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Research report

Electrical high frequency stimulation in the dorsal striatum: Effects on response learning and on GABA levels in rats

Anett Schumacher[a,*], Anne Pereira de Vasconcelos[b], Lucas Lecourtier[b], Andreas Moser[a], Jean-Christophe Cassel[b]

[a] Neurochemical Research Group, Department of Neurology, University of Lübeck, Ratzeburger Allee 160, D-23538 Lübeck, Germany
[b] LINC – UMR 7237, Université de Strasbourg–CNRS, 12 rue Goethe, F-67000 Strasbourg, France

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ABSTRACT

Electrical high frequency stimulation (HFS) has been used to treat various neurological and psychiatric diseases. The striatal area contributes to response learning and procedural memory. Therefore, we investigated the effect of striatal HFS application on procedural/declarative-like memory in rats. All rats were trained in a flooded *Double-H* maze for three days (4 trials/day) to swim to an escape platform hidden at a constant location. The starting place was the same for all trials. After each training session, HFS of the left dorsal striatum was performed over 4 h in alternating 20 min periods (during rest time, 10 a.m. to 3 p.m.). Nineteen hours after the last HFS and right after a probe trial assessing the rats' strategy (procedural vs. declarative-like memory-based choice), animals were sacrificed and the dorsal striatum was quickly removed. Neurotransmitter levels were measured by HPLC. Stimulated rats did not differ from sham-operated and control rats in acquisition performance, but exhibited altered behavior during the probe trial (procedural memory responses being less frequent than in controls). In stimulated rats, GABA levels were significantly increased in the dorsal striatum on both sides. We suggest that HFS of the dorsal striatum does not alter learning behavior in rats but influences the strategy by which the rats solve the task. Given that the HFS-induced increase of GABA levels was found 19 h after stimulation, it can be assumed that HFS has consequences lasting for several hours and which are functionally significant at a behavioral level, at least under our stimulation (frequency, timing, location, side and strength of stimulation) and testing conditions.

1. Introduction

Electrical high frequency stimulation (HFS) of several brain regions has been used as an effective treatment for different neurological diseases, including those characterized by movement disorders (e.g., Refs. [1,2]). Indeed, HFS of the subthalamic nucleus (STN) has positive effects on Parkinson's disease (PD) symptoms [3,4]. When applied to the thalamus, HFS is also used successfully to treat epilepsy and Tourette's syndrome [5,6]. However, the mechanisms by which HFS ameliorates the symptoms of these diseases are still a large area of experimental and clinical research. In several studies it could be shown that HFS of STN altered various neurotransmitter systems. For example, Temel et al. [7] demonstrated that bilateral HFS of STN had a decreasing effect on the firing rate of dorsal raphe 5-hydroxytryptamine (5-HT) neurons in rats and elicited a depressive-like behavior that could be linked to the weakening of 5-HT functions. Using an in vivo enzyme-linked glutamate biosensor in rats, Lee et al. [8] showed that unilateral HFS of the left STN increased glutamate levels. Despite different results on the mechanism of HFS there is evidence that GABAergic neurons play an essential role in the efficacy of HFS [9]. It has been demonstrated that HFS of slices from the striatum increased extracellular GABA levels in vitro [10]. Further studies supported the assumption that effects on the GABAergic system contributed to the efficacy of HFS [11,12]. In freely moving rats, as assessed by microdialysis, HFS of the striatum enhanced local GABA outflow without affecting other neurotransmitter systems [13]. Whereas increased GABA levels in the striatum might have consequences on motor functions, it could also interfere with memory functions in which the striatum is also involved.

Indeed, especially the dorsal striatum (DS) is known to contribute to response learning and procedural memory [14]. Procedural memory derives from e.g., repetitive motor responses or sequential movements resulting in behavioral automatisms, which can be modeled in laboratory rodents [15–18]. Considering that GABA and glutamate exert opposite synaptic effects (inhibitory and excitatory, respectively), and that intrastriatal infusions of glutamate were shown to strengthen procedural learning in a maze

* Corresponding author. Tel.: +49 0451 5002486, fax: +49 0451 5002936.
E-mail address: anett.schumacher@neuro.uni-luebeck.de (A. Schumacher).

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task [19], it is conceivable that HFS effects on GABA may contribute to alter such memory functions. Interestingly, in monkeys, intrastriatal infusions of a GABA agonist (muscimol) impaired learning of sequential movements [20]. In rats, intrastriatal infusions of GABA antagonists (e.g., bicuculine or picrotoxin) disrupted memory consolidation [21,22]. These findings indicate that changes in the GABAergic tonus of the DS could affect memory processes, perhaps particularly consolidation, without pointing to a particular prediction as to whether the effects could be beneficial or detrimental. To evaluate response learning/procedural memory in rats subjected to unilateral HFS of the DS, we used a water-escape task in a novel testing device called the *Double-H* maze [23]. Beside its simplicity, a main advantage of this task is the fact that procedural and declarative-like memory processes can be assessed in a within-subject design using a single probe trial (for detail, see Ref. [23]). Besides the behavioral level, we also focused on possible changes of neurotransmitter systems following HFS. Since previous studies could already demonstrate short-term effects (during stimulation and within 10 min post-stimulation) of HFS on neurotransmitter systems [11–13], our current approach focused on more long-term effects of HFS.

2. Materials and methods

2.1. Subjects

In this study 45 male Wistar rats (Centre d'Elevage René Janvier, Le Genest-St-Isle, France) were used. They weighted 300 ± 50 g at their arrival at the laboratory. All rats were housed individually in transparent Makrolon cages (42 cm × 26 cm × 15 cm) with food and water *ad libitum* in an enclosed room with constant temperature (22 ± 1 °C) and humidity (55 ± 5%) under a 12–12 h light–dark cycle (light on at 8:00 a.m.). To study the effects of electrical HFS in the DS, rats were randomly allocated to one of three groups: operated and stimulated rats (STIM, $n = 15$), operated but non-stimulated rats (SHAM, $n = 15$) and non-operated, control rats (CONT, $n = 15$). Experimental protocols and animal care were in compliance with the national (council directive 87-848, 19 October 1987, Ministère de l'Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animale; authorization no. 67-215 for J.-C.C. and no. 67-7 for APV) and international (directive 86-609, 24 November 1986, European Community) laws and policies.

2.2. Surgery

Surgery was performed after five days of habituation on the laboratory conditions and three days of handling (10 min/day). Each rat was anesthetized with an intraperitoneal injection of a mixture of ketamine (82.5 mg/kg) and xylazine (11.0 mg/kg), and body temperature was kept constant (37 °C) during surgery with an insulating blanket. All rats were secured in a stereotaxic frame (Stoelting Co., Wood Dale, Illinois) and a small scalp incision was made to visualize the cranial landmarks Lambda and Bregma. Just above the left DS a guide cannula (CMA/11 Guide Cannula, CMA Microdialysis, Solna, Sweden) was placed and fixed to the skull with dental cement. Stereotaxic coordinates for the guide cannula were AP −0.26 mm and ML +3.0 mm relative to bregma, and DV −1.0 mm from dural surface in accordance with Paxinos and Watson [24]. Animals were allowed to recover from surgery for at least 10 days before starting behavioral training and the first out of three electrical stimulation sessions.

2.3. Behavioral test

2.3.1. Double-H maze

The Double-H maze is a new behavioral test to analyze memory in rats; it was conceived by J.-C.C. The testing device and procedures, as well as data validating the approach, are described in the work of Pol Bodetto et al. [23]. One of the advantages of the Double-H compared to established memory tests like the Morris Water Maze or the Object Recognition Test is the possibility to distinguish performance relying upon declarative-like spatial memory processes from that based on procedural memory, and to evaluate, within a single probe trial, whether rats can or cannot switch from the procedural to the other memory system. The Double-H maze is a 160 cm × 160 cm water maze apparatus consisting of six maze arms – North (N), North-East (NE), North-West (NW), South (S), South-East (SE), South-West (SW) – which are connected with one central maze arm, as illustrated in Fig. 1. The maze was made of transparent Plexiglas; water had a constant temperature of 21 ± 1 °C and was made opaque by addition of powdered milk. The escape platform was hidden, 1 cm below the water surface, at the end of the NE arm during training; it was removed for the probe trial. The testing room contained different extra-maze cues (e.g., chair, table, computer, water heater, several patterns stuck on the walls, etc.) in order to ease orientation and navigation. A webcam connected to a computer was used to record each rat's behavior and to enable off-line analyses of the swim tracks. The rats were moved to the experimental room just after their arrival at the laboratory and were kept therein until the end of the experiment. Behavioral testing was performed over four consecutive days, always between 10:00 a.m. and 11:00 a.m. The Double-H testing protocol comprised two phases: acquisition and probe trial. During acquisition, the rat had to learn the fixed way from the start position to the escape platform (in egocentric terms: turn right and then turn left; in allocentric terms: go to NE). All rats were given 4 trials a day during 3 consecutive days. For each trial, the N arm was blocked and the rat was placed at the extremity of the S arm, from where it was given a maximum of 60 s to reach the escape platform immersed in the NE arm. When the rat found the platform, it was given 10 s before being removed from the maze. If a rat failed to find the platform within 60 s, it was placed at the entry of S arm by the experimenter, but was now gently guided to the platform and left there for 10 s. The probe trial was performed on day 4. The platform was removed, the N arm was opened, and the S arm was blocked. The rat was placed at the end of the SW arm and was given a single trial lasting 20 s. The start of the probe trial was different from that used for acquisition trials as done by Packard and McGaugh in their cross maze task [17]. According to recent experiments in the Double-H maze [23], when rats are released with a 180° switch between the acquisition and probe trials (which had been the case if they had been released from the N), most of them immediately switch to a declarative-like memory-based strategy. This is why we have chosen to release the rats from the SW arm, a protocol yielding a high proportion of initial procedural memory-based responses in the Pol Bodetto et al.'s study [23]. The probe trial was used to check if the rat had developed a procedure (turn right, then left; if so, its first choice led it into the N arm, and thus an incorrect one) or a declarative-like representation of the platform location (if so, its first choice led it into the appropriate target arm, namely that where the platform had been immersed during training, i.e., NE). Data analyses considered several variables. For acquisition performance, we analyzed the latencies to the platform, as well as the number of errors made. An error was counted each time a rat entered a wrong arm (i.e., any other arm than NE). For the probe trial, we considered the latency to the first entry into the NE arm, the number of errors (as defined for acquisition), the time spent in the target arm, but also the number of rats in each group having first chosen the N arm (a choice based on procedural memory), having swum directly to the NE (a choice based on declarative-like memory) or having swum to the NW. The time spent in the target arm was also compared to chance (i.e., the theoretical time derived from the relative surface of the target arm [14.5%] under the hypothesis that the rats have swum for 20 s without any focused research pattern: 14.5% of 20 s = 2.9 s).

2.4. Electrical deep brain stimulation

On each of the training days, right after the fourth trial, the rat was placed in a box (Plexiglas, 30 cm × 30 cm) with food and water *ad libitum* and the left DS was stimulated after insertion of a concentric bipolar electrode (CBCPG30, FHC Inc., Bowdoin) through the guiding tube into a depth of DV −5.0 mm from dural surface in accordance with Paxinos and Watson [24]. Unilateral stimulation was used to remain as close as possible to the protocol of previous work [13] and to preserve the possibility of a within-subject control by comparing the stimulated with the non stimulated side. Due to the fact that the Double-H maze is a water test and the rat had to swim a fixed way to the platform, stimulation of the DS could not be performed during training to avoid electric shocks. We have chosen to perform it after training given that the data available in rats suggest a striatal GABAergic contribution to memory consolidation processes [21,22]. The stimulation electrode consisting of Platinum/Iridium had a cathode in the centre (diameter 75 μm) and was concentrically surrounded by the anode (diameter 250 μm). Stimulation was performed over 4 h in alternating 20 min periods while the rat was sitting quite in its cage or sleeping (10:00 a.m. to 3:00 p.m.). Stimulation parameters were as follows: monopolar positive rectangular pulses of 124 Hz (Isostim A320D stimulator, WPI, Berlin, Germany), duration of 60 μs and constant current of 0.5 mA. These parameters were chosen on the basis of previous experiments [13]. It is noteworthy that the onset or offset of the stimulation did not alter the behavioral state of the rat. Given that no studies exist about the exact characteristics of the stimulation area for the electrode used in this work, we were not able to define the area of the DS directly affected by the HFS. It can only be assumed, by referring to the work of Butson et al. [25], that the signal strength is limited by the VTA (volume of tissue activated).

2.5. Neurochemical analysis

2.5.1. High performance liquid chromatography (HPLC)

After the probe trial (day 4 of behavioral testing), and thus 19 h after the last electrical stimulation, ten randomly selected rats in each group were euthanized by exposure to carbon dioxide and immediately decapitated. The brain was quickly removed, separated in its two hemispheres, and left as well as right DS were dissected out and stored at −80 °C until HPLC measurement. After pre-column derivatization with *o*-phthaldialdehyde and sodium sulphite for 10 min, GABA and glutamate values were measured using HPLC with electrochemical detection [10]. The HPLC-system consisted of a C18 column (Eurospher 100, 5 μm, column size 250 mm × 4 mm) and a precolumn (30 mm × 4 mm). The isocratic mobile phase 1 (0.1 M sodium phosphate buffer, pH 4.5, containing 0.5 mM EDTA and 25% methanol)