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Research report

Overshadowing in conditioned taste aversion or in conditioned emotional response after neonatal ventral hippocampal lesions in rats

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ABSTRACT

The neonatal hippocampus lesion thought to model schizophrenia should show the same modifications in behavioural tests as other models, especially pharmacological models, namely decreased latent inhibition, blocking and overshadowing. The present study is set out to evaluate overshadowing in order to complement our previous studies, which had tested latent inhibition. "Overshadowing" refers to the decreased conditioning that occurs when the to-be-conditioned stimulus is combined with another stimulus at the conditioning stage. We used the same two Pavlovian conditioning paradigms as in our previous works, namely conditioned taste aversion (CTA) and conditioned emotional response (CER). A sweet taste overshadowed a salty conditioned stimulus, and a tone overshadowed a flashing light. Totally different stimuli were used to counter possible sensory biases. The protocols were validated with two groups of Sprague Dawley rats. The same two protocols were then applied to a cohort of rats whose ventral hippocampus had been destroyed when they were 7 days old. Only rats with extended ventral hippocampus lesions were included. The overall effect of Pavlovian conditioning was attenuated, significantly so in the conditioned emotional response paradigm, but overshadowing appeared not to be modified in either the conditioned emotional response or the conditioned taste aversion paradigm.

1. Introduction

For schizophrenic patients, weak predictors of events can be abnormally salient [12]. Salience, which determines the memory strength of learning cues, was manipulated experimentally by a variety of means, for example by rendering the cue irrelevant or redundant (as in latent inhibition and blocking), or by pairing it with a cue that is physically more salient (overshadowing) [31]. A low dose of amphetamine which enhances psychotic manifestations in schizophrenic patients was shown to reduce both latent inhibition, blocking and overshadowing [20,24–26,29,31]. Studying these effects in lower animals helped to model what occurs in the disease because it could be considered representative of how the salience of events is mismanaged in schizophrenia and it was suggested that the brain mesolimbic dopaminergic system is involved [27,31,34].

Other lower animal models have been proposed. For example, Wood et al. demonstrated that excitotoxic damage of the ventral hippocampus 7 days after birth resulted in abnormal behaviour and hypersensitivity to dopaminergic drugs that are especially visible after puberty [35]. It has been suggested that this may be because of maturation of the mesolimbic dopaminergic system is disturbed because the latter was deprived of the glutamatergic axons coming from the hippocampus [6,9,10]. In other words, the developmental consequence of the lesion is more relevant to this model than the absence of the ventral hippocampus in adult animals. Initial observations were complemented by a large number of subsequent studies which gave rise to what has become the standard "Lipska–Weinberger" model of schizophrenia [15]. However, few studies have addressed this model directly in terms of learning and memory impairments with protocols, such as those mentioned above, where the salience of the stimuli and management of the mesolimbic dopaminergic system are crucial. Having previously tested latent inhibition with two different conditioning paradigms, namely conditioned taste aversion (CTA) and conditioned emotional response (CER), we decided to document this aspect further with overshadowing, using the same general conditioning methods [1].

Hypersensitivity to dopamine is the residual consequence of the reorganisation of the nucleus accumbens that occurs after neonatal hippocampus lesions. This heightened sensitivity to dopamine

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should strengthen the salience attributed to target stimuli in learning procedures or, conversely, attribute salience to stimuli that are usually ignored—the so-called "over-selectivity effect" [8,27,31]. So, there could be two opposite modifications of overshadowing in lesioned rats, reinforced or reduced overshadowing. In the present experiments, overshadowing was tested both in respect of conditioned taste aversion and conditioned emotional response according to the "Lipska–Weinberger" model, with minimal modification of the conditioning methods previously used to test latent inhibition in our laboratory [1]. The corresponding protocols had to be slightly modified however. They had to fit with the methods that were reported to elicit overshadowing in conditioned taste aversion and conditioned emotional response in other such studies [16,22–26]. Owing to the difficulty of obtaining appropriate lesions in rat pups we used the same cohort of rats for both protocols. Since this meant there might be a possible interference between the two experiments we decided to test the protocols first on two independent groups of naïve non-operated Sprague Dawley rats to determine the reference level of conditioning and overshadowing in these protocols [21,32]. Once this verification was complete, a number of Sprague Dawley rats whose ventral hippocampus had been lesioned at the age of 7 days, and the extent of whose lesions was assessed by magnetic resonance imaging (MRI) before the tests started, were submitted to both protocols and compared with sham lesioned rats. The rats had to be selected prior to the experiments because it is difficult to conduct conditioning experiments with a large number of rats simultaneously, which is what we would have had to do had we tested all the rats. Having their ventral hippocampus impaired at the time of testing should not be of concern as it was shown that rats whose hippocampus had been damaged in adulthood still showed overshadowing [7,11].

2. Materials and methods

2.1. Animals

Subjects were 48-male-Sprague Dawley rats, purchased as adults (3 month old, 350 g), and 48 rats of the same strain selected among a set of rats born in the animal care facility at the Faculty of Medicine of Louis Pasteur University in Strasbourg, and submitted to the surgical procedure explained below. Adult rats and dams had been purchased from Janvier rat farm, France. They were housed on a 14/10 h light/dark cycle (lights on at 7 am) with food and water available *ad libitum*, except during the experiments as indicated in the description of the corresponding procedures. Prior to the beginning of the behavioural tests, they were handled for about 3 min/day for 3 days.

2.2. Ethical considerations

All procedures comply with the European Communities Council Directive of November 24, 1986 (86/609/EEC) and the National Council Directive of October 19, 1987 (87848, "Ministère de l'Agriculture et de la Forêt, Service Vétérinaire de la Santé de la Protection Animale").

2.3. Surgery

Dams arrived at the animal housing facility of the Faculty of Medicine of Strasbourg 13 days after mating. After delivery, litters of 5 or 6 males were constituted. When 7 days old, weight being 15–20 g, 112 rats were submitted to the surgical procedure (72 lesioned and 40 sham operated). These rats were scheduled for several series of experiments, the present ones being the first one using these rats. Anesthetized by isoflurane inhalation (5% for induction and 3% for maintenance), they were placed over a heating pad. Either 0.3 μl of ibotenic acid (Sigma, France, 10 μg/μl, pH adjusted to 7.4 by addition of NaOH, 10 M) or artificial cerebrospinal fluid was bilaterally infused over a 3 min period by a 1-μl Hamilton syringe driven by a microinjection device (type 5000, David Kopf Instruments). The basic commercially available microinjection device was driven by a constant speed electrical motor. The tip of the injection needle was aimed at the ventral hippocampus (antero-posterior -3.0 mm, medio-lateral ± 3.5 mm and ventro-dorsal -5.0 mm relative to bregma). After infusion, the needle was left immobile during 3 min, then withdrawn. The skin was sutured and rats were allowed to recover on a heating pad before being returned to their dam (within 10 min after the end of the surgery). Three weeks after surgery, pups were weaned and housed two per cage in a temperature controlled room on a 12/12 h light/dark cycle (lights on at 8 am).

2.4. MRI imaging method

Two-month-old lesioned rats were anesthetized for 15–20 min by groups of seven, using an injection of diazepam, 5 mg/ml (0.4 ml i.p.) followed 10 min later by an injection of ketamine chlorhydrate (50 mg/ml, 0.6 ml i.p.). This MRI procedure was not painful but we needed rats to remain motionless during it. An MRI was performed at 2 T (BRUKER S200). It is a standard MRI device constructed for MRI imaging on humans. An image was obtained for seven rats together, each one placed in a vinyl polychlorure tube (diameter: 6 cm), seven tubes being glued together as a large honeycomb. Two small tubes filled with water were glued on each side of one of the rat containing tubes which served to identify the position of the rats. The tubes were placed in an MRI head coil. Rat's brains were scanned from the frontal cortex to the cerebellum as consecutive 2-mm-thick coronal slices with a T_2-weighted, spin-echo fast imaging method sequence. A fast spin echo (FSE) with FOV 18 cm, 2 mm slice thickness, and four excitations was used to image the seven rats simultaneously.

2.5. Selection of the subjects and organisation of the tests

Each protocol was first tested with a set of 3-month-old naïve (300–400 g), and not operated rats in order to get the level of overshadowing in non-operated independent groups of rats. Then a group of lesioned and sham lesioned rats was submitted successively to the two protocols at the same age. Only bilaterally lesioned and sham operated rats were used. Lesioned rats were selected by inspection of the lesions on MRI pictures. This was done before the beginning of the experiments in order to avoid post hoc debates about the experimental groups, i.e., after having got information on the individual behavioural performances. Only rats with large bilateral lesion were included as in our previous studies [1]. A selection of 48 rats (24 lesioned and 24 sham) was used successively in a conditioned taste aversion and a conditioned emotional response paradigm, each one with an overshadowing condition.

2.6. Histological verifications

At the end of the experiment, 13 of the 24 lesioned rats were given a fatal overdose of pentobarbital (the brains of the other rats have been provided to partner laboratories for biochemical studies). The brains that were processed for histological verifications were collected as follows. As soon as the spontaneous respiration of the rats stopped they were given an intra-cardiac perfusion of saline then 4% paraformaldehyde. Brains were removed and stored in this fixative. Two days before being cut, the brains were transferred into 30% saccharose. Coronal sections (40 μm thickness), one every 0.5 mm, were cut using a freezing microtome, then mounted on gelatin-coated glass slides and stained with cresyl violet to allow visual inspection and drawing of the lesioned surfaces.

2.7. Behavioural tests

2.7.1. Overshadowing in conditioned taste aversion

Apparatus. Testing was carried out in eight transparent plastic cages (base: 25 cm × 25 cm; height: 35 cm), with a sawdust floor and no ceiling. There were two holes on one of the walls (diameter: 2 cm; distance from one another: 9 cm; height above grid 2.5 cm), into which graduated glass tubes (Richter tubes) were inserted to provide the rats free access to drinking solutions. *Procedure*. One factor of the experiment was the conditioning protocols used, namely conditioning with saline alone (NaCl–water) or with saline and sucrose (NaCl–sucrose), the same conditioning method being used in both groups. All groups comprised 12 rats. The other factor (absent in the initial validation experiment) was the surgical treatment (lesion/sham). Rats were placed on a water-deprivation schedule for 4 days prior to the experiments. The duration of access to water was progressively reduced and the rats' weight was monitored daily to ensure how they were adapting to this schedule. Water was available in their home cage for 2 h at the end of the afternoon, after the tests. Rats were tested in groups of eight. Each rat was tested in the same test cage throughout the experiments. The procedure comprised the following four stages: (1) *Shaping (5 days)*. Rats were trained to drink tap water from a single tube inserted into one of the holes in the test cage in a counterbalanced pseudorandom sequence. All beverages were given to rats at room temperature. The drinking duration was gradually reduced. From 5 min on day 1, it was reduced to 3 min 45 s on day 2, 2 min 30 s on day 3 and, finally, 1 min 15 s on days 4 and 5. (2) *Conditioning (1 day)*. All rats were first allowed to drink 5 ml of 1% saline. This was immediately followed either by 5 ml of sucrose (3.5%) in NaCl–sucrose groups or by 5 ml of water in the control groups. Rats drank the two beverages in less than 15 min. Within 5 min of the end of this drinking session, rats were injected i.p. with LiCl (63.5 mg/kg, dissolved in water and injected at a volume of 15 ml/kg). (3) *Reshaping (2 days)*. Rats had access to tap water for 5 min from two tubes in the test cage. (4) *Testing (2 days)*. Rats were placed in the test cage for 15 min, with access to a tube containing 1% saline, on two consecutive days. Volumes of fluid drunk by each rat were measured to the