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Lesions of the rat entopeduncular nucleus further deteriorate N-methyl-D-aspartate receptor antagonist-induced deficient prepulse inhibition

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ABSTRACT

Lesions of the rat entopeduncular nucleus (EPN), the equivalent to the human globus pallidus internus (GPi), have been shown to improve deficient prepulse inhibition (PPI) induced by the dopamine agonist apomorphine. We here tested the effect of EPN lesions on the PPI-disruptive effect of the non-competitive NMDA receptor antagonist dizocilpine in rats. Neurotoxic bilateral lesions of the EPN were induced by ibotenic acid (4 μg in 0.4 μl). Rats were tested for PPI and locomotor activity after systemic injection of dizocilpine (vehicle and 0.15 mg/kg). Bilateral EPN lesions further deteriorated the PPI deficit induced by dizocilpine, while locomotion was not affected. This work indicates that the EPN is an important brain region within the neuronal circuit responsible for NMDA receptor antagonist-induced PPI deficits.

1. Introduction

Deficient sensorimotor gating, measured as prepulse inhibition (PPI) of the acoustic startle response (ASR), reflects the inability of an organism to filter out irrelevant sensory information, a phenomenon seen in certain neuropsychiatric disorders, such as schizophrenia and Tourette's syndrome [30]. Dopamine (DA) receptor agonists and glutamate N-methyl-D-aspartate (NMDA) receptor antagonists disrupt PPI in rodents and induce psychotomimetic symptoms in humans [5,11,12]. Evaluation of the neuronal circuitries involved may lead to a better understanding of the neuronal mechanisms underlying psychotic conditions and may help to identify candidate targets for therapeutic interventions [18,31].

Prepulse inhibition is regulated by sequential and parallel processes in the cortico-pallido-striato-thalamic system (CPST) [30,31]. The nucleus accumbens and ventral pallidum play important roles in the modulation of PPI with regard to DA function, while not affecting PPI deficits induced by NMDA receptor antagonists [20,21]. Instead, lesions of the prefrontal cortex prevented NMDA receptor antagonist-induced PPI deficits, while not affecting a deficit induced by DA agonists [27].

The rat entopeduncular nucleus (EPN), i.e., the equivalent to the human globus pallidus internus (GPi), receives GABAergic inhibitory input from the striatum, nucleus accumbens and ventral pallidum. Additionally, it receives glutamatergic excitatory input from the subthalamic nucleus and probably from the frontal cortex [3,4,10,23,29]. We recently showed that lesions of the EPN ameliorate deficient PPI and hyperlocomotion induced by the DA receptor agonist apomorphine [22] and also deficient PPI induced by selective breeding [13,28]. We here tested the effect of bilateral excitotoxic lesions of the EPN on the PPI-disruptive and locomotor enhancing effect of the non-competitive NMDA receptor antagonist dizocilpine.

2. Materials and methods

Male Sprague-Dawley rats ($n = 23$, Charles River Laboratories, 180–200 g) were used in this study [22]. Rats were kept in standard Macrolon Type IV S (Techniplast, Hohenpeissenberg, Germany) cages in groups of 3–4 under controlled ambient conditions (22 °C, 14 h light/10 h dark cycles with lights on at 7 a.m.). They received water and rat chow ad libitum. Experiments were done in accordance with the internationally accepted principles in the care and use of experimental animals.

Rats were anaesthetized with chloral hydrate (360 mg/kg, i.p.) and placed into a stereotactic frame, tooth bar set at −3.3 mm. After preparation of bregma an injection canula (5 μl syringe, SGE Analytical Science Pty. Ltd., Victoria, Australia) was inserted through a burr hole at the following coordinates relative to bregma according to the atlas of Paxinos and Watson [24]: −2.5 mm rostro-caudal, ±2.8 mm lateral, and 8.6 mm ventral. Ibotenate (Sigma–Aldrich,

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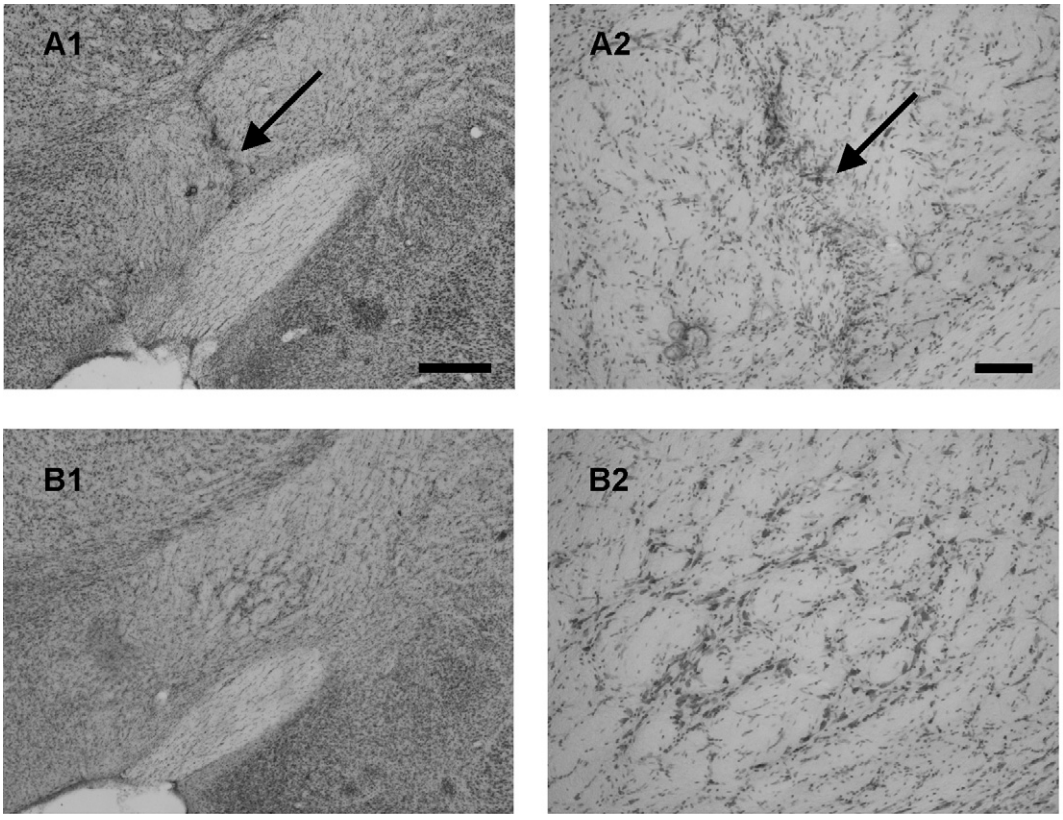

Fig. 1. Ibotenate-induced loss of neurons in the EPN (A1) with minute gliosis along the tract of the injection cannula (indicated by a black arrow). No tissue damage was seen after vehicle injection (sham-lesion; B1). A2/B2 are higher magnifications of the EPN area in A1/B1. Calibration bar A1/B1 500 μm, A2/B2 100 μm.

Steinheim, Germany, 10 μg/μl, 0.4 μl, pH 6.5) for lesions and phosphate buffered saline (PBS, 0.4 μl) for sham-lesions were injected into the EPN with an injection velocity of 0.1 μl/min. To prevent severe motor deficits seen after simultaneous bilateral lesioning of the EPN [28], the EPN was first lesioned on one side, and the other side was lesioned one week later. For behavioral testing we used 14 lesioned and 9 sham-lesioned rats. After one week of recovery all rats were tested for locomotor activity and for PPI of ASR after challenge with dizocilpine or vehicle.

2.1. PPI of ASR

A startle response system (SR-LAB, San Diego Instruments, San Diego, USA) was used for testing. Each box is equipped with a motion sensitive platform with a pizoelectronic accelerometer underneath and a loudspeaker mounted above the platform. For testing the rat was placed in a small tube made of Plexiglas that was connected to the platform. Rats were first acclimatized for 5 min followed by 5 startle stimuli (20 ms white noise of 105 dB sound pressure level (SPL)). Thereafter, 4 trials were presented that all lasted 500 ms with a continuous white noise background set at 60 dB SPL: (1) startle stimulus only, (2) prepulse stimulus only (20 ms white noise of 72 dB SPL), (3) startle stimulus proceeded by a prepulse of 64 dB, 68 dB or 72 dB SPL, with 80 ms between startle and prepulse, and (4) control trial of white noise background. All trials were presented 10 times each in a randomized order with an intertrial interval randomized within a range of 20–30 s. Tests were performed after subcutaneous injection with the NMDA-receptor antagonist dizocilpine (0.15 mg/kg) and for control saline in a randomized order with at least two days in between. The ASR magnitudes of each different trial were averaged and PPI was calculated as the percent decrease of the ASR in pulse-alone trials compared to an ASR in prepulse-pulse trials ($100 \times$ (pulse-alone trial − prepulse-pulse trial)/pulse-alone trial). Further, no-stimulus trials served as an indication for spontaneous activity, and prepulse-only trials for orienting response to the prepulse.

2.2. Locomotor activity

A black square plastic box (60 cm × 60 cm) with 30 cm high walls was used. Rat's activity was recorded by a camera installed above the box and analyzed by using a video tracking system (TopScan 1.0, Clever Sys. Inc., Reston, VA, USA). Rats were first accustomed to the open field for 30 min. On the following days rats were twice recorded for 30 min after either injection of dizocilpine (0.07 mg/kg) or vehicle (saline) in a randomized order with at least two days in between.

2.3. Statistics

For statistical analysis all measures were analyzed by two-way ANOVA with lesion as independent and drug injection as dependent factor, followed by post hoc Tukey's test for pair-wise comparison in case of significance. All tests were performed two-sided and $p < 0.05$ was considered statistically significant.

2.4. Histology

After behavioral testing the rats were overdosed with anaesthetic, transcardially perfused with 4% formaldehyde–PBS and the brains were sectioned. The extent of the lesions (loss of neurons and gliosis) was determined in Nissl-stained sections using a Zeiss light-microscope.

3. Results

Five rats were excluded due to sufficient lesion only unilaterally, no response to the startle stimulus, or unexpected death, which