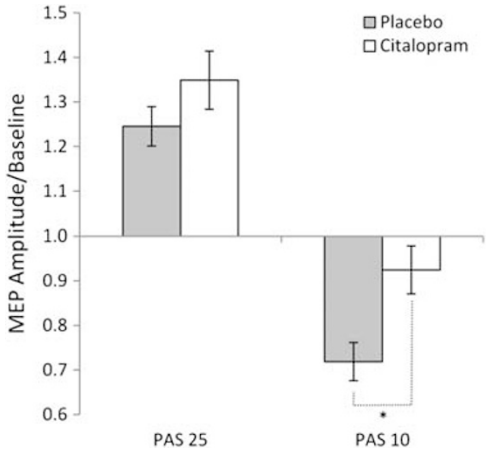

Figure 3 Citalopram enhances PAS25-induced excitatory plasticity and abolishes PAS10-induced inhibitory plasticity. Each column represents the mean of baseline-normalized MEP ± SEM amplitudes until 30 min after stimulation. Asterisks indicate significant differences between drug and placebo conditions (Student's *t*-test, two-tailed, paired samples, $p < 0.05$).

animal studies have shown that serotonin can enhance LTP (Huang and Kandel, 2007; Kojic *et al*, 1997; Machacek *et al*, 2001; Mori *et al*, 2001; Ohashi *et al*, 2002; Park *et al*, 2012), block the stress-caused inhibition of LTP (Ryan *et al*, 2008) or block LTD (Normann *et al*, 2007). Activation of 5-HT receptors was furthermore shown to reverse LTD induction or convert it into LTP (Costa *et al*, 2012; Kemp and Manahan-Vaughan, 2005).

However, activation of serotoninergic receptors also had opposing results on plasticity in other studies. Some studies have shown negative or no effect of serotonin on LTP induction (Edagawa *et al*, 1998; Huang and Kandel, 2007; Kojima *et al*, 2003; Normann *et al*, 2007; Sanberg *et al*, 2006), which can be explained by activation of different serotoninergic receptor subtypes, stage of brain development or dosage and frequency of application of 5-HT agonists or antagonists (Mori *et al*, 2001; Park *et al*, 2012; Staubli and Otaky, 1994). To explore the reasons of such opposing results, future studies should address specific serotoninergic receptor subtypes, using different 5-HT-receptor agonist or antagonist drugs. It might also make sense to explore different dosages of serotoninergic receptor agonists, as it has been demonstrated that other neuromodulators, such as dopamine, have dose-dependent effects on focal and non-focal plasticity in humans (Monte-Silva *et al*, 2009; Monte-Silva *et al*, 2010).

Proposed Mechanisms of Action

After-effects of tDCS and PAS are NMDA- and Ca^{2+}-dependent (Nitsche *et al*, 2003a; Stefan *et al*, 2002; Wolters *et al*, 2003). It has been shown that serotonin facilitates NMDA receptor-dependent LTP (Park *et al*, 2012). Furthermore, serotonin affects K^{+}-channels and reduces membrane potassium conductance (Andrade and Chaput, 1991; Bockaert *et al*, 1992; Choi and Hahn, 2012; Jeong *et al*, 2012; Panicker *et al*, 1991). In case of enhanced serotonin level, these factors could result in membrane depolarization and enhanced Ca^{2+} influx into the postsynaptic neurons through calcium channels and NMDA receptors (Gu, 2002). The direction of induced plasticity depends on the amount of intracellular calcium, with low concentration inducing LTD, high concentration inducing LTP and medium concentration resulting in no plasticity (Cho *et al*, 2001; Lisman, 2001). Therefore, the above-mentioned serotonin-triggered enhancement of calcium influx could have resulted in a tendency towards facilitation of PAS25-induced LTP-like plasticity, similar to that accomplished by anodal tDCS in a previous study (Nitsche *et al*, 2009). Unlike for cathodal tDCS, where neuroplastic excitability diminutions were converted to facilitation by citalopram (Nitsche *et al*, 2009), the drug abolished PAS10-induced LTD-like plasticity in the present study. This can be explained by differences of the respective plasticity induction protocols. Plasticity induced by tDCS is accomplished by long, tonic depolarization of large neuronal populations and activation of voltage-dependent calcium channels, whereas depolarization caused by PAS is short-lasting and affects only small groups of neurons. Therefore the increase of intracellular calcium might be smaller after PAS administration, as compared with tDCS. Given the dependency of plasticity induction from intracellular calcium level, thus the calcium increase accomplished by citalopram might have been sufficient to induce LTP-like plasticity in case of cathodal tDCS, but not for PAS10. This also explains why the shift in excitability toward PAS25-induced excitatory plasticity enhancement is not as clear as in case of anodal tDCS. This hypothesis should however be tested more directly in future experiments.

The role of specific 5-HT receptors in the impact of citalopram on PAS-generated plasticity is not clear. 5-HT2 and 5-HT3 are candidate receptors. The 5-HT3 receptor enhances Ca^{2+} conductance, leading to neuronal depolarization, while the 5-HT2 receptor induces Ca^{2+} release from intracellular stores (Reiser *et al*, 1989). Accordingly, activation of 5-HT2 receptors has a facilitatory effect on NMDA receptor-dependent LTP induction in the visual cortex of adult rats (Park *et al*, 2012). Finally, serotonin affects cholinergic (Consolo *et al*, 1994; Matsumoto *et al*, 2001; Yamaguchi *et al*, 1997), GABAergic (Roerig and Katz, 1997; Waider *et al*, 2012), nicotinergic (Zaniewska *et al*, 2009), and dopaminergic (Gobert and Millan, 1999; Wood and Wren, 2008) systems, which have a major impact on stimulation-induced plasticity in humans (Kuo *et al*, 2007; Kuo *et al*, 2008; Monte-Silva *et al*, 2009; Monte-Silva *et al*, 2010; Nitsche *et al*, 2004; Thirugnanasambandam *et al*, 2012). While it cannot be ruled out completely that serotonin enhancement affected plasticity partially by its impact on one of these neuromodulatory systems, a profound contribution seems unlikely, because the impact of citalopram on tDCS-, and PAS-induced plasticity differs relevantly from those of other neuromodulators. Specifically the above-mentioned studies show that dopamine, acetylcholine, and nicotine have a focusing effect on LTP-like motor cortex plasticity, which is hypothesized to be advantageous for task performance if stable information processing is needed (eg, a simple task which requires uniform action). In contrast, de-focusing—as obtained by citalopram, which enhances focal and non-focal LTP-like plasticity, as shown in the present study, and in a previous study of our group (Nitsche *et al*, 2009) might be advantageous when a task requires flexible information

processing (eg, complex problem solving) (Seamans and Yang, 2004). This hypothetical specific impact of serotonin on ask performance should be explored in future experiments.

General Remarks

PAS is assumed to be related to learning processes as it shares some characteristics with STDP, such as timing and synchronization of two pulses as a requirement to induce plasticity. Therefore, the results of the present and other studies, which show an enhancement of LTP-like PAS-induced plasticity, and a reduction of LTD-like plasticity by SSRIs (Nitsche *et al*, 2009; Normann *et al*, 2007), make these drugs interesting substances for improving learning and motor performance in several clinical conditions (eg, in motor or speech rehabilitation after stroke). Especially with regard to stroke and depression, where LTP-like plasticity seems to be reduced, and/or LTD-like plasticity enhanced by disease-related processes (Foy *et al*, 1987; Schaechter, 2004; Traversa *et al*, 1997; Traversa *et al*, 1998; Turton *et al*, 1996; Xu *et al*, 1997), the results of the present study can at least partially explain why SSRIs can reduce symptoms. In accordance with the LTP-enhancing effect of SSRI with regard to stimulation-induced plasticity (Nitsche *et al*, 2009), a synergistic effect of tDCS and SSRI medication on major depression has been described recently, most probably related to the increased efficacy of anodal tDCS-induced LTP-like plasticity under SSRI (Brunoni *et al*, 2013).

One possible limitation to our study could be that 1-week intersession interval might not be sufficient to rule out any interference effects definitely, as suggested by the results of a recent study (Rajji *et al*, 2011), where PAS25 and PAS10 had a significant impact on motor task performance 1 week after PAS administration. In our study, MEP amplitudes recorded the day after plasticity induction however show no effect of PAS with or without citalopram on motor cortex excitability. Moreover, because of the randomized order of conditions, we would not expect a systematic impact of any minor carryover effect on the results. Finally, previous studies of our group in which a similar procedure was performed showed PAS plasticity effects in the placebo medication conditions, which are comparable to the experiments of other groups, in which not such a frequent repetition of sessions was performed (Kuo *et al*, 2008; Monte-Silva *et al*, 2009; Monte-Silva *et al*, 2010; Stefan *et al*, 2000; Stefan *et al*, 2004; Thirugnanasambandam *et al*, 2012). Therefore, late-phase plasticity is unlikely to have compromised the results of the present experiments.

Interestingly, chronic application of SSRI has different effects on cortical excitability as compared wth single-dose application, although both conditions resulted in functional improvement of motor performance (Gerdelat-Mas *et al*, 2005; Loubinoux *et al*, 2002a; Loubinoux *et al*, 2002b). Clinical studies show that it takes several weeks to obtain therapeutic effects of SSRIs. This suggests an involvement of different mechanisms, such as desensitization and down-regulation of receptors, or reduction of serotonin synthesis in the effects of chronic administration of SSRIs (Blier and Bouchard, 1994; Pineyro *et al*, 1994; Yamane *et al*, 2001), which should be explored in larger detail in future studies.

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