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Single session contextual fear conditioning remains dependent on the hippocampus despite an increase in the number of context-shock pairings during learning

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ABSTRACT

We examined if the strength of contextual fear learning determines whether remote memories become independent of the hippocampus. Rats received 3 or 10 shocks in a single contextual fear conditioning session and then received sham or complete neurotoxic lesions of the hippocampus 7, 50, or 100 days later. Following recovery from surgery, the rats were returned to the conditioning context for a 5-min retention test. During this test, freezing, complete immobility except for breathing, was used as an index of memory. Regardless of the learning-to-surgery interval, the rats with hippocampal damage from the 3-shock condition showed little and significantly less freezing than their respective control group, suggesting profound flat graded retrograde amnesia. Similarly, each group of hippocampal-damaged rats from the 10-shock condition froze significantly less than their respective control group. However, the rats that received hippocampal damage 50 days after learning froze significantly more than the rats that received the damage 7 days after learning. The latter gradient to the retrograde amnesia did not increase with more time as the freezing was not as high in the most remote memory group (100 days). Combined, these findings suggest that a contextual fear memory acquired in a single session under stronger learning parameters remains dependent on the hippocampus.

1. Introduction

Extensive evidence suggests that damage to the hippocampus (HPC) causes retrograde amnesia – a loss of memory for information acquired before the onset of the damage (Kim & Fanselow, 1992; Lehmann, Lacanilao, & Sutherland, 2007; Martin, de Hoz, & Morris, 2005; Rempel-Clower, Zola, Squire, & Amaral, 1996; Scoville & Milner, 1957; Sutherland et al., 2001; Zola-Morgan & Squire, 1990). The retrograde amnesia may, in some instances, be temporally graded, meaning that long-term memories acquired soon before the onset of the HPC damage are lost, whereas long-term memories acquired long before are intact (Kim, Clark, & Thompson, 1995; Kim & Fanselow, 1992; Lesburgueres et al., 2011; Scoville & Milner, 1957; Zola-Morgan & Squire, 1990). Theoretical accounts for temporally graded retrograde amnesia following HPC damage suggest that long-term memories are initially dependent on the HPC, but that with time the memories are strengthened in neocortical structures and become independent of the HPC – a process called long-term systems consolidation (Frankland & Bontempi, 2005; McClelland, McNaughton, & O'Reilly, 1995; Nadel & Hardt, 2010; Nadel & Moscovitch, 1997; Squire, Stark, & Clark, 2004; Winocur, Moscovitch, & Bontempi, 2010).

Patients with HPC damage may display temporally graded retrograde amnesia for memories that are termed declarative or episodic (Rempel-Clower et al., 1996; Rosenbaum et al., 2000). Rodents with HPC damage may also display temporally graded retrograde amnesia in several tasks (Broadbent, Gaskin, Squire, & Clark, 2010; Kim et al., 1995; Parsons & Otto, 2010; Quinn, Ma, Tinsley, Koch, & Fanselow, 2008; Tse et al., 2007; Winocur, McDonald, & Moscovitch, 2001) and commonly in contextual fear conditioning (Alvares Lde et al., 2012; Anagnostaras, Maren, & Fanselow, 1999; Goshen et al., 2011; Kim & Fanselow, 1992; Maren, Aharonov, & Fanselow, 1997; Restivo, Vetere, Bontempi, & Ammassari-Teule, 2009; Wang, Teixeira, Wheeler, & Frankland, 2009; Ward, Oler, & Markus, 1999; Winocur, Frankland, Sekeres, Fogel, & Moscovitch, 2009). This task involves learning and remembering that a specific context (i.e., configuration of static cues) is associated with an aversive stimulus such as foot-shock. When tested for retention, the rats or mice are returned to the conditioning context in which they previously received shock. They then normally display several species-specific defensive responses, including freezing or complete immobility, which is typically used

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as an index of memory. Kim and Fanselow (1992) were the first to report temporally graded retrograde amnesia for contextual fear conditioning following HPC damage. Specifically, they found that damage to the dorsal HPC impaired context fear that was acquired 1-day but not 28-days prior to the damage. They, thus, concluded that context fear memories initially depend on the HPC, but that over the course of a month they come to rely on other structures. This pattern of amnesia for contextual fear conditioning following HPC damage has not been consistently observed. In other studies flat gradients have been observed (Lehmann et al., 2007; Quinn et al., 2008; Sparks, Lehmann, Hernandez, & Sutherland, 2011; Sutherland, O'Brien, & Lehmann, 2008). Flat gradients imply that the HPC remains permanently involved in context fear memory. For instance, Lehmann et al. (2007) found that damage to the hippocampus, whether partial or complete, impaired contextual fear conditioning that was acquired as long as 180-days prior to the onset of the damage. Therefore, 6-months was insufficient to enable the memory to become independent of the HPC.

The mixed findings across studies suggests that there may be procedural differences during the conditioning that determine if a context fear memory becomes independent of the HPC over time. When contrasting the procedures used in the studies that find temporally graded retrograde amnesia for contextual fear conditioning following permanent HPC damage with the studies that do not, a distinction emerges: in the studies reporting temporally graded retrograde amnesia the number of context-shock pairings during conditioning is often substantially higher. Indeed, 10 or more context-shock pairings were given to the rats or mice in the majority of studies that found temporally graded retrograde amnesia (Anagnostaras et al., 1999; Kim & Fanselow, 1992; Winocur et al., 2009). Some studies involved fewer context-shock pairings (Wang et al., 2009; Maren et al., 1997; Restivo et al., 2009; Ward et al., 1999), but there are complicating features with each of these studies. First, Maren et al. (1997) claim that dorsal HPC damage caused temporally graded retrograde amnesia for contextual fear conditioning acquired with three context-shock pairings, but if one inspects the freezing data for the entire retention test, the HPC damaged rats with even the longest conditioning-to-surgery interval (i.e., the remote memory group) show a significant freezing deficit, consistent with flat graded retrograde amnesia. The claim that the memory became independent of the HPC in this group is derived from extracting the single minute over the 8-min test that shows peak freezing for each individual rat; only then does the performance of the lesion group approximate that of the control group. This is not conclusive evidence for a gradient. Second, Restivo et al. (2009) report a gradient with a conditioning procedure that involved five context-shock pairings, but the HPC damage causing retrograde amnesia was induced 1 h after learning. This is an unusually short learning-to-surgery interval for a study aiming at finding evidence of long-term systems consolidation. Inducing damage within 1 h of learning likely disrupted shorter-term, cellular consolidation processes outside the HPC *per se*. This consideration undermines the conclusion that the context fear memories became independent of the HPC because of a protracted interaction between the HPC and other networks (Rudy & Sutherland, 2008). Third, despite the fear conditioning only involving few context-shock pairings in the Ward et al. (1999) and Wang et al. (2009) studies, more than a single conditioning session were given and recent evidence suggests that repeated conditioning sessions make context fear memories more resistant to HPC damage (Lehmann & McNamara, 2011; Lehmann et al., 2009). Therefore, temporally graded retrograde amnesia for contextual fear conditioning following HPC damage is mostly found in studies that administered 10–15 context-shock pairings during conditioning. In contrast, the studies that find flat graded retrograde amnesia typically gave 2–5 context-shock pairings (Lehmann et al., 2007; Sparks et al., 2011; Sutherland et al., 2008), but see Quinn et al. (2008) who gave 10 shocks. Here we can even include the Maren et al. (1997) paper, described above, that reports retrograde amnesia for damage caused even 100-days after learning which involved 3 shocks. Hence, when considering all these studies, it is possible that long-term systems consolidation requires a very strong context fear memory which is more likely to be established when the conditioning involved many rather than few context-shock pairings.

In the current study, we investigated whether the number of shocks given during conditioning affects whether context fear memories become independent of the HPC over a protracted time period. Rats received, in a single conditioning session, three or 10 context-shock pairings. Rats then either received complete HPC damage seven, 50, or 100 days later. Given that only the 10-shock condition was more likely to undergo long-term systems consolidation, it was predicted that all groups with HPC damage should be impaired on the retention test with the exception of the 10-shock groups with the long learning-to surgery interval (50-days and 100-days).

2. Methods

2.1. Subjects

Eighty-one Male Long-Evans rats (Charles River, Quebec), of approximately 120 days of age at the beginning of the experiment, were housed in groups of two in standard laboratory cages, and were on a 12:12 light–dark cycle (lights on at 7:00). They were provided with ~40 g of rat chow daily food and water *ad libitum*. All procedures were approved by the Trent University Animal Care Committee, which follows the standards of the Canadian Council on Animal Care.

2.2. Apparatus

The fear conditioning chambers measured 30.5 × 26 × 26 cm and were made of 2 aluminium side walls, a Plexiglas rear wall, ceiling, and hinged door. The floor consisted of 18 stainless steel rods (2 mm diameter), spaced 1.5 cm apart centre to centre. The chambers were placed side by side on a table with the aluminium walls facing each other, so the rats could not see each other. The rest of the room included standard laboratory furniture. Shocks (0.75 mA; 2 s) were delivered through the stainless steel rods in the floor, which were connected to a shock generator and scrambler (Tech Serv, Beltsville, Maryland). The chambers were cleaned with 70% ethanol before and after each rat underwent conditioning and testing. All conditioning and testing sessions were video recorded using a webcam placed in front of the chambers and attached to a notebook computer.

2.3. Behavioural procedures and surgery

2.3.1. Conditioning

Rats were assigned to either the 3-shock or 10-shock group. Rats were transported individually to the apparatus and placed inside the fear conditioning chamber. The 3-shock group was placed in the chamber for 8 min, and received 3 foot-shocks at 1 min intervals with the first shock occurring 5 min after being placed in the chamber. The 10-shock group was placed in the chamber for 15 min, and received 10 foot-shocks at 1 min intervals with the first shock occurring 5 min after being placed in the chamber. Following conditioning rats were returned to their home cages.