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Abbreviations: 5-HIAA, 5-hydroxyindolacetic acid; 5-HT, serotonin; CT, computed tomography; DA, dopamine; DLPFC, dorsolateral prefrontal cortex; ECT, electroconvulsive therapy; EMG, electromyography; [^{18}F]-FDG, 2-deoxy-2-(^{18}F)fluoro-D-glucose; HF-rTMS, high-frequency repetitive transcranial magnetic stimulation; MEP, motor evoked potential; MT, motor threshold; PET, positron emission tomography; sgACC, subgenual anterior cingulate cortex; SUV_{glu}, glucose corrected standardized uptake value; TRD, treatment-resistant depression; VOI, volume of interest.

Abstract

High-frequency repetitive transcranial magnetic stimulation (HF-rTMS) is currently accepted as an evidence-based treatment option for treatment resistant depression (TRD). Additionally, HF-rTMS showed beneficial effects on psychomotor retardation in patients. The classical HF-rTMS paradigms however are unlikely to replace electroconvulsive therapy, a more potent alternative for TRD albeit with important side-effects. Therefore, recent studies have investigated 'accelerated' HF-rTMS protocols demonstrating promising clinical responses in patients with TRD. Since the neuronal effects of accelerated HF-rTMS are underinvestigated, we evaluate here the possible metabolic and neurochemical effects of this treatment alternative. More specifically, we measured the effect on brain glucose metabolism and monoamines/metabolites, as well as on the spontaneous motor activity in rats. We found that brain glucose metabolism and monoamines remained generally unaffected after accelerated HF-rTMS, with the exception of reduced total striatal 5-hydroxyindolacetic acid (a metabolite of serotonin) levels. Interestingly, when compared to sham stimulation the velocity, the total distance travelled as well as the percentage of movement, as measured by the open-field test, were significantly enhanced after accelerated HF-rTMS showing an increased motor activity.

Our current results indicate that the accelerated HF-rTMS-induced increase in motor activity in rats, may be related to the striatal neurochemical effect.

Introduction

Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive technique where no anesthetics are required and side-effects are barely induced. This technique has wide applications in neurology and psychiatry (Lefaucheur et al., 2014). In patients, it is currently accepted as an evidence-based treatment option for major depression, as up to 15% of patients suffering from major depression do not respond to the available antidepressant drugs (Berlim and Turecki, 2007a). High-frequency (HF)-rTMS is thereby mostly applied to the left dorsolateral prefrontal cortex (DLPFC) (Berlim and Turecki, 2007b) however, response rates are rather limited (Mitchell and Loo, 2006). Electroconvulsive therapy (ECT) has been proven to be a more potent alternative for treatment-resistant depression (TRD) but the use of anesthetics and the risk for cognitive impairments increase the need for alternative treatments.

According to the meta-analysis performed by Berlim and coworkers, the classical HF-rTMS paradigms are unlikely to replace ECT (Berlim et al., 2013; Micallef-Trigona, 2014). Therefore, in order to increase the clinical response of TRD, recent studies have investigated 'intensified' or 'accelerated' HF-rTMS protocols (Baeken et al., 2013; Hadley et al., 2011; Holtzheimer et al., 2010; Zeeuws et al., 2011). A case report described successful treatment of an ECT-resistant depressed woman with accelerated HF-rTMS consisting of 20 HF-rTMS sessions administered during 4 consecutive days (daily 5 sessions of 1560 stimuli each and a total amount of 31200 pulses after 4 days) instead of classical protocols consisting of a daily single session with a duration varying from 2 to 9 weeks (Zeeuws et al., 2011). Furthermore, the same HF-rTMS paradigm, in a placebo-controlled study consisting of 20 unipolar patients with TRD, was found to be safe and well-tolerated and proved its benefits since 35% of the patients showed immediate clinical response (Baeken et al., 2013).

Several studies have also explored the neuronal impact of rTMS treatment in both healthy subjects and patients suffering from major depression (Baeken et al., 2011; Baeken et al., 2009; Kito et al., 2012; Knoch et al., 2006; Sibon et al., 2007). For example Knoch et al. demonstrated in healthy men that changes in the regional cerebral blood flow induced by prefrontal rTMS differ upon hemisphere stimulated and vary with stimulation frequency (Knoch et al., 2006). Recently, we demonstrated for the first time a decreased metabolic activity of the subgenual anterior cingulate cortex (sgACC) after accelerated HF-rTMS of the left DLPFC which was associated with a beneficial clinical outcome in patients with TRD (Baeken et al., 2015; Baeken et al., 2014). The latter brain area is reported to be metabolically hyperactive during depressive episodes (Drevets et al., 2008), and successful treatments in general attenuate this sgACC metabolic activity, indicating that HF-rTMS affects disturbed cortico-limbic pathways when clinically depressed. Since the investigation possibilities in human studies are ethically restricted, research on the effects of rTMS in laboratory rats has been performed demonstrating, amongst others, changes in neurotransmitter systems (Ben-Shachar et al., 1997; Ben-Shachar et al., 1999; Kanno et al., 2004; Keck et al., 2000; Keck et al., 2002). In this pilot study we investigated possible metabolic and neurochemical effects of such an accelerated HF-rTMS paradigm using a circular TMS coil on healthy rats. Since the therapeutic effects of most antidepressants are associated with alterations of brain monoamines, we investigated the effect of accelerated HF-rTMS on the total content of dopamine (DA), serotonin (5-HT) and their metabolites in different brain areas. Additionally, given that brain glucose metabolism is an indirect indication of neuronal activity, we also measured the uptake of the positron emission tomography (PET) tracer 2-deoxy-2-(^{18}F)fluoro-D-glucose ([^{18}F]-FDG) in different brain structures. Moreover, besides the typical depressive mood and lack of interests, major depression also comprises psychomotor retardation. Indeed, prior research in medication-free patients reported decreased motor activity during

wakefulness (van Londen et al., 1998; Volkers et al., 2003). Interestingly, we previously reported that a classical HF-rTMS paradigm (10 HF-rTMS sessions spread over 10 days), resulted in decreased psychomotor retardation in patients with TRD (Baeken et al., 2010). Moreover, we also noticed increased motor activity in patients with TRD after accelerated HF-rTMS treatment (20 HF-rTMS sessions spread over 4 days) (C Baeken, Department of Psychiatry, Universitair Ziekenhuis Brussel, Center for Neurosciences, Vrije Universiteit Brussel, personal communication). Besides, prefrontal rTMS significantly improved the psychomotor speed after active stimulation of the right DLPFC in healthy females (Baeken et al., 2012). Therefore, besides the aforementioned metabolic and neurochemical assessments, the motor activity was also explored following this accelerated stimulation paradigm in rats.

Experimental Procedures

Animals

Twelve male Sprague-Dawley rats (Janvier, France), weighing 250 – 275g (7 weeks old) at the start of the experiment, were used. Rats were housed in our animal housing facilities and had *ad libitum* access to water and food. Experiments were carried out according to the European Ethics Committee (86/609/EEC) and were approved by the Antwerp University Ethical Committee for Animal Experiments (ECD 2011 30). All efforts were made to minimize animal suffering and the minimal number of animals necessary to produce reliable scientific data was used.

Experimental procedure

All rats were first handled and trained during 5 consecutive days to remain immobile in a conical cylinder to minimalize stress during the experiment and to increase reproducible

positioning of the coil as well as to habituate to the acoustic effect of the coil during stimulation (coil is placed at a distance) (Fig. 1A). Immediately after the handling session on the 4th day of the handling period, rats were transported to the open field room (15min) and were allowed to acclimatize during 1h. This was followed by the open field test during which the spontaneous motor activity was measured (section 2.4) (delay between the training session and open field test was 1h and 15min). The next day, in order to assess the brain glucose metabolism, animals were subjected to [^{18}F]-FDG-µPET imaging (section 2.5) immediately after the handling session. Thereafter, animals were randomly divided into the rTMS and sham group. During the HF-rTMS/sham stimulation period (section 2.3), each animal received daily 5 suprathreshold HF-rTMS sessions or sham stimulation during 4 consecutive days. On the 4th day of the HF-rTMS/sham stimulation, after the last stimulation session, rats were allowed to acclimatize during 1h in the open field room and were subsequently subjected to the open field test. The following day, brain glucose metabolism post HF-rTMS/sham treatment was assessed using [^{18}F]-FDG-µPET imaging. After the final scans, rats were sacrificed using a guillotine (after being shortly anesthetized by inhalation of a mixture of isoflurane and medical oxygen). The brains were removed from the skull and both left and right motor cortices, medial prefrontal cortices, striata and hippocampi were dissected, snap frozen and finally kept at - 80°C until the neurochemical measurements.

Accelerated HF-rTMS stimulation

Before receiving HF-rTMS or sham stimulation, rats were fixed in a conical cylindric restrainer. Rats of the rTMS group (n = 6) received daily 5 suprathreshold HF-rTMS sessions during 4 days (1560 stimuli per session and 31200 stimuli after the complete stimulation period in 20 sessions (Baeken et al., 2013; Zeeuws et al., 2011)) using a MagPro R100 stimulator connected to a C-100 circular coil (MagVenture, Farum, Denmark). The coil was

kept in a constant position by hand on top of the restrainer above the cerebral cortex. Particularly, the center of the coil was placed over the midline and approximately 14mm anterior from the interaural line by means of an image of the prefrontal cortices obtained by a CT scan of a rat placed in the restrainer. The TMS coil used in this study is a human coil with an outer diameter of 110mm and an inner diameter of 20mm. This was obligatory since the amount of stimuli that we had to administer to achieve the accelerated rTMS protocol could not be produced by the at that time available smaller coils due to overheating issues. The stimulation parameters used in this study, as mimicked from the clinical protocol (Baeken et al., 2013; Zeeuws et al., 2011), were as follows: frequency = 20Hz, train duration = 1.95s, # pulses per train = 39, intertrain interval = 12.05s, # trains = 40, total session duration = ca. 9min, intersession interval = 15min, intensity = 110% of the individual motor threshold (MT) (this corresponds to a mean output of 29% of the stimulator) (Fig. 1B-C). The group of sham animals (n = 6) were placed in proximity (ca. 50cm) of the HF-rTMS setup in order to experience the same acoustic effect made by the stimulator while not receiving any electrical stimulation during 4 days.

Motor threshold determination

The MT, i.e. the lowest stimulation intensity required to evoke a reproducible motor evoked potential (MEP) of ≥ 50μV at the hind limb biceps muscle, was determined for each animal before the start of the stimulation period by means of electromyographic (EMG) measurements during propofol anesthesia. The method of the MT determination has been recently described in detail by Parthoens et al. (Parthoens et al., 2016). In summary, the tail vein of rats were catheterized (after a brief anesthesia with a mixture of medical oxygen and isoflurane (5%)) for continuous infusion of propofol. The latter anesthetic is used since it has no or a neglible effect on the MEP and it maintained stable MEP responses over a period of

4h when given at low doses compared to e.g. halothane (Luft et al., 2001). Next, a disposable monopolar EMG needle ground electrode (Technomed, Europe) was inserted in the tail followed by the placement of EMG surface electrodes (Ambu Neuroline 700, 20 x 15mm) on the depilated left hind limb. Fifteen minutes after the start of propofol infusion, single pulses where administered to the right hemisphere while the MEPs were recorded with a MEP Monitor (2 x 10^4 samples/s, 100Hz – 5kHz, MagVenture A/S, Denmark). The MT was determined by first stimulating at 20% of the maximum machine output followed by increasing the intensity in 10% steps until a positive MEP response was measured. A positive response corresponds here with an amplitude of 50µV. Then the lower threshold (MT_{low}) was determined by decreasing the intensity in 1% steps and is defined as the maximum intensity at which 5 consecutive pulses all produced no response. Similarly, the minimum intensity at which 5 stimuli all produced a positive response was determined by increasing the intensity in 1% steps and is defined as the upper threshold (MT_{high}). As proposed by Mills and Nithi, the MT is defined as (MT_{low} + MT_{high})/2 (Mills and Nithi, 1997). When administering pulses during the MT determination, an interpulse interval of minimum 8s was introduced in order to be certain no low frequency rTMS effects would be elicited that may influence cortical excitability. Also, this interval allowed the coil to cool down before administration of the next pulse.

Open-field test

Before and after the treatment (for timeline see Fig. 1A), animals were subjected to the open-field test for measuring spontaneous motor activity (Maurice et al., 2015; Tadaiesky et al., 2008; Wu et al., 2016). Therefore, each animal was placed in a black plexiglass arena of 60 x 60 x 40cm followed by the monitoring of the motor behavior during a 10-min test period with the Noldus EthoVision video tracking system (Wageningen, the Netherlands). The following

motor parameters, velocity (cm/s), total distance travelled (cm) and percent of movement (%) were recorded (Smolders et al., 2008). The different parameters are expressed as the ratio of the open-field test results obtained post-treatment and the open-field test results obtained at baseline (fold change). The two-tailed unpaired t-test was applied to compare data obtained from the sham group with those obtained from the HF-rTMS-treated group. Besides, the data were also subjected to a one-sample t-test. For all statistical analyses $\alpha = 0.05$.

[^{18}F]-FDG-µPET-CT imaging

The method and materials used for µPET-computed tomography (CT) acquisition on rats were described in detail in previous reports (Parthoens et al., 2014a; Parthoens et al., 2014b). In summary, in order to normalize blood plasma glucose levels, the animals were fasted at least 12h before the scan (Deleye et al., 2014). On the days of the scan, animals received a bolus injection of 1mCi of [^{18}F]-FDG in the tail vein after being shortly anesthetized by inhalation of a mixture of isoflurane and medical oxygen. After 20min of awake [^{18}F]-FDG uptake, rats were again anesthetized by inhalation of a mixture of isoflurane and medical oxygen (5% induction and 1.5% maintenance) and placed on a thermostatically heated bed of a µPET-CT scanner (Siemens Preclinical Solution, Knoxville, TN, USA) until they reached a total of 30min of [^{18}F]-FDG uptake. Thereafter, a 20-min static PET acquisition was started followed by a 10-min CT scan.

For analysis, each PET image was spatially normalized into the space of an [^{18}F]-FDG template using brain normalization in PMOD v3.3 (PMOD Technologies, Zurich, Switzerland) followed by a count normalization for the injected dose, animal weight and plasma glucose level measured before the scans. The images were thus expressed as glucose corrected standardized uptake value (SUV_{glu}) (Deleye et al., 2014). For two animals (one in

each condition) both the pre-treatment and post-treatment images were left out of the analysis since one of both images were considered unusable due to a hardware failure or moving of the animal's head during the scan acquisition. For each experimental group (pre-sham stimulation, post-sham stimulation, pre-HF-rTMS and post-HF-rTMS), the average SUV_{glu} images over all animals (n = 5 per group) were calculated. For volume of interest (VOI) based analysis, the average SUV_{glu} of each VOI (amongst others motor cortices, medial prefrontal cortices, striata and hippocampi) was calculated. Significant repeated measures analysis of variances were followed-up by *post-hoc* tests ($\alpha = 0.05$) to compare the different experimental groups (pre-sham stimulation, post-sham stimulation, pre-HF-rTMS and post-HF-rTMS). In addition, a voxel-based statistical parametric mapping analysis was performed for further exploration of the imaging data consisting of repeated measure analysis of variances followed by *post-hoc* tests for comparisons between the different experimental groups (pre-sham stimulation, post-sham stimulation, pre-HF-rTMS and post-HF-rTMS) with a minimum cluster threshold of 50 voxels ($\alpha = 0.05$).

Analysis of the total monoamine/metabolite content

The total DA and 5-HT content as well as their metabolites (3,4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindolacetic acid (5-HIAA)) in the different brain structures were measured based on previously reported methods (El Arfani et al., 2014; Izurieta-Sánchez et al., 1998). In summary, after weighing striatal or hippocampal tissue, 760µl of an antioxidant solution (0.1mM acetic acid, 3.3mM L-cysteine, 0.27mM Na_2EDTA and 12.5mM ascorbic acid) and 40µl of a an internal standard solution (3,4-dihydroxybenzylamine solution 100ng/100µl in antioxidant) were added to the brain structures. Similarly, after weighing cortical tissue, 380µl of the same antioxidant solution and 20µl of the internal standard solution were added. The samples were centrifuged (20min, 9500g, 4°C) after

homogenization. The supernatant was diluted 5-fold in 0.5M acetic acid and 20µl was injected automatically on a reversed phase liquid chromatography system (autosampler ASI-100 and HPLC pump P680 A HPG/2, Dionex, Amsterdam, The Netherlands) with electrochemical detection (potential = + 700mV) (Amperometric Detector LC-4C, BAS, Indiana, USA). The separation was achieved using a narrowbore C_{18} column (Alltech®, Alltima™, 5µm, 150 x 2.1mm, Grace, Deerfield, IL, USA). The mobile phase buffer contained 0.1M sodium acetate, 20mM citric acid, 1mM sodium octane sulfonic acid, 1mM dibutylamine and 0.1mM Na_2EDTA adjusted to pH 3.7 (mobile phase composition: 97 buffer / 3 methanol (v/v)). Tissue concentration was expressed as ng monoamine (or metabolite)/g wet tissue (ng/g). The two-tailed unpaired t-test was used to compare data obtained from the sham group with those obtained from the HF-rTMS-treated group ($\alpha = 0.05$).

Results

Effect of accelerated HF-rTMS on the [^{18}F]-FDG uptake and on the total monoamine/metabolite content in the brain

Accelerated HF-rTMS was well tolerated by the animals and no sign of discomfort nor any abnormal behavior was observed during or after the HF-rTMS/sham treatment.

The average SUV_{glu} images over all animals (n = 5 per group) for each experimental group (pre-sham stimulation, post-sham stimulation, pre-HF-rTMS and post-HF-rTMS) are shown in Fig. 2A. VOI-based PET analysis as well as voxel-based analysis revealed no significant differences in glucose metabolism between 1) the pre- and post-images for either sham or accelerated HF-rTMS treatment and 2) the sham and HF-rTMS condition per time point in the brain as a whole (Fig. 2B) nor in the different brain regions such as the striatum (Fig. 2C).

The total monoamine/metabolite content in all studied brain regions was not significantly affected by the accelerated stimulation protocol as illustrated here for the striatal DA and DOPAC content as well as the DA turnover (DOPAC/DA) (Fig. 3A-C). Although the striatal 5-HT content was not significantly affected by accelerated HF-rTMS (Fig. 3D), its metabolite 5-HIAA was moderately but significantly decreased in the striatum (-14%) of rats receiving this treatment compared to sham-stimulated animals (t_{22} = 2.364, $p \leq 0.05$) (Fig. 3E). Moreover, a significant correlation between striatal 5-HT and 5-HIAA levels was found ($F_{1,22}$ = 18.66, r^2 = 0.4589, $p \leq 0.001$) (Fig. 3F).

Enhanced motor activity induced by accelerated HF-rTMS

Of interest here, a significant enhancement of the spontaneous motor activity after accelerated HF-rTMS was observed. In particular, the velocity (t_{10} = 5.995, $p \leq 0.0001$) (Fig. 4A), the total distance (t_{10} = 5.996, $p \leq 0.0001$) (Fig. 4B) as well as the % of movement (t_{10} = 5.727, $p \leq 0.001$) (Fig. 4C), expressed as ratios (post/pre; fold change), were significantly enhanced after accelerated HF-rTMS compared to the sham treatment (two-tailed unpaired t-test). The significant improvement of the spontaneous motor activity is confirmed by the one-sample t-test showing HF-rTMS ratios being significantly higher than 1 for the velocity (t_5 = 3.249, $p \leq 0.05$) and the total distance (t_5 = 3.249, $p \leq 0.05$).

Discussion

Our rat study shows unaffected brain glucose metabolism after accelerated HF-rTMS treatment which is not in line with our initial assumptions. However, a previous report in patients with depression also showed unaltered brain glucose metabolism after ECT when compared with their baselines (Yuuki et al., 2005). These authors speculated that a certain

period of time after successful ECT is necessary before changes in brain glucose metabolism could be observed. A [^{18}F]-FDG-PET study at a later time point after treatment would therefore be useful. Furthermore, our study shows unaltered monoamine levels in motor and depression-related brain regions after the accelerated stimulation paradigm. On the other hand, a modest but significant reduction of striatal 5-HIAA level was measured after intensive stimulation. It has been demonstrated that acutely-induced changes in the monoamine/metabolite contents in healthy rats by classical HF-rTMS were reestablished to normal after chronic HF-rTMS which might be the result of compensation mechanisms (Ben-Shachar et al., 1999). Such compensation could have occurred in this study explaining the lack of effect on monoamine levels as well as the modest 5-HIAA change.

Interestingly, an increased spontaneous motor behavior was observed in our rats after intensive stimulation. Although the current results are seen in healthy animals, it is worthy to mention that the latter effect has been previously observed in patients receiving this accelerated treatment as well as in patients after less intensive HF-rTMS paradigms (Baeken et al., 2010; Hoeppner et al., 2010). Besides, prefrontal rTMS significantly improved the psychomotor speed in healthy females (Baeken et al., 2012). The striatal DA-ergic system is possibly involved in the mechanism of psychomotor retardation in patients with major depression, as some studies demonstrated rTMS-induced DA release (Buyukdura et al., 2011; Pogarell et al., 2006; Strafella et al., 2001). As the total striatal DA content, as measured in the present study, might not reflect DA neurotransmission, we cannot confirm nor deny that accelerated HF-rTMS-induced increase in motor activity is associated with enhanced DA release. Future research with for instance [^{11}C]-Raclopride PET may be clarifying.

Previous research also demonstrated the involvement of the 5-HT-ergic system in psychomotor retardation (Sabbe et al., 2001). Indeed, 5-HT agonists were able to reduce spontaneous motor activity in animals and, inversely, lesioning of 5-HT-ergic neurons

resulted in hyperactivity in rats (Brus et al., 2004). It is possible that impaired striatal 5-HT metabolism induced by accelerated HF-rTMS, as seen in the present study, could be related with enhanced motor activity in rats and with improved psychomotor retardation in patients. Finally, this accelerated HF-rTMS-induced increase in motor activity could also partly be the consequence of direct stimulation of the motor cortical areas due to the limited spatial resolution of rTMS in rodents.

One limitation in this study is the use of a human TMS coil. As mentioned previously, the use of the latter was obligatory since the accelerated HF-rTMS paradigm cannot be obtained by the available smaller coils. Consequently, one could question about the focality and expected size of the affected brain tissue. Based on first order electromagnetic simulations and according to the observation described here further, the region that is targeted in this study (the prefrontal cortices) received the strongest stimulation. Indeed, during the determination of the MT, we observed that when the human coil was very slightly shifted from its place (target motor cortex), no muscular contraction could be measured anymore showing that we still can obtain a satisfying focus with the human coil on rodents. Besides, also other parts of the rat brain were probably affected by the stimulation. However, the stimulation intensity diminishes exponentially with increasing distance from the center of the coil. These limitations have led us to develop a new rodent, which is able to allow for high field electromagnetic potentials with a high number of stimuli before overheating. The latter coil will be used for future rTMS studies in rodents as it meets the requirements for an adequate TMS coil for rodents (Parthoens et al., 2016).

Important to take into consideration is that this study has been conducted in healthy rats and not in ‘depressive-like’ rats. Nevertheless, it is - to the best of our knowledge - the first time that this accelerated HF-rTMS paradigm has been explored for its neuronal and behavioral

impact on laboratory animals. Future work is to investigate the neurochemical, metabolic and behavioral effects after this intensive stimulation protocol in rats exhibiting 'depressive-like' behavior.

Taken together, although additional research will be required to substantiate our current findings in rats, the present study demonstrates clearly enhanced motor activity in rats after accelerated HF-rTMS and the latter could possibly be related to the striatal neurochemical alteration.

Authors' roles

C. Baeken, S. Staelens, I. Smolders, A. El Arfani, J. Parthoens and T. Wyckhuys are responsible for the design of the study. The experimental work was mainly performed by A. El Arfani. J. Parthoens and T. Demuyser also contributed to the experimental work. The analysis of the data was performed by A. El Arfani, J. Parthoens, S. Staelens, S. Servaes, M. De Coninck and D. Van Dam. A. El Arfani wrote the report. All authors, including P.P. De Deyn also contributed to the writing of the report.

Disclosures

The authors have no conflicts of interest to declare.

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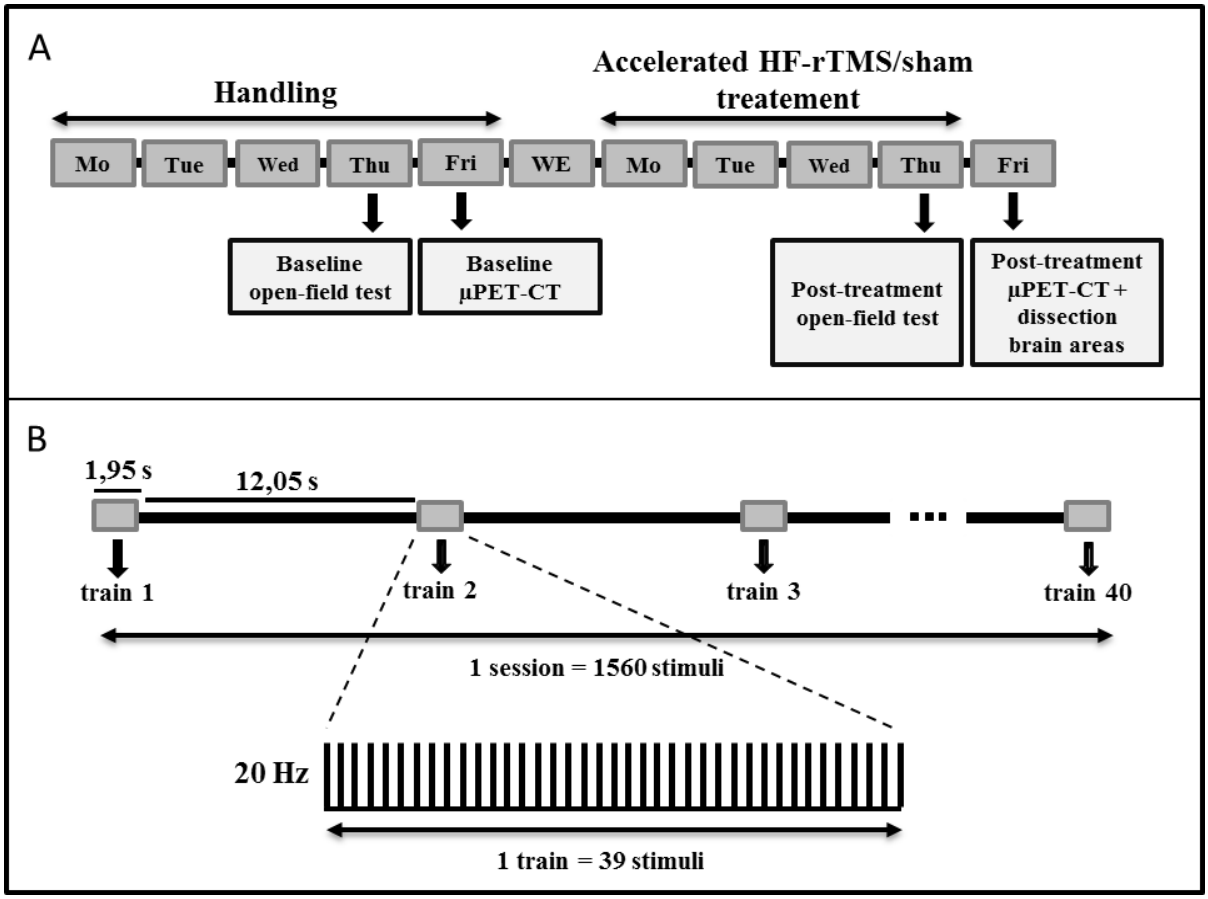

Fig. 1. Experimental protocol and stimulation protocol and parameters used in this study. (A) The 5 days during handling period is followed by accelerated HF-rTMS/sham stimulation during 4 consecutive days. The baseline open-field test was performed after the handling sessions on the 4th day of the handling period (= 4 days before the stimulation period). The baseline µPET-CT imaging was executed immediately after the handling sessions (= 3 days before the stimulation period). On the 4th day of the stimulation period the post-treatment open-field test was performed after the last HF-rTMS session, followed by the post-treatment µPET-CT imaging executed on the next day. (B) The stimulation protocol used in this study as mimicked from the clinical protocol. (C) The stimulation parameters used in this study as mimicked from the clinical protocol.

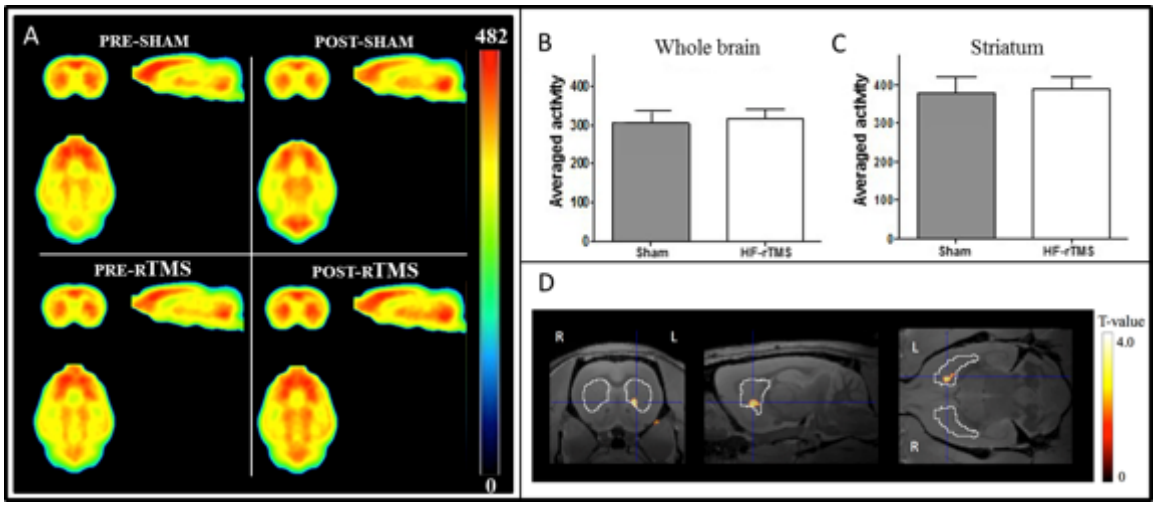

Fig. 2. Metabolic effects following accelerated HF-rTMS (n = 5 per group). (A) Averaged standardized uptake values images with plasma glucose correction for all groups. The color bar on the right displays the range of standardized uptake values. Quantified average glucose corrected standardized uptake value for the whole brain (B) and the striatum (C) after sham/accelerated HF-rTMS treatment. All data are expressed as means + standard error of the mean.

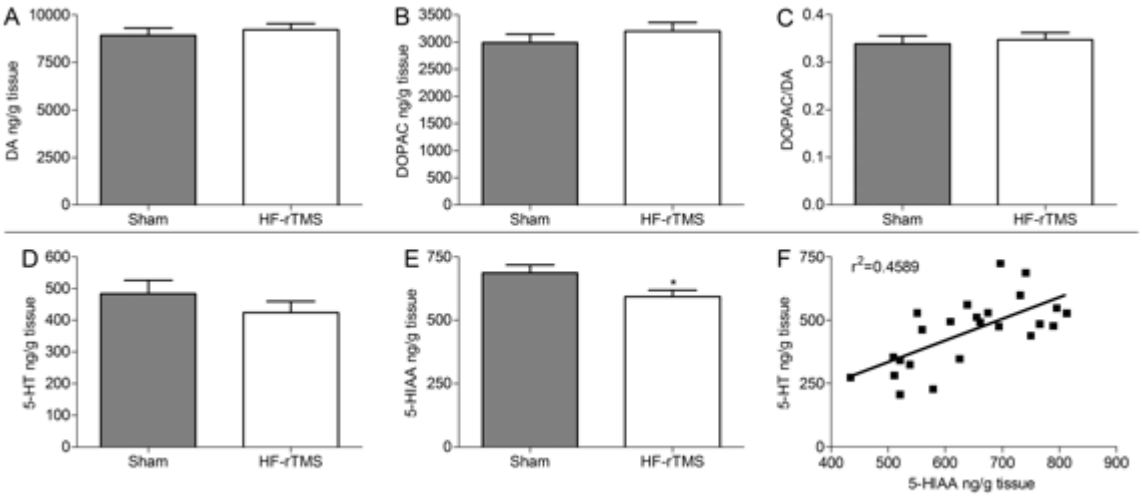

Fig. 3. Neurochemical effects following accelerated HF-rTMS (n = 6 per group). The total striatal DA (A) and DOPAC (B) content as well as the striatal DOPAC/DA ratio (C) in sham and HF-rTMS-treated animals. The total striatal 5-HT (D) and 5-HIAA (E) content in sham

and HF-rTMS-treated animals. (F) Linear regression analysis performed with the striatal 5-HT and 5-HIAA data of the two experimental groups revealed significant correlation between the 5-HT and 5-HIAA levels ($F_{1,22}$ = 18.66, $p \leq 0.001$). All data are expressed as means + standard error of the mean. Significantly different from the corresponding sham (two-tailed unpaired t-test): * $p \leq 0.05$.

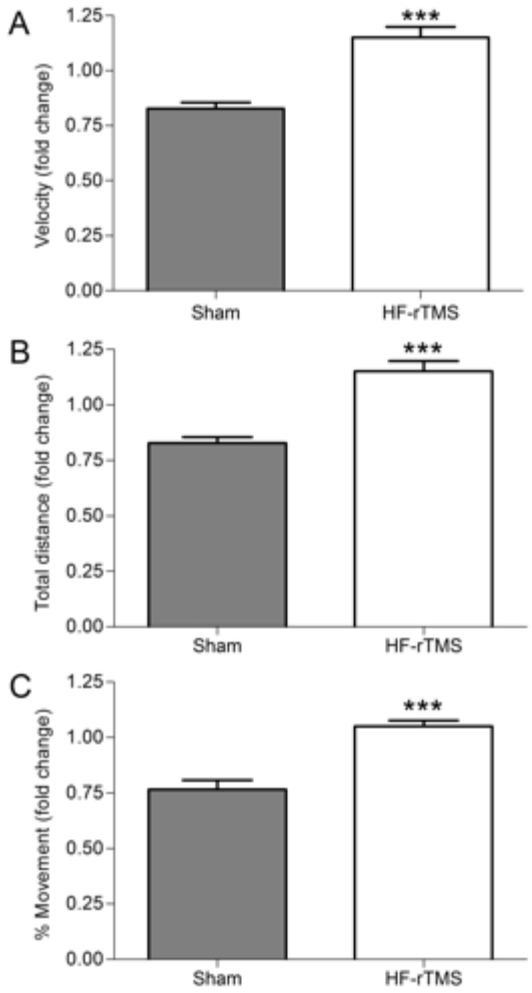

Fig. 4. Behavioral effects following accelerated HF-rTMS (n = 6 per group). The effect of accelerated HF-rTMS on the different parameters of the open-field test: velocity (A), total distance (B) and % movement (C). The different parameters are expressed as the ratio of the open-field test results obtained post-sham/HF-rTMS and the open-field test results obtained pre-sham/HF-rTMS (fold change). All data are expressed as means + standard error of the

mean. Significantly different from the corresponding sham (two-tailed unpaired t-test): *** $p \leq 0.001$. The significant improvement of the spontaneous motor activity is confirmed by the one-sample t-test showing HF-rTMS ratios being significantly higher than 1 for the velocity ($t_5 = 3.249$, $p \leq 0.05$) and the total distance ($t_5 = 3.249$, $p \leq 0.05$).