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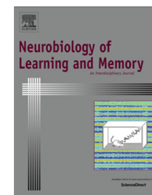

Consolidation of spatial memory in the rat: Findings using zeta-inhibitory peptide

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ABSTRACT

Whether or not spatial memories reorganize in the rodent brain is an unanswered question that carries the importance of whether the rodent provides a suitable animal model of human retrograde amnesia. The finding of equally impaired recent and remote spatial memory could reflect the continued importance of the hippocampus for spatial memory or a performance deficit (for example, hippocampal lesions may impair the rat's ability to use distal spatial cues to navigate to a specific point in space). In the current study, we tested recent and remote spatial memory in rats following hippocampal ZIP (zeta-pseudosubstrate inhibitory peptide) infusion to inhibit PKMzeta. Hippocampal ZIP infusion has previously been shown to impair spatial and nonspatial memory soon after learning, presumably by reversing late-phase long-term potentiation, allowing us to disrupt memory without damaging hippocampal tissue. We used a stereotaxic approach for infusing ZIP throughout the dorsal, intermediate, and ventral hippocampus following spatial memory training. Although rats showed intact memory retrieval on the standard Morris watermaze task and trace fear conditioning, rats infused with ZIP 24 h after training on the annular watermaze task exhibited impaired spatial memory compared to control rats (those infused with aCSF) and performed no different than chance. In contrast, rats infused with ZIP 1 month after training performed similar to control rats and both groups performed above chance. Additionally, the ability to form new memories after ZIP infusions remained intact. Thus, ZIP infusions into the hippocampus after learning impaired retrieval of recently formed spatial memories while sparing remote spatial memories.

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1. Introduction

A fundamental question in neuroscience concerns how long-term memory is organized and stored in the brain. It is widely accepted that the process by which new memories are gradually transformed from a labile state to a more permanent state involves time-dependent modifications in circuits that support memory storage and retrieval and is known as systems consolidation. Evidence of consolidation was derived from the observation that patient H.M. appeared to have retrograde amnesia for a few years immediately prior to his surgery, but normal-appearing memory for earlier years (Milner, Corkin, & Teuber, 1968; Scoville & Milner, 1957). More formal testing firmly established this phenomenon in H.M. (Marslen-Wilson & Teuber, 1975) and in other patients, including those with damage restricted to the hippocampus (Manns, Hopkins, & Squire, 2003). This form of retrograde memory impairment is known as temporally graded retrograde amnesia (TGRA) and is the primary evidence supporting systems consolidation.

Although a model of TGRA was successfully established in the experimental animal (Kim, Clark, & Thompson, 1995; Kim & Fanselow, 1992; Squire, Clark, & Knowlton, 2001; Winocur, 1990; Zola-Morgan & Squire, 1990), TGRA has not been observed in studies of spatial memory in rodents. Instead, recent and remote memory are impaired to the same extent following hippocampal damage in the rodent (Broadbent, Squire, & Clark, 2006; Clark, Broadbent, & Squire, 2005a, 2007; Clark & Martin, 2005; Martin, de Hoz, & Morris, 2005). These findings are suggestive that, unlike non-spatial memories, spatial memories are organized differently and permanently stored in the hippocampus. However, other

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possibilities are that the hippocampus may need to be functional in order to express a consolidated spatial memory (Clark, Broadbent, & Squire, 2005b; Clark et al., 2005a, 2007) or that hippocampal lesions could cause extra-hippocampal damage in the very structures that ultimately support consolidated long-term spatial memory. One way to evaluate these possibilities is through reversible disruption of the hippocampus, which has typically involved infusing pharmacological compounds through implanted cannulas (e.g., Broadbent et al., 2006; Holahan & Routtenberg, 2011; Kesner & Warthen, 2010). However, this method has three complicating limitations: (1) only a portion of the hippocampus can be disrupted due to cannula placement constraints, (2) the implantation of the cannula and volume of fluid necessary to inactivate a large enough area to impair memory causes substantial, irreversible mechanical damage, and (3) pharmacological agents need to be infused and active during the retention test in order to impair memory. Here we introduce a more powerful method to study spatial memory and systems consolidation in the rodent. We used stereotaxic infusions of ZIP (zeta-pseudosubstrate inhibitory peptide), a compound which has been shown to erase memory by reversing late-phase long-term potentiation (LTP) (Sacktor et al., 1993). ZIP is thought to work by specifically inhibiting PKMzeta (an atypical isoform of protein kinase C). Although there is controversy as to whether ZIP specifically inhibits PKMzeta or produces some nonspecific mechanism of action (for reviews of these issues see LeBlancq, McKinney, & Dickson, 2016; Tsokas et al., 2016), the important point for this study is that ZIP infusion can impair established memory. Critically, ZIP does not need to be infused through an infusion cannula, as previous studies have done (Pastalkova et al., 2006; Serrano et al., 2008; Shema, Sacktor, & Dudai, 2007). Given that ZIP infusion is effective even days after learning (Serrano et al., 2008), following training, ZIP could be infused stereotaxically throughout the hippocampus using a small 30-g syringe (which is substantially smaller than the infusion and guide cannula normally used in infusion experiments). Thus, we could avoid the substantial mechanical damage caused by implanting an infusion and guide cannula, disrupt the entire hippocampus in a single stereotaxic surgery, and, later, present a retention test to animals with a fully intact and functional hippocampus.

We trained rats on three spatial memory tasks: (Experiment 1) standard Morris watermaze, the benchmark task of spatial memory in the rodent, (Experiment 2) annular watermaze, a spatial recognition memory task without navigational demands, and (Experiment 3) trace fear conditioning, a hippocampus-dependent task which involves forming a memory for a tone-shock pairing as well as a memory for spatial context in which the shock occurred. Following training, rats received bilateral hippocampal infusions of ZIP or aCSF and were tested on each task after recovering from surgery. The characteristics of ZIP allow us to disrupt memory without damaging hippocampal tissue, thereby allowing the hippocampus to be functional during retrieval.

2. Experiment 1: Morris water maze

The Morris watermaze is the benchmark test for hippocampus-dependent spatial memory in the rodent. After rats received infusions of ZIP or aCSF into the hippocampus, we examined the amount of time rats spent in the quadrant where the platform had been located during training.

2.1. Methods

2.1.1. Subjects

All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of California, San Diego. Subjects were 28 male, Long-Evans rats weighing between 300 and 350 g at the beginning of the study. Rats were individually housed and maintained on a 12:12 h light:dark cycle. Food and water were freely available. Rats were randomly assigned to receive bilateral infusions of ZIP or aCSF 3–6 days post-training (ZIP n = 14; aCSF n = 14). One aCSF rat died before the retention test.

2.1.2. Apparatus

Testing was conducted in a pool of water (1.8 m diameter at the water level) that was rendered opaque by the addition of powdered milk. The testing room contained a number of constant, salient visual cues (posters, objects, and equipment). A video camera mounted on the ceiling directly above the pool was used in conjunction with a video tracking system (San Diego Instruments) to record the swim path of each rat. An Atlantis platform (12.7 cm diameter) was used which could be raised or lowered remotely (Spooner, Thomson, Hall, Morris, & Salter, 1994). When the platform was in the lowered position, the rat could neither detect the platform nor escape from the water. When the platform was in the raised position (1.5 cm below the surface of the water), it remained invisible to the rat but provided a means to escape the water. At the end of a probe trial, the platform was raised so that the rat could escape and be rewarded for searching in the correct location.

2.1.3. Acquisition

Rats began each of the 10 acquisition days with a reinforced probe trial followed by four standard training trials (with the same platform location for all trials). During the reinforced probe trial, rats were placed in the water facing the pool wall at one of four start points (counterbalanced across animals). The platform remained lowered for the first 60 s of the probe trial. The platform was then raised, and the rat had an additional 60 s to reach the platform before being guided to it by the experimenter. After escaping the water, the rat remained on the platform for 30 s. Performance on the probe trial was calculated by measuring, within the first 60 s, the percentage of time that a rat spent in the quadrant of the pool where the platform had been located during training (chance performance = 25%) and the percentage of time that a rat spent in a circular zone directly above the platform location (chance performance = 4%). During the remaining four standard training trials, the platform remained in its raised position to permit escape from the water. Rats were given a maximum of 2 min to find the platform before being guided to the platform by the experimenter. After escaping, the rats remained on the platform for 30 s before they were returned to their home cage. Following training, rats were divided into ZIP and aCSF infusion groups. Rats underwent surgery 3–6 days post-training.

2.1.4. Surgery

Anesthesia was maintained throughout surgery with isoflurane gas (0.8–2.0% isoflurane delivered in O_2 at 1 L/min). The rat was placed in a Kopf stereotaxic instrument, and the incisor bar was adjusted until Bregma was level with Lambda. ZIP or aCSF were infused stereotaxically throughout the hippocampus (bilateral) by local microinjections with a 10 μL Hamilton syringe mounted on a stereotaxic frame and held with a Kopf Microinjector (model 5000). Biotinylated ZIP (1 mg) was reconstituted in 100 μL of sterile water with a resulting stock solution concentration of 10 μM ZIP. 10 μL of the 10 μM ZIP was then diluted in 9.99 mL of aCSF to provide a solution with a concentration of 10 nM ZIP/μl aCSF (Serrano et al., 2008). The syringe needle was lowered to the target coordinate and left in place for 1 min before beginning the injection. A total of 4.8 μL of ZIP or aCSF was injected into 8 sites within each hippocampus. All coordinates are in millimeters anteroposte-

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