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Loss of hippocampal function impairs pattern separation on a mouse touch-screen operant paradigm

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ABSTRACT

The hippocampus is heavily involved in the learning and memory processes necessary to successfully encode environmental stimuli and representations over time. Impairment of hippocampal function is associated with numerous neuropsychiatric diseases and can lead to detriments in the quality of life. In order to take full advantage of preclinical models of these disorders, there is a need for the development of more refined measures of clinically relevant hippocampal behaviors. While arena-based navigation tasks have provided fundamental information regarding the role of the hippocampus in spatial memory, the development of automated operant variants have had mixed results. Recently, an automated touch-screen paradigm has been shown to be highly sensitive to hippocampal function in the rat and eliminated mediating strategies that arose in previous tasks. Here we show that mice with lesions encompassing the entire ventral portion of the dorsal hippocampus are impaired on pattern separation behavior using a delayed nonmatching-to-location (TUNL) adapted for mice. Lesioned mice readily acquired the task at control rates when separations were maximal and delay periods were short while decreasing separations significantly impaired lesion mice. However, in contrast to previously reported results in the rat, consistently increasing delays did not significantly impair performance in the lesion group. Presentation of a variable delay within a session significantly impaired performance in lesion mice across delay periods. The current results demonstrate the utility of a touch-screen paradigm for measuring hippocampal-dependent pattern separation in the mouse and establish the paradigm as an important platform for future studies in disease models.

1. Introduction

There is a wealth of data demonstrating the involvement of the hippocampus in learning and memory processes (Deadwyler, Bunn, & Hampson, 1996; Bannerman et al., 1999; Burgess, Maguire, & O'Keefe, 2002). The ability to successfully encode and discriminate distinct environmental stimuli is essential for survival and the hippocampus has been shown to be essential for these processes across species (Scoville & Milner, 1957; Dunnett, Wareham, & Torres, 1990; Gilbert, Kesner, & Lee, 2001; Sloan, Dobrossy, & Dunnett, 2006). Loss of hippocampal function has been associated with neuropsychiatric disease such as Alzheimer's disease, schizophrenia and neurodevelopmental insults and can have major impacts on quality of life (Braak, Braak, & Bohl, 1993; Daenen, Wolterink, Gerrits, & Van Ree, 2002; Brady, Allan, & Caldwell, 2012). The development of more refined behavioral endpoints in order to increase the translational potential of data generated in animal models has become an increasing focus in biomedical research. Traditionally, studies examining insults and disease models targeting the hippocampus have relied primarily on arena-based tasks such as the radial arm and Morris water mazes. These tasks have provided important information regarding the role of the hippocampus in spatial location memory, but also have disadvantages (Xavier, Oliveira-Filho, & Santos, 1999). While automated tracking and analysis can decrease experimenter demands and potential bias in these tasks, they still require a high level of motor response in the animals. Further, tasks utilizing escape from aversive environments can cause undue stress in subjects which may lead to confounded results. With the increasing use of techniques to examine and control neuronal activity, such as *in vivo* electrophysiology and optogenetic stimulation/inhibition, there is also an increasing need for assays that allow for easy integration with these systems.

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In an attempt to address these issues, operant tasks have been developed that require both spatial and delay dependent memory. These paradigms, categorized as matching and nonmatching-to-position tasks, require an animal to respond (typically via lever press) in either a novel or familiar spatial location for reward while ignoring a non-rewarded lever. Studies examining the effects of hippocampal lesion on delay nonmatching-to-position (DNMTP) paradigms have had mixed results. While several studies have reliably shown that loss of hippocampal function impairs DNMTP (Dunnett et al., 1990; Aggleton, Keith, Rawlins, Hunt, & Sahgal, 1992; Hampson, Jarrard, & Deadwyler, 1999), others suggest that these behaviors may not require intact hippocampal functioning (Sloan, Good, & Dunnett, 2006). It has been suggested that these conflicting results may stem from the nature of the tasks. Due to the limited number of response locations, animals can develop mediating behaviors (positioning themselves on the side where the correct choice will appear) that allow the animals to subvert the need to recall the information after a delay (Herremans, Hijzen, Welborn, Olivier, & Slangen, 1996; Chudasama & Muir, 1997; Talpos, McTighe, Dias, Saksida, & Bussey, 2010).

Recently, Talpos and colleagues developed an operant task designed to exclude mediating behaviors and increase dependency on hippocampal function (Talpos et al., 2010). This task, trial unique nonmatching-to-location (TUNL), is similar to the DNMTP task, but with several crucial differences. First, it utilizes a touch-screen in order to provide an array of possible locations for visual stimuli, causing a wide variety of patterns. This circumvents an animal's ability to use mediating behaviors to "cheat" during a delay period and allows for the ability to test spatial memory rigorously in a pattern specific manner by varying the distance between the two stimuli causing gradients of impairment. Secondly, by using visual stimuli and direct response (touch) to the stimuli, it more closely models clinically relevant measures of memory currently used, such as the Cambridge Neuropsychological Test Automated Battery (CANTAB). Importantly, it was demonstrated that loss of hippocampal function via targeted lesion impaired TUNL performance in a separation and delay specific manner in rats (Talpos et al., 2010).

Given the increasing reliance on the mouse as a preclinical model of multiple neuropsychiatric and developmental disorders that can alter hippocampal function, we wished to examine the effects of hippocampal loss on pattern separation behavior using the touch-screen TUNL paradigm in mice. We found that mice with loss of hippocampal function can learn the TUNL task when task demands were sufficiently low. Additionally, we show that excitotoxic lesions of the hippocampus impair the ability to perform the TUNL task when decreased separation or variable delay conditions make the cognitive demands high. Together, these results demonstrate the involvement of the mouse hippocampus in delay-dependent memory and pattern-separation learning, as well as the utility of the touch-screen TUNL task for screening these behaviors.

2. Materials and methods

2.1. Subjects

Male C57BL/6J mice ($n = 18$ at beginning of pre-training) were used in this study (Jackson Labs). Mice were housed in groupings of 2 per cage in a temperature and humidity-controlled vivarium under a reverse 12 h light/dark cycle (lights off 0800 h) and tested during the dark phase. Mice were aged 6 weeks at the onset of behavioral testing. All experimental procedures were performed in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the University of New Mexico Health Sciences Center Institutional Animal Care and Use Committee.

2.2. Operant apparatus

All operant behavior was conducted in a chamber measuring 21.6 × 17.8 × 12.7 cm (model # ENV-307W, Med Associates, St. Albans, VT, USA) housed within a sound- and light-attenuating box (Med Associates) as previously described (Marquardt, Saha, Mishina, Young, & Brigman, 2014; Marquardt, Sigdel, Caldwell, & Brigman, 2014). The standard grid floor of the chamber was covered with a solid acrylic plate to facilitate ambulation. A pellet dispenser delivering 14 mg dustless pellets (#F05684, BioServ, Frenchtown, NJ, USA) into a magazine, a house-light, tone generator and an ultra-sensitive lever was located at one end of the chamber. At the opposite end of the chamber there was a touch-sensitive screen (Conclusive Solutions, Sawbridgeworth, UK) covered by a black acrylic aperture plate allowing 2 rows of 5 touch areas measuring 2.5 × 2.5 cm separated by 0.6 cm and located at a height of 1.6 cm from the floor of the chamber. Stimulus presentation in the response windows and touches were controlled and recorded by the K-Limbic Software Package (Conclusive Solutions, Sawbridgeworth, UK).

2.3. Pre-training

Mice were first slowly reduced and then maintained at 85% free-feeding body weight. Prior to testing, mice were acclimated to the 14 mg dustless pellet food reward (Bioserv, Flemington, NJ) by provision of ~10 pellets/mouse in the home cage for 3–5 days. After becoming acclimated to the reward pellets mice were then habituated to the operant chamber and eating out of the pellet magazine by being placed in the chamber for 30 min with 10 pellets available. Mice retrieving 10 pellets within 30 min were moved to a pre-training regimen. First, mice were able to obtain reward by pressing a lever within the chamber. Mice pressing and collecting 30 rewards in under 30 min were moved to touch training. In touch training, a lever press led to the presentation of a white square stimulus in 1 of the 10 response windows (spatially pseudorandomized). The stimulus remained on the screen until a response was made. Touches in the blank response window had no response. Criterion for touch training was touching, retrieving and eating 30 pellets within 30 min.

2.4. Excitotoxic lesions of the hippocampus

After 1 week of feeding to facilitate recovery, mice were assigned to lesion or sham groups via matched-pair random assignment. Mice were anesthetized with isoflurane and fixed in a stereotaxic apparatus (1900 Stereotaxic Alignment System, David Kopf Instruments, Tujunga, CA) as previously described (Brigman et al., 2013). A 33-gauge infusion cannula (Plastics One, Roanoke, VA) attached with polyurethane tubing to a Hamilton syringe (Hamilton, Reno NV) was directed at 6 sites bilaterally targeting the hippocampus (−1.50, −1.80 and −2.25 mm AP, ±1.00, ±1.40 and ±1.75 mm ML, −2.00, −2.00 and −2.25 mm DV to Bregma). 0.2 μL *N*-methyl-D-aspartate (12.5 mg/mL, Sigma–Aldrich, St. Louis, MO) or saline vehicle was infused over 5 min using a pump (GenieTouch, Kent Scientific, Torrington, CT), with the cannula left in place for an additional 2.5 min to allow full diffusion. On completion of the last infusion, mice were sutured, given .05 mL Diazepam (.5 mg/mL), with an additional .025 ml as needed, to control seizures and returned to their home cages. Mice were given 1 week of recovery before being returned to food restriction. Mice began TUNL testing approximately 2 weeks after completion of surgery.

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