Transcranial magnetic stimulation induces increases in extracellular levels of dopamine and glutamate in the nucleus accumbens

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Transcranial magnetic stimulation (TMS) is a non-invasive approach used for stimulating the human brain. Repetitive stimulation over the prefrontal cortex has proven effective in the treatment of major depression, however the mechanism of the antidepressant action is unknown. Since the nucleus accumbens is a major region implicated in reward circuitry and depressive disorders, we used the microdialysis technique to study some of the neurochemical changes induced in that region during and after acute TMS. Magnetic stimulation was applied over the frontal or the caudal cortex of the rat brain using a special coil design and microdialysis samples were collected before, during and after the stimulation session. The extracellular levels of both dopamine and glutamate in the nucleus accumbens were increased during the stimulation while the extracellular levels of acetylcholine were not affected. Stimulation over the caudal cortex caused a greater increase in dopamine levels than the stimulation over the frontal cortex, while such difference was not observed for glutamate levels. The changes in dopamine and glutamate extracellular levels in the nucleus accumbens may play a role in the antidepressant effect of TMS and it is therefore suggested that the effect of stimulation over caudal cortical sites on depressive patients will be examined. *NeuroReport* 13:2401–2405 © 2002 Lippincott Williams & Wilkins.

Key words: Acetylcholine; Dopamine; Glutamate; Microdialysis; Nucleus accumbens; Transcranial magnetic stimulation

INTRODUCTION

Transcranial magnetic stimulation (TMS) is a non-invasive tool used for application of magnetic pulses to the brain *in vivo*. The pulses are applied by passing high currents through an electromagnetic coil placed upon the scalp that can induce electrical currents in the underlying cortical tissue, thereby producing a localized axonal depolarization. In the last 5 years, this technique has been applied to the study and treatment of various neurobehavioral disorders, primarily mood disorders [1,2]. Several groups around the world have recently demonstrated that repetitive TMS over the prefrontal cortex can affect mood in healthy control subjects, as well as potentially treat depressive disorders [2] and be as effective as electroconvulsive therapy in the treatment of non-delusional major depression [3]. The mechanism of the antidepressant action of TMS is not clear but it may involve local alterations within the prefrontal cortex or distal effects mediated by neuronal interactions between the prefrontal cortex and other brain regions [4].

Release of dopamine in the nucleus accumbens is strongly associated with mediation of reward and motivation [5–9]. Therefore, it was suggested that several antidepressant treatment approaches involve alterations in the release of dopamine within the nucleus accumbens [10–12]. Moreover, descending pathways from the frontal cortex modulate the release of dopamine in the nucleus accumbens [13–15]. Such modulation is mediated both directly, via glutamatergic corticostriatal projections [14], and indirectly by an effect on mesolimbic dopamine neurons in the midbrain [13,15]. In order to learn about the acute neurochemical alterations induced by TMS we used the microdialysis technique for measuring extracellular levels of several neurotransmitters within the nucleus accumbens *in vivo* and a special TMS coil that better fit a rat brain.

MATERIALS AND METHODS

Animal and experimental preparation: *In vivo* TMS and microdialysis experiments were carried out on male Sprague–Dawley rats (340–470 g, Charles River, Japan). The rats were housed under conditions of constant temperature (22°C) and humidity (50%), with a 12:12 h light:dark cycle. Food and water were provided *ad lib*. All animal procedures were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals and all efforts were made to minimize the number of animals

used and their suffering. During the surgery for a microdialysis guide cannula implantation, the rats were anesthetized with ketamine (72.8 mg/kg) and xylazine (10.9 mg/kg), and their body temperature was maintained by a temperature controller. Their skulls were exposed and a 1 mm hole was drilled through the bone, then an intracerebral guide cannula (BAS, USA) was implanted above the nucleus accumbens (1.4 mm anterior to bregma, 1.0 mm lateral to the middle suture and 5.8 mm ventral from the dura). The guide cannulae were fixed permanently with acrylic resin. The surgery for the intracerebral guide cannula setup was performed one week before the TMS experiment. A non-metal microdialysis probe (0.5 mm diameter with a 2 mm membrane length, 20 kDa cut off, BR2, BAS, USA) was inserted into the guide cannula 1 day before the experiment. During the TMS and microdialysis experiment, the microdialysis probe was continuously perfused, at a constant rate of 2.0 μl/min using a microinjection pump (CMA100, CMA, Sweden), with artificial cerebrospinal fluid (mM: Na^+ 147, K^+ 2.7, Ca^{2+} 1.2, Mg^+ 0.85 and Cl^- 153.8). Samples were collected every 15 min with a fraction collector (CMA142, CMA, Sweden) and kept at −80°C until analysis.

Transcranial magnetic stimulation and microdialysis sampling: TMS was administrated to the rats using 98% of the maximal output of a magnetic stimulator (SMN1200, Nihon Kohden, Japan) and the smallest available circular coil (19 mm i.d., 54 mm o.d.; prototype, Nihon Kohden, Japan) which generated maximum electric field intensity of about 500 V/m, 90 μs width monophasic pulse. The coil was placed horizontally above the rat's head in a clockwise wind direction in such way that the windings were placed over either the frontal or the caudal cortex. Control sham stimulation was given over the rat's back. In order to maintain the location of the coil during the TMS session, the rats were extensively handled for 2 weeks before the experiment and were mildly restrained by hand during the stimulation in all cases (including the sham condition). Three microdialysis samples (30 μl, each) were collected for baseline level detection, and during the fourth sampling period, 200 stimuli at 2 Hz were administrated. Then, another three samples were collected to monitor the neurochemical change in the nucleus accumbens after the TMS administration.

Determination of catecholamines: Dopamine and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) and the serotonin metabolite 5-hydroxy-indoleacetic acid (5-HIAA) were measured with HPLC coupled to electrochemical detection as described previously [11]. The detection limit was 0.2 nM for dopamine, DOPAC, and 5-HIAA and 1 nM for HVA.

Determination of glutamate: Glutamate was measured by HPLC with precolumn derivatization with a o-phthaldialdehyde/mercaptoethanol reagent and fluorescence detection, as described previously [16]. The detection limit for glutamate was 10 nM.

Determination of acetylcholine: Acetylcholine (ACh) was measured by liquid chromatography/tandem mass spectrometry (LC/MS/MS), as described previously [17] and optimized in our laboratory. The microdialysates were mixed with 0.5 ng of the internal standard, acetyl-fl-methylcholine, and measured against a duplicate calibration curve (concentration range 0.01563–1.08 ng). The LC/MS/MS system employed for analysis consisted: (1) a Series 200 autosampler (Perkin-Elmer, Norwalk, CT) (2) two Perkin-Elmer Series 200 pumps connected by a 10-1 tee mixer (Lee Company, Westbrok, CT); a Rheodyne Model 7725 switching valve; and (3) an API 3000 tandem quadrupole mass spectrometer with a TurboIonSpray interface (PE-Sciex, Foster City, Canada) operated in positive ion, MRM mode. The LC column employed for chromatographic separation was a Chrompack 10 × 2 mm RP-guard column followed by a 100 × 3 mm i.d., 3 μm particle size Chrompack Inertsil ODS-3 column (Varian, Walnut Creek, CA). Aqueous and organic mobile phases consisted of 20 mM heptafluorobutyric acid with 20 mM ammonium acetate in H_2O (A) and acetonitrile (B). Reversed phase gradient elution is performed at a flow rate of 0.7 ml/min. Gradient elution is performed as follows: 93% A for 0.1 min, linear decrease to 70% in 6 min, step increase to 93% at 6.1 min with a 5 min equilibration. Column effluent was diverted to waste for the first 2.5smin in order to reduce the amount of salt entering the mass spectrometer. In initial experiments, characteristic spectra for ACh were acquired in single quadrupole scan mode. Parent ions were mass selected, and product ion scanning was performed with flow injection of single standards. Appropriate multiple reaction monitoring experiments were then selected to optimize sensitivity and selectivity for ACh and acetyl-fl-methylcholine. Collision induced fragmentation was performed in PCI mode with nitrogen as the reagent gas.

Histology: At the end of the experiment, rats were overdosed, an Evans Blue solution was injected through the microdialysis probe and brains were removed and soaked in 4% paraformaldehyde. The brains were then frozen, sliced into 40 μm coronal sections, and examined under the microscope for verification of probe placement.

Statistical analysis: Data are expressed as the mean ± s.e.m. of values obtained from the indicated number of rats. Significance was determined by repeated ANOVA coupled with application of the Student–Newman–Keuls *post-hoc* test as indicated. $p < 0.05$ was considered significant.

RESULTS

Effect of TMS on extracellular levels of monoamines in the nucleus accumbens: The basal levels of dopamine in the dialysates were 0.76 ± 0.07 nM in the caudal TMS group ($n = 8$) and 0.84 ± 0.12 nM in the frontal TMS group ($n = 8$). TMS over the caudal cortex induced a significant increase (41 ± 4%) in the dialysate levels of dopamine in the nucleus accumbens ($F(6,42) = 15.05$, $p = 0.0001$). Dopamine levels returned to baseline within 30 min after TMS administration (Fig. 1a). TMS over the frontal cortex induced a smaller but significant increase (20 ± 5%) in the dialysate levels of dopamine in the nucleus accumbens ($F(6,42) = 3.06$, $p = 0.01$), which returned to baseline within 15 min after TMS administration. Two-way ANOVA (within time-

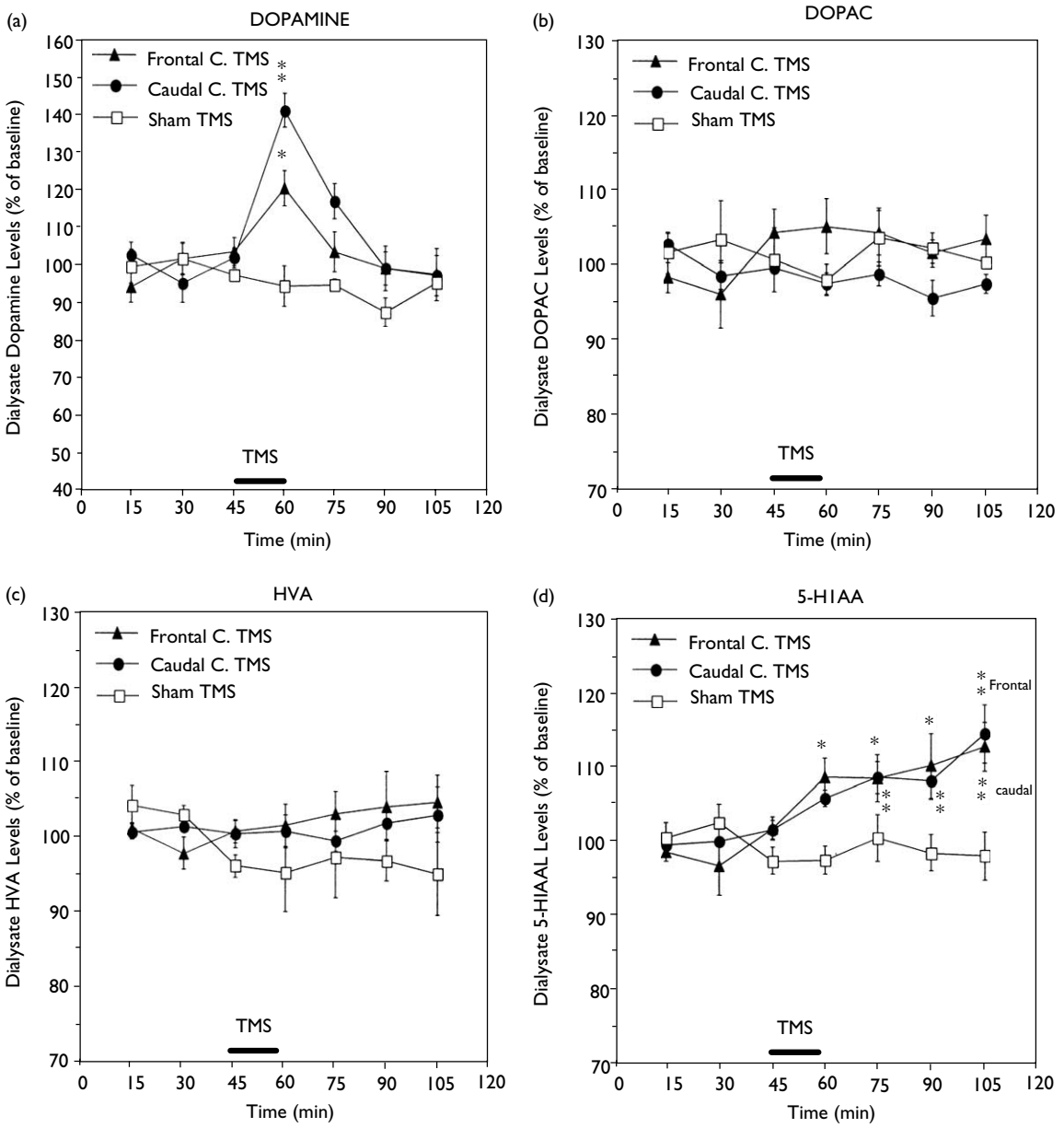

Fig. 1. Effect of TMS over the frontal or the caudal cortex on extracellular levels of dopamine, DOPAC, HVA and 5-HIAA in the nucleus accumbens. After three baseline collections TMS was applied for 15 min, as indicated by the bar on the x-axis. The mean baseline level was calculated for each rat separately and all values for that rat were calculated as percentage of the mean baseline level. Mean ± s.e.m. percentage values of eight rats stimulated over the frontal or the caudal cortex and three rats stimulated over the back (sham TMS) are presented. Values statistically different from baseline were determined by one-way ANOVA with repeated measures over time followed by the Student–Newman–Keuls *post-hoc* test. $*p < 0.05$, $**p < 0.005$.

× between site of TMS application) revealed no significant effect of site but a significant site × time interaction ($F(6,84) = 2.31$, $p = 0.04$). Sham stimulation (over the rat's back) did not affect extracellular levels of dopamine in the nucleus accumbens ($n = 3$; Fig. 1a).

The basal levels of the dopamine metabolites DOPAC and HVA in the dialysates were 693 ± 134 and 348 ± 46 nM in the caudal TMS group and 724 ± 149 and 365 ± 49 nM in the frontal TMS groups, respectively ($n = 8$). The dialysate levels of DOPAC and HVA were not significantly altered by TMS (Fig. 1b,c). The basal levels of the serotonin metabolite, 5-HIAA, in the dialysates were 363 ± 62 in the caudal TMS group ($n = 8$) and 347 ± 48 in the frontal TMS group ($n = 8$). Dialysate levels of 5-HIAA gradually increased following the TMS session when applied either over the caudal cortex ($F(6,42) = 9.10$, $p = 0.0001$) or the frontal cortex ($F(6,42) = 5.34$, $p = 0.0004$) and reached 13–15% over baseline 1 h after TMS administration. Two-way ANOVA (within time x between site of TMS application) revealed no significant effect of site and no significant site × time

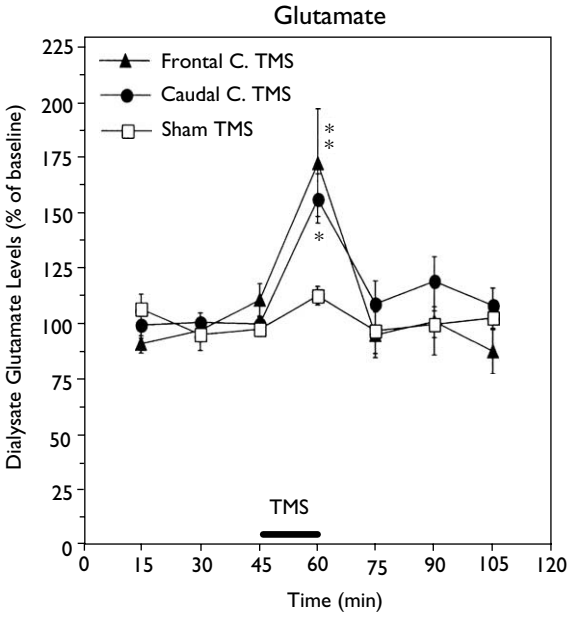

Fig. 2. Effect of TMS over the frontal or the caudal cortex on extracellular levels of glutamate in the nucleus accumbens. After three baseline collections, TMS was applied for 15 min, as indicated by the bar on the x-axis. Mean ± s.e.m. percentage values of seven rats stimulated over the frontal or the caudal cortex and three rats stimulated over the back (sham TMS) are presented. Values statistically different from baseline were determined by one-way ANOVA with repeated measures over time followed by the Student–Newman–Keuls *post-hoc* test. $*p < 0.001$, $**p < 0.0001$.

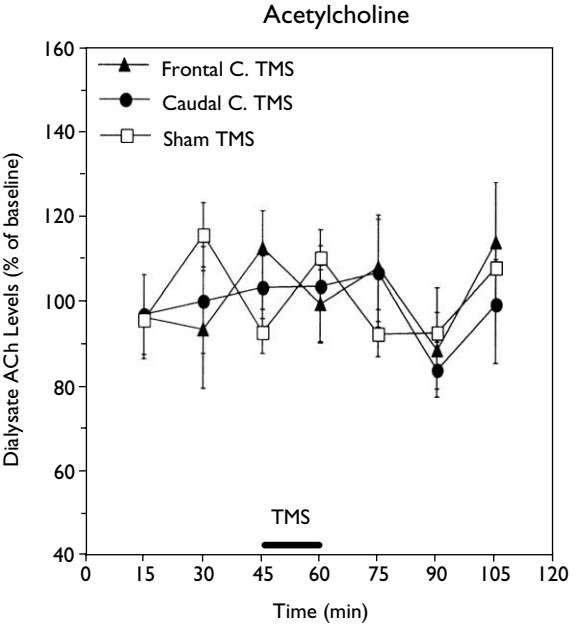

Fig. 3. Effect of TMS over the frontal or the caudal cortex on extracellular levels of acetylcholine in the nucleus accumbens. After three baseline collections, TMS was applied for 15 min, as indicated by the bar on the x-axis. Mean ± s.e.m. percentage values of eight rats stimulated over the frontal cortex, seven rats stimulated over the caudal cortex and three rats stimulated over the back (sham TMS) are presented.

interaction. Sham stimulation ($n = 3$) did not affect 5-HIAA levels (Fig. 1d).

Effect of TMS on extracellular levels of glutamate in the nucleus accumbens: The basal levels of glutamate in the dialysates were 827 ± 81 nM in the caudal TMS group ($n = 7$) and 845 ± 86 nM in the frontal TMS group ($n = 7$). TMS over the caudal cortex induced a significant increase (56 ± 11%) in the dialysate levels of glutamate ($F(6,36) = 5.22$, $p = 0.0006$) in the nucleus accumbens. Glutamate levels returned to baseline within 15 min after TMS administration (Fig. 2). TMS over the frontal cortex also induced an increase (72 ± 24%) in dialysate levels of glutamate in the nucleus accumbens ($F(6,36) = 6.51$, $p = 0.0001$), which returned to baseline within 15 min after TMS administration. Two-way ANOVA (within time- × between site of TMS application) revealed no significant effect of site and no significant site × time interaction. Sham stimulation ($n = 3$) did not a significant change in the extracellular levels of glutamate in the nucleus accumbens (Fig. 2).

Effect of TMS on extracellular levels of acetylcholine in the nucleus accumbens: The basal levels of ACh in the dialysates were 1.72 ± 0.16 nM in the caudal TMS group ($n = 8$) and 1.96 ± 0.21 in the frontal TMS group ($n = 7$). No change in dialysate levels of ACh was observed during or after TMS applied over either the caudal cortex ($F(6,42) = 0.61$, $p = 0.71$) or the frontal cortex ($F(6,36) = 1.02$, $p = 0.42$; Fig. 3).

DISCUSSION

The present study indicates acute neurochemical alterations induced by TMS. The extracellular levels of both dopamine and glutamate were increased in the nucleus accumbens when TMS was applied either over the frontal or the caudal cortex, while ACh levels were not altered. Since dopamine release in the nucleus accumbens is associated with reward and motivation [5–9], and to some extent with depressive disorders [10–12], the therapeutic effect of chronic TMS in depressive disorders may involve attenuation of dopaminergic neurotransmission in the nucleus accumbens. Therefore, stimulation over the caudal cortex, which had a greater effect on dopaminergic neurotransmission, may potentially have a greater therapeutic effect. However, it should be pointed out that although a relatively small circular coil (specially designed for a better fit to a rat brain) was used, the distribution of the magnetic field induced by standard coils in the human brain would be different.

The fact that extracellular levels of ACh were not altered in the nucleus accumbens indicates that this region was not stimulated directly either by the frontal or by the caudal stimulation since the nucleus accumbens itself contains a large number of ACh interneurons [18], and direct stimulation of these neurons would have affected extracellular levels of ACh.

Since TMS induced increases in both glutamate and dopamine extracellular levels in the nucleus accumbens, it is possible that stimulation of the excitatory corticostriatal

projections induced dopamine release in the nucleus accumbens by a local effect of glutamate on adjacent dopaminergic nerve terminals [19]. Such an effect may be mediated by local ionotropic [20] or metabotropic [14] glutamate receptors. The existence of this mechanism is supported by the fact that cortical neurons originating in the prefrontal cortex and dopamine neurons from the ventral tegmental area synapse in close proximity to one another on the spines of nucleus accumbens medium spiny neurons [21]. An alternative mechanism for the TMS-induced dopamine release is the stimulation of the excitatory projections from the cortex to the ventral tegmental area (VTA), where mesolimbic dopamine cells project to the nucleus accumbens [13–15]. A similar mechanism may take place in the case of caudal stimulation: projections from the cerebellum and the superior colliculus to the VTA [22] may be stimulated by the caudal TMS and activate the dopaminergic projections from the VTA to the nucleus accumbens.

It is unlikely that the VTA was stimulated directly by the caudal stimulation (thereby inducing a greater increase in dopamine levels in the nucleus accumbens) since the distance between the VTA and the caudal coil placement is greater than the distance between the nucleus accumbens and the frontal coil placement and our data show that ACh interneurons in the nucleus accumbens where not stimulated by TMS over the frontal cortex.

The gradual increase in the levels of the serotonin metabolite, 5-HIAA, in the nucleus accumbens after either frontal or caudal TMS, indicate that TMS also affects serotonergic neurotransmission. While the effects on dopamine and glutamate were observed only during the TMS session (and no changes were detected in DOPAC and HVA), the 5-HIAA levels continued to increase > 60 min later. Although extracellular levels of 5-HIAA do not necessarily correlate with serotonin itself [23], this finding may also be relevant to the therapeutic effect of TMS in depressive disorders, since alteration in serotonergic neurotransmission is strongly associated with both the etiology of these disorders and with the mode of action of various antidepressant drugs [24], including chronic TMS [25].

Further studies will determine whether the acute neurochemical changes induced in the nucleus accumbens by one TMS session still occur after chronic treatment and whether baseline extracellular levels of these neurotransmitters are altered as a result of chronic TMS treatment.

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