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Original Research

Effect of Deep Brain Stimulation in Rats Selectively Bred for Reduced Prepulse Inhibition

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ABSTRACT

Background: Sensorimotor gating, measured as prepulse inhibition (PPI) of the acoustic startle reaction (ASR), is disturbed in certain neuropsychiatric disorders, such as schizophrenia, obsessive compulsive disorder, and Tourette's syndrome (TS). Deep brain stimulation (DBS) of the centromedian–parafascicular complex (CM-Pf), globus pallidus internus (in rats the entopeduncular nucleus – EPN), and the ventral striatum (in rats the nucleus accumbens – NAC) has been used for treatment in TS.
Objective: We tested whether DBS of these regions would alleviate breeding-induced low PPI in rats.
Methods: Rats with breeding-induced *low* and *high* PPI were bilaterally implanted with electrodes in the CM-Pf, the EPN, or the NAC. After two weeks, they were stimulated or sham stimulated for epochs of 6 days (in the EPN with a current of 20% below the individual threshold for stimulation-induced side effects, in the NAC or CM-Pf with 100 μA and 150 μA). On the 6th day the rats were tested for PPI of ASR.
Results: Stimulation in the CM-Pf with 150 μA significantly alleviated PPI, while NAC stimulation was less effective. In PPI *low* rats electrode implantation in the EPN already improved PPI, while subsequent stimulation had no additional effect. Startle reaction of PPI *low* rats was not affected by stimulation of either region.
Conclusion: The CM-Pf and the EPN are important for the modulation of sensorimotor gating in rats with breeding-induced low PPI. These rats may therefore be useful to further investigate the pathophysiological mechanisms of deficient sensorimotor gating and also mechanisms of action of DBS in these circumstances.

Introduction

Some features of certain neuropsychiatric disorders are in part explained by a dysfunction of sensorimotor gating processes, which can be operationalized by prepulse inhibition (PPI) of the acoustic startle response (ASR), i.e., the reduction of the startle response to an intense acoustic stimulus when this stimulus is shortly preceded by a weaker non-startling stimulus [1]. Reduced PPI has been demonstrated in patients with schizophrenia, Tourette's syndrome (TS) and obsessive compulsive disorder [2–5]. PPI is regulated by a neuronal circuitry that includes portions of the basal ganglia (BG) also implicated in the pathophysiology of these disorders [1,6,7]. Experimentally induced PPI-deficits in rodents have therefore been suggested to be useful to investigate the pathophysiological mechanisms of neuro-modulative treatment in these disorders [5].

High frequency deep brain stimulation (DBS) is currently under investigation for treatment of pharmacoresistant TS patients. So far, various regions have been targeted, but the centromedian parafascicular nucleus (CM-Pf) and the globus pallidus internus (GPi) have been used in the majority of cases to treat tics, while the ventral striatum is regarded useful to treat comorbid obsessive compulsive symptoms [8–12].

Selective breeding for *high* and *low* PPI-levels in Wistar rats leads to segregation of two rat lines with significantly different PPI [13]. Modulation of neuronal activity by lesions or DBS of the entopeduncular nucleus (EPN, the equivalent to the human GPi) improves the breeding-induced and the apomorphine-induced PPI deficit [14]. Additionally, lesions [15] and DBS [16] of the EPN prevent apomorphine-induced deficient sensorimotor gating. We investigated whether DBS of three different targets of the basal

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ganglia circuitry - the EPN, the CM-Pf and the ventral striatum/nucleus accumbens (NAC) would improve PPI in rats selectively bred for reduced PPI.

Materials and methods

Animals

For this study 64 male rats selectively bred for *high* and *low* PPI were used. The animals were kept in groups of 3–4 in standard Macrolon Type IV S cages (Techniplast, Hohenpeissenberg, Germany) under controlled ambient conditions (22 °C, 12 h light/dark cycle, lights on at 07:00 am.). After surgery, each rat was kept in a standard Macrolon Type III cage. They received tap water ad libitum and 12 g rat-chow/animal/day. The experimental protocols used in this study were in accordance with the national and international ethical guidelines, conducted in compliance with the German Animal Welfare Act and approved by the local authorities, which includes approval by an animal ethics committee.

Selective breeding

The parental generation consisted of male and female outbred adult Hannover-strain Wistar rats from the breeder Harlan-Winkelmann, Borchen, Germany. The TSE Startle Response System™ (Bad Homburg, Germany) was used to test the rats for their PPI. The animals were placed into wire-mesh cages (27 × 9 × 10 cm) on piezoelectric accelerometers in sound attenuated chambers with two loudspeakers mounted at a distance of 4 cm from the test cage. For assessment of PPI, animals received four different trial types (10 trials each) in a random order: (1) no-stimulus, (2) pulse-alone: 20 ms white noise pulse of 105 dB SPL, (3) prepulse-alone: 20 ms 80 dB tone pulse of 10 kHz, and (4) prepulse-pulse trials: prepulse of 80 dB followed by a 105 dB pulse with the onset–onset interval between the prepulse and the pulse set at 100 ms. PPI was calculated as a percentage score and the two females and males with the highest and the lowest level of PPI were chosen for selective breeding of two lines with either *high* or *low* level of PPI. The offspring of these rats (F1–F17 generation) was tested for PPI and again selected for subsequent breeding. After the 11th generation the SR-LAB startle response system (San Diego Instruments, USA) was used with 20 ms 68 dB white noise as prepulse. For the present study, rats from the 13th to 17th generation were used.

Experimental design

For each of the three nuclei tested, animals with *high* and *low* PPI were bilaterally implanted with electrodes: CM-Pf – $n = 21$; EPN – $n = 30$ and NAC – $n = 13$. The operation was followed by two weeks of postoperative recovery. Each animal implanted in either the CM-Pf or the NAC was tested three times – one epoch with 0 μA (sham-stimulation), one with 100 μA and one with 150 μA stimulation in a randomized crossover design to account for any order effects (such as persistent effects of DBS in the group that received it first, or changing effects of the surgery over time). The animals implanted in EPN, received only one type of stimulation (20% under the individual motor reaction threshold) and sham stimulation, also in a random order. There were two days between each stimulation epoch with no stimulation and no cable connection. The stimulation or sham-stimulation epochs were always six days long with two days rest between them. On the 6th day of each epoch, the rats were tested for PPI of ASR under ongoing stimulation (Fig. 1). For statistical analysis we used a two way ANOVA for repeated measure.

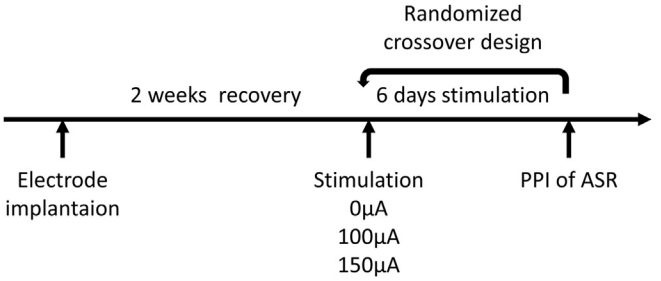

Figure 1. Shows the experimental design for surgery, stimulation and behavioral testing of the PPI *high* and PPI *low* rats.

Surgery

The rats were intraperitoneally anaesthetized with chloral hydrate (360 mg/kg) and fixed into a stereotactic frame. Additionally, the surgical site was infiltrated with a local anesthetic (prilocaine hydrochloride 2%). After incision and defining of bregma, two burr holes were drilled bilaterally above the target and bipolar electrodes were implanted into one of the targets using the following coordinates (in mm) relative to bregma: CM-Pf – AP: −4.1, ML: ±1.4, DV: −6.4; EPN – anteroposterior (AP): −2.5, mediolateral (ML): ±2.8, dorsoventral (DV): −8.2; NAC – AP: +1.7, ML: ±1.6, DV: −7.2; The tooth bar was set to −3.3 mm.

Bipolar electrodes were made of two parallel platinum-iridium (90:10) wires insulated with Teflon ($d = 0.0055''$ with insulation and $d = 0.003''$ uninsulated; Science-Products GmbH, Hofheim, Germany), placed in a 0.55 × 17 mm stainless steel tube cut from a syringe needle 24G. At the contact end, both wires were uninsulated leaving 500 μm long bare surface with about 250 μm intercontact distance. The other end was welded to a socket. Four screws (1 × 2 mm) were wound to the skull as reinforcement. The electrodes and the socket were fixed to the skull with dental acrylic cement (Paladur®, Heraus Kulzer GmbH, Hanau, Germany). Antibiotics were applied for five days postoperatively (0.1 mL i.m. 5% Baytril® (Enrofloxacin), Bayer HealthCare, Leverkusen, Germany).

Deep brain stimulation

After two weeks of postoperative recovery, continuous electrical stimulation was applied via a cable that was bite-protected by a metal spring-like shield. One side of the cable was connected to the socket on the rat's skull and the other to a stimulation device (Multichannel Systems STG2008, Software: Mc-Stimulus II). A swivel (Plastics one Inc, Roanoke, VA, USA) in the stimulation line allowed free movement of the rat without twisting the cable. For electrical stimulation 130 Hz symmetric, biphasic, rectangular waves with duration of 160 μs and polarization change after 80 μs were used. For sham-stimulation the cable was attached to the swivel but no stimulation was applied. During the continuous stimulation, each rat was single housed in a standard Macrolon Type III cage. A 2 × 25 cm slot in the cage lid allowed free movement of the animal with the cable attached.

For EPN stimulation, the threshold for stimulation-induced behavioral side effects (such as clonic movement of the contralateral forepaw or rotation to the contralateral side) was determined for each electrode separately in a stair-step procedure (40–500 μA, 20 μA steps). Thereafter, continuous stimulation started with a current of 20% below the individual threshold. If rats seemed disturbed by the stimulation, i.e. did not start grooming and/or did not respond to feeding within 30 min after onset of stimulation, the current was further reduced by 20%. Thereafter the rats were