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Cholinergic deafferentation of the hippocampus causes non-temporally graded retrograde amnesia in an odor discrimination task

Jenny R. Köppen[a], Sarah L. Stuebing[a], Megan L. Sieg[a], Ashley A. Blackwell[a], Philip A. Blankenship[a], Joseph L. Cheatwood[b], Douglas G. Wallace[a,*]

[a] Psychology Department Northern Illinois University De Kalb, IL, USA
[b] Department of Anatomy Southern Illinois University School of Medicine, Carbondale, Illinois, USA

HIGHLIGHTS

- Infusion of 192-IgG-Saporin into the medial septum significantly reduced cholinergic input into the hippocampus.
- Removal of cholinergic projections from the medial septum produced retrograde amnesia, but there was no evidence for a temporal gradient.
- Infusion of 192-IgG-Saporin into the medial septum did not produce anterograde amnesia.
- Using the string pulling task, mnemonic function can be evaluated, and may allow for the development of therapeutic assessment for neurodegenerative disorders.

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ABSTRACT

Dementia of the Alzheimer's type (DAT) is a neurodegenerative disorder marked by loss of hippocampal cholinergic tone and significant memory impairments, specifically for memories acquired prior to disease onset. The nature of this relationship, however, remains debated. The current study used the string pulling task to evaluate the temporal effects of odor discrimination learning in animals with selective cholinergic lesions to determine the role of the septohippocampal cholinergic system in mnemonic function. Rats with 192-IgG-Saporin lesions to the medial septum had a higher number of correct responses in the reversal training when compared to sham rats, suggesting an inability to retrieve the previously learned discrimination; however, no temporal gradient was observed. Furthermore, there were no group differences when learning a novel odor discrimination, demonstrating the ability for all rats to form new memories. These results establish a role for the cholinergic medial septum projections in long-term memory retrieval. The current study provides a behavioral assessment technique to investigate factors that influence mnemonic deficits associated with rodent models of DAT.

1. Introduction

Impairments in mnemonic function have been seen in those with neurodegenerative disorders, such as Dementia of the Alzheimer's type (DAT). As DAT progresses, the ability to retrieve information about people, places, or events becomes more difficult [1]. The loss of cholinergic basal forebrain neurons that project to the hippocampal formation has been posited as one factor contributing to these mnemonic deficits [2,3]. This relationship has led to the "cholinergic hypothesis" associated with DAT (For a review, see Craig et al. [4]). One component of this deficit is retrograde amnesia or the inability to recall previously learned information [5]. Interestingly, many DAT patients who develop retrograde amnesia experience a temporal gradient, in which older memories are more stable, and recent memories are more susceptible to loss [6]. This pattern of mnemonic deficits may reflect the differential involvement of hippocampal formation in memory retrieval [7]. It is important to understand the characteristics of retrograde amnesia in hopes of identifying early warning signs for memory dysfunction seen in neurodegenerative disorders, such as DAT.

* Corresponding author at: Psychology Department Northern Illinois University De Kalb, IL 60115-2892, USA. Fax: +1 815 753 8088.
E-mail address: dwallace@niu.edu (D.G. Wallace).

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Animal models provide a unique opportunity to investigate mnemonic function. Previous work has shown that hippocampal damage disrupts performance on tasks that depend on previously learned discriminations [8–14]. In particular, Epp et al. [14] have shown that full hippocampal lesions can produce retrograde amnesia in a visual discrimination task. Interestingly, this large lesion did not produce anterograde amnesia, such that rats were able to learn a new discrimination from a pair of novel patterns. This work has demonstrated that full hippocampal lesions are sufficient to produce retrograde amnesia. More selective lesions may shed light on the role of specific neurotransmitter systems in mnemonic function. Previous research has found that cholinergic lesions to the medial septum do not impair performance on traditional spatial tasks [15–18], however, recent work using spontaneous behaviors have demonstrated a role for this system in information processing [19,20]. These selective lesion techniques allow for the further investigation of whether cholinergic projections originating in the medial septum are necessary for normal mnemonic function.

The current study uses string pulling behavior to examine the effects of 192-IgG-Saporin infusion into the medial septum on mnemonic function. The development of 192-IgG-Saporin has made it possible to observe the effects of cholinergic deafferentation on memory. Using a selective cholinergic lesion will allow for the accurate evaluation of the cholinergic hypothesis, which as discussed, has been used to describe the memory deficits seen in DAT. Many species have been observed to engage in the spontaneous behavior of string pulling [21–24] and rats can easily discriminate between strings based on odor or tactile cues [25]. This task can be adapted to examine several features of mnemonic function. First, the strength of a memory can be inferred from performance associated with a reversal in reinforcement relative to a previously learned discrimination. Next, varying the time between initial learning and 192-IgG-Saporin infusion can be used to evaluate the temporal gradient frequently associated with retrograde amnesia [10,11]. Finally, exposure to a novel odor discrimination can be used to determine the effects of this lesion on memory encoding. In summary, the string pulling task can be used to assess various characteristics associated with mnemonic function.

2. Materials and methods

2.1. Animals

Fifty-one female (90 days old) hooded Long Evans rats bred at Northern Illinois University were used in the current study. The rats were housed at 20–21 °C and on a 12-h light/dark cycle. The rats were food deprived to 85% of their free feeding weight and water was provided ad libitum. All experimental protocols were approved by the NIU Institutional Animal Care and Use Committee (IACUC).

2.2. Surgery

A total of 13 rats failed to engage in string pulling behavior during the shaping phase; therefore, 38 rats received either 192-IgG-saporin ($n = 20$) or saline ($n = 18$) infusions into the medial septum. First, rats were anesthetized with a mixture of isoflurane and oxygen. Lesions were made using standard stereotaxic techniques with the aid of a surgical microscope. The skin over the skull was opened, and the surface of the skull was exposed. Using a fine dental burr (0.4 mm), two holes (one per hemisphere) were drilled through the skull; each hole was drilled to a depth such that the dura was exposed but not damaged. Cholinergic lesions were produced by micro injections of 192-IgG-Saporin (Advanced Targeting Systems, San Diego, CA) into the medial septum. There were two lesion sites per hemisphere, using coordinates with respect to Bregma and the surface of the dura: AP: +1.30, ML: ±0.20, DV1: −6.9 [0.20 μL], DV2: −5.9 [0.15 μL]. The injection volumes were infused at a rate of 0.10 μL/min. After each injection, the cannula was left in place for 3 min to limit the diffusion of solution up the needle tract. After suturing the surrounding skin with silk, the incision was treated with an antibiotic salve.

Table 1
Experimental methods.

	Observer	Strings	Cashew	Weight	String length
Shaping (D1)	Yes	Unscented	Both	No	33 cm
Shaping (D2)	Yes	Unscented	Both	No	75 cm
Shaping (D3)	Yes	Unscented	Both	4 g	107 cm
Acquisition	Yes	Scented	+A/B	20 g	107 cm
Reversal	Yes	Scented	+B/A	20 g	107 cm
Novel pair	Yes	Scented	+C/D	20 g	107 cm

2.3. Apparatus

The string pulling apparatus was a transparent, rectangular cage (46 cm × 26 cm). The cage sat upon a table that was located 75 cm above the floor in a small room with many cues, including posters, a chair, and a door. In between trials, the rat was transferred to an opaque holding cage (46 cm × 26 cm) while the testing cage was prepared for the next trial. Two strings (shaping: 33 cm and 75 cm; testing: 107 cm) were placed in the front of the testing cage, one on either side. One end of the string was held in the cage with a weight, to keep it in place. The other end extended out the top and hung below the cage, with an unseen cashew (and potentially a weight, depending on the stage) attached to the end. The testing cage was wiped down after each rat. To scent the strings, strings were soaked in flavor extracts for 10 min and then dried for 2–4 h. The strings were stored separately in jars and re-scented every day to ensure no contamination from other scents. The scents used were anise, lemon, mint and rum (Watkins Incorporated, Winona, MN). A camera was positioned on a tripod at the same level as the testing cage.

2.4. Procedure

2.4.1. Shaping

The string pulling task involved four stages: shaping, acquisition, reversal, and novel pair training (see Table 1). During the shaping stage, the testing cage contained two unscented strings. A trial consisted of placing the rat into the cage opposite of the strings and waiting for the rat to pull up a string. The trial ended when the rat pulled up a string and consumed the cashew. The rats were given five trials for all shaping sessions. Once both strings were pulled up and the cashews were consumed, the researcher removed the rat from the testing cage and placed it in the holding cage while he or she prepared for the next trial (e.g., re-baiting the strings, switching string position). The rats were given 20 min to perform all five trials. Rats that failed to pull each string in five times would repeat the session the following day. Rats that still failed to pull each string in five times were excluded from the study. Shaping occurred for approximately three days, in which the string length progressed from the initial length (33 cm), to a medium length (75 cm), and then to the final string length (107 cm). A light weight (4 g) was added to the end of the strings once they were lengthened to 107 cm. Once a rat was fully shaped to pull in both strings, it moved on to the acquisition stage.

2.4.2. Acquisition

During the acquisition stage, a 20 g weight was added to the ends of the strings. This weight was used for the duration of the experiment. From this point on, the rats were split up into two groups, in