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Research report

Automated dissection of permanent effects of hippocampal or prefrontal lesions on performance at spatial, working memory and circadian timing tasks of C57BL/6 mice in IntelliCage

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ABSTRACT

To evaluate permanent effects of hippocampal and prefrontal cortex lesion on spatial tasks, lesioned and sham-operated female C57BL/6 mice were exposed to a series of conditioning schemes in IntelliCages housing 8–10 transponder-tagged mice from each treatment group. Sequential testing started at 51–172 days after bilateral lesions and lasted for 154 and 218 days in two batches of mice, respectively. Spontaneous undisturbed behavioral patterns clearly separated the three groups, hippocampals being characterized by more erratic hyperactivity, and strongly impaired circadian synchronization ability. Hippocampal lesions led to deficits in spatial passive avoidance, as well as in spatial reference and working memory tasks. Impairment was minimal in rewarded preference/reversal schemes, but prominent if behavioral responses required precise circadian timing or included punishment of wrong spatial choices. No differences between sham-operated and prefrontally lesioned subjects in conditioning success were discernible. These results corroborate the view that hippocampal dysfunction spares simple spatial learning tasks but impairs the ability to cope with conflicting task-inherent spatial, temporal or emotional cues. Methodologically, the results show that automated testing and data analysis of socially kept mice is a powerful, efficient and animal-friendly tool for dissecting complex features and behavioral profiles of hippocampal dysfunction characterizing many transgenic or pharmacological mouse models.

1. Introduction

Behavioral correlates of hippocampal malfunction induced by genetic or experimental lesions have become a gold standard in documenting cognitive changes in laboratory rodents. Deficits in spatial learning tasks such as the water maze are taken as a proxy of hippocampal malfunction, and considered to reflect impaired or missing spatial reference memory as evidenced by prolonged escape latencies and reduced searching over the position of removed escape platforms (probe trials). Likewise, the hippocampus is thought to be critical for short-term spatial working memory as observed in radial maze tasks requiring win-shift strategies to visit rewarded locations that may vary according to a first choice and shifting spatial cues. However, the history of testing hippocampal lesion effects has revealed two important findings. For one, lesion experiments have shown that spatial reference memory can recover to some extent [1,2]. In addition, many rodent studies found changes in anxiety [3], increased behavioral stereotypies [4], increased locomotor activity [5,6], impaired species-typical behavior [3,7,8], diminished sociability [9–11] and include, unsurprisingly, hormonal or physiological changes as well [12]. Thus, lesions generate a complex hippocampal syndrome whose components and their interaction are still poorly understood. This situation hampers a comprehensive understanding of hippocampal function(s), specifically after prolonged recovery times or transgenic impairment of hippocampal functions as often observed in constitutive knockout mice.

Likewise, lesioning or inactivating the rodent prefrontal cortex faces

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the same problem, as this structure interacts strongly with the hippocampus [13], being embedded in a larger forebrain-to-midbrain network governing cognitive and executive processes. However, unlike cytotoxic hippocampal lesions in mice [7], technically similar lesions of the mouse prefrontal cortex entailed hardly any cognitive deficits in a variety of tasks but appeared anxiolytic and revealed a deficit in food burrowing [14].

Obviously, dissecting a hippocampal or prefrontal lesion syndrome requires extensive behavioral testing which is mostly beyond the capacities of behavioral laboratories working with traditional apparatus. In order to establish reference behavioral profiles characterizing hippocampal and other brain area malfunction efficiently, we conducted several IntelliCage studies with mice subjected to hippocampal and other brain area lesions (e.g., pre-frontal cortex, striatum) to assess spontaneous behavior [15], and performance in spatial and non-spatial learning tasks including both reward and punishment [2].

IntelliCage by NewBehavior (TSE Systems GmbH, Germany) is a programmable automated behavioral test system designed for high-throughput behavioral analysis of transponder-tagged mice living in social groups in a large standardized home-cage wherein each mouse can visit 4 operant conditioning chambers. It thus permits to combine analysis of spatial behavior with operant conditioning, thereby covering a large cognitive spectrum. To analyze the large data volumes typically produced by such systems we developed novel software (FlowR, available at XBehavior GmbH, Switzerland) for automated statistical data analysis. This enabled us to analyze efficiently the experimental raw data by extracting common spontaneous behavioral patterns as well as conditioning success in various forms of learning paradigms, following both hippocampal and prefrontal lesions in grouped mice

Using female C57BL/6 mice and long postoperative recovery times to identify lasting deficits, we report here that bilateral large hippocampal lesions impaired only marginally the ability to solve simple rewarded spatial preference and reversal tasks. However, lesioned mice showed massive problems when the task required precise circadian timing, putative working memory, or when wrong choices of locations were punished by airpuffs. Lesions of the medial prefrontal cortex did not entail deficits in any cognitive task applied, notwithstanding apparent separation of spontaneous activity phenotypes of all three groups.

2. Material & methods

All behavioral tests were carried out at the Institute of Anatomy, University of Zürich, under standard laboratory conditions with inverted light/dark cycle. Adult female C57BL/6J mice were lesioned in the hippocampal region (HIPP), the prefrontal cortex (PFC), or sham-operated (CTR), and subsequently exposed to a series of fully automated conditioning schemes in IntelliCage (NewBehavior/TSE-Systems GmbH, Bad Homburg, Germany). Two batches of mice (Les1, Les2) were tested in a sequence of protocols using 3 and 4 cages, respectively, each containing mice from each of the three lesion groups. All experiments were carried out in accordance with the guidelines of the European Communities Council Directive of 24 November 1986 (86/609/EEC) and were approved by the veterinary office of the Canton of Zürich.

2.1. Animals and lesions

Sixty-five female C57BL/6JRccHsd mice at 70 days of age were obtained from Harlan Laboratories (Füllinsdorf, Switzerland). Animals were kept in groups of 8–10 mice under standard laboratory conditions (temperature 21 ± 1 °C, humidity 50 ± 5%, inverse 12/12 h light/dark cycle) in an animal facility at the institute. The animals were housed in groups of 8–11 mice in standard Type III cages (Tecniplast, Buguggiate, Italy) with water and food (Kliba Nafag 3430; Provimi Kliba AG, Kaiseraugst, Switzerland) ad lib. For the first batch, Les1, 30 mice were randomly assigned to be lesioned either in prefrontal cortex (PFC, n = 10), or hippocampal regions (HIPP, n = 11), or sham-operated (CTR, n = 9). At 192 days of age, 51–116 days after surgical intervention, Les1 mice were transferred to the IntelliCages. For the second batch, Les2, 35 mice were lesioned (PFC, n = 12; HIPP, n = 10; CTR, n = 13) and transferred to IntelliCages at 250 days of age, 150–172 days after lesioning.

2.2. Surgical procedure and brain preparation

Preoperatively, each subject was injected (i.p.) with the anesthetic and analgesic Avertin (tribromethanol, 20 ml/kg), the anticonvulsive chlordiazepoxide hydrochloride (10 mg/kg in 10 ml saline) and the anti-inflammatory Rimadyl (carprofen, Pfizer Animal Health, 0.1 ml/kg in 10 ml saline). Following the premedication, mice were placed in a stereotaxic frame (David Kopf Instruments, Tujuga, California). The incisor bar was set to −1 mm to ensure a level head. After incision of the scalp the skull was exposed. The coordinates for the lesion sites were calculated using a reference brain atlas [16]. The interaural line was used as reference for all coordinates, except for the vertical ones.

After verification of the lesion site coordinates using three pilot surgeries, we chose four injection sites for the bilateral hippocampus lesion and three injection sites for the bilateral lesion of the prefrontal cortex on each side of the sagittal suture (Table 1). By means of a 5 µl syringe equipped with a 34 gauge stainless steel needle (Hamilton, Bonaduz, Switzerland) an N-methyl-D-aspartic acid solution in phosphate buffered saline (NMDA, 10 mg/kg)) was injected on each site for the respective lesions (Table 1). After subcutaneous injection of the transponders (Datamars SA, Bedano, Switzerland) through the scalp incision, the scalp was sutured. Control mice were sham operated, after application of the same pre-operative drugs as the lesioned groups. The sham operation consisted in a scalp incision, subcutaneous injection of the transponders and suture of the skin.

All operated mice (lesion and sham) were placed under a heating lamp (temperature controlled at 30 °C ± 2 °C) for recovery. Postoperatively all mice were single housed and regularly screened for seizures and later for locomotor deficits. Conscience was regained after three to four hours post-op. During four days following the surgery all mice had the possibility to drink water with an addition of 10% glucose to sustain recovery.

Perfusion and histology was conducted after experiments, when mice were injected (i.p.) with pentobarbital (100 mg/kg) and transcardially perfused with paraformaldehyde (PFA) 4%, their brains extracted and postfixed for four hours in PFA 4%. The brains were cryoprotected with 30% sucrose to avoid freezing artefacts until sinking of the brains. Freezing of the brains was obtained by CO_2 application. A sliding microtome (Jung CM 300) was used for cutting (10 series, 40 µm coronar slices). Every 10th series was stained with toluidin-blue. Subsequent lesion scoring was done using a light microscope (Leitz, dialux 20 EB). In the hippocampus as well as in prefrontal cortex most

Table 1
Coordinates and injected volumes (µl) for the brain lesions as depicted in Fig. 2. AP: distance (mm) from interaural line; L: from sagittal suture; V: from skull surface at bregma level. Overjet (mm) refers to the lowering of the needle past the V coordinate. The latter procedure ensures a diffusion of the cytotoxic agent into ventral parts of the targeted structure.

Site	AP(+)	L	V(−)	Overjet	Volume
HPP1	2.1	1.2	1.9	0	0.1
HPP2	1.5	1.7	1.9	0	0.15
HPP3	1.0	2.2	2.0	0.3	0.1
HPP4	0.7	2.8	4.0	0.2	0.2
PFC1	6.3	0.5	2.0	0.5	0.1
PFC2	5.5	0.5	2.0	0.5	0.1
PFC3	4.7	0.5	1.5	0	0.1