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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

which one group of rats experienced a short delay between learning the first discrimination and the reversal probe (recent: $n = 18$) and another group that experienced a longer delay (remote: $n = 20$). The discrimination contained one scented string that was reinforced with a cashew (+A) and one differently scented string that did not have a cashew on the end of it (B). The scents (lemon and anise) were randomly assigned to each rat. The rats were given eight trials a day until they reached criterion. Criterion was met when the correct string (+A) was chosen seven out of eight trials (88% correct) for three days. If an incorrect string was pulled, the researcher gave one correction trial per day, which consisted of allowing the rat to also pull in the correct string after pulling in the incorrect string. If the rat again did not pull up the correct string, it was removed from the testing cage and placed back into the holding cage, thus concluding the trial. Once criterion was met, the remote group was not tested for 6 weeks, in which rats received either cholinergic ($n = 10$) or sham lesions ($n = 10$) after the first 4 weeks and allowed to recover for the remaining 2 weeks. The recent group received either cholinergic ($n = 10$) or sham lesions ($n = 8$) immediately following criterion, which resulted in a short delay of only 2 weeks.

2.4.3. Reversal training

After each group's surgery and delay, the reversal stage began. A new set of scented strings was used for the reversal stage. During reversal, the rats experienced the same scented strings; however, the cashew was at the end of the string that was not reinforced previously. For example, string B now had a cashew at the end of it, not A. Reversal training was given for four days, regardless of correct responses. There were no correction trials given. Within each day, the procedure was similar to that of acquisition.

2.4.4. Novel pair training

Following the reversal phase, rats were given a novel odor pairing (+C/D) to evaluate the effects of 192-IgG-Saporin infusion into the medial septum on the encoding of new information. A new set of odors (mint and rum) were randomly assigned and presented, one being reinforced with a cashew at the end. New strings were scented in a manner similar to acquisition and reversal. Novel pair training was given for four days, regardless of correct responses. There were no correction trials given. Within each day, the procedure was similar to that of acquisition and reversal. After the four days of being exposed to the new discrimination, the rats were sacrificed for histological analysis.

2.5. Analysis of string pulling behavior

The number of days to reach criterion was used to assess the ability of rats to learn the discrimination during acquisition. For the reversal and novel pair training phases, the number of correct responses were summed per day for the four days and divided by the total number of trials (i.e., 8). This yielded a percent correct for each day. Data from the acquisition phase was analyzed with a univariate ANOVA to determine if there were any differences between groups in days to reach criterion, which would suggest that all rats were able to learn the discrimination before the surgery. In addition, percent correct during the reversal was analyzed to determine if there was an effect of lesion or time. A repeated-measures ANOVA consisting of three levels: two between-subjects comparisons (group: sham vs. SAP; time: remote vs. recent) and one within-subject comparison (day: D1-D4), was conducted to determine if there were any differences. Last, performance from the novel pair training was analyzed with a repeated-measures ANOVA to determine if there was an effect of percent correct across days (i.e., evaluating for anterograde amnesia). Effect size for each analysis of percent correct was conducted and reported as Cohen's d.

In addition, latency to reach the string was evaluated across the first four days of acquisition and reversal stages to evaluate whether groups differ in motivation before and after surgery. Specifically, latency was considered the time from the point of placement in the cage to the point of contact with the string. Furthermore, latency to pull up the string was measured across the first four days of acquisition and reversal stages to evaluate if groups differed in terms of motor function before and after the surgery. Due to the fact that the number of correct versus incorrect responses was unequal across days, data for both latency to approach the string and latency to pull up the string was collapsed across all four days. For acquisition, a univariate ANOVA was conducted to evaluate latency to approach and latency to pull up the string to determine if there were any main effects of lesion or delay or if there was a lesion by delay interaction. For the reversal phase, an independent-samples t test was conducted for each latency measure to evaluate any differences. Effect size for each latency analysis was conducted and reported as Cohen's d.

2.6. Histology

After behavioral testing, animals were deeply anesthetized and perfused with phosphate-buffered saline, followed by 4% paraformaldahyde. Brains were stored in a paraformaldehyde solution for 24 h, then moved to a 30% sucrose solution for approximately 48 h. The brains were sliced into 40 μm sections, and stained for acetylcholinesterase at the level of the dorsal hippocampus (for description of procedure, see Karnovsky and Roots [26]). The optical density was calculated for a square area (52×52 pixels) on one single section of the dorsal hippocampus (Scion Image; Scion Corporation). Examined areas included the dentate gyrus, CA1 and CA3 regions of the hippocampus, the retrosplenial and motor cortices and the lateral posterior thalamic nucleus. The optical density values were relative to the gray values associated within the sampled area (white: 0, 0; black: 255). Analysis of acetylcholinesterase activity in the hippocampus was used to determine whether the lesion was successful in reducing the population of cholinergic cells in the medial septum (MS) for each case.

3. Results

3.1. Histology

Acetylcholinesterase-stained sections exhibited reduced staining in the dorsal hippocampus (see Fig. 1). Independent-samples t tests conducted on optical density scores yielded a significant difference between groups in hippocampal and cortical sections (for optical density scores, t-scores and significance values, see Table 2). There was no significant difference detected in sections from the lateral posterior thalamic nucleus ($p = 0.151$). Saporin (SAP) rats exhibited a lower optical density score relative to sham rats in the dorsal hippocampus and cortex. These results demonstrate a significant decrease in the marker for cholinergic function in the hippocampus ($p < 0.001$). There was a significant difference in optical densities in the motor cortex and retrosplenial granular cortex, as well ($p < 0.001$). Two rats had non-selective lesions that affected the entire MS; therefore, these rats were removed from any further analyses (final $n = 36$; sham = 18 and SAP = 18).

3.2. Acquisition phase

Days to criterion, latency to approach the string and latency to pull up the string were analyzed in the acquisition phase. The number of days it took rats to reach criterion was analyzed

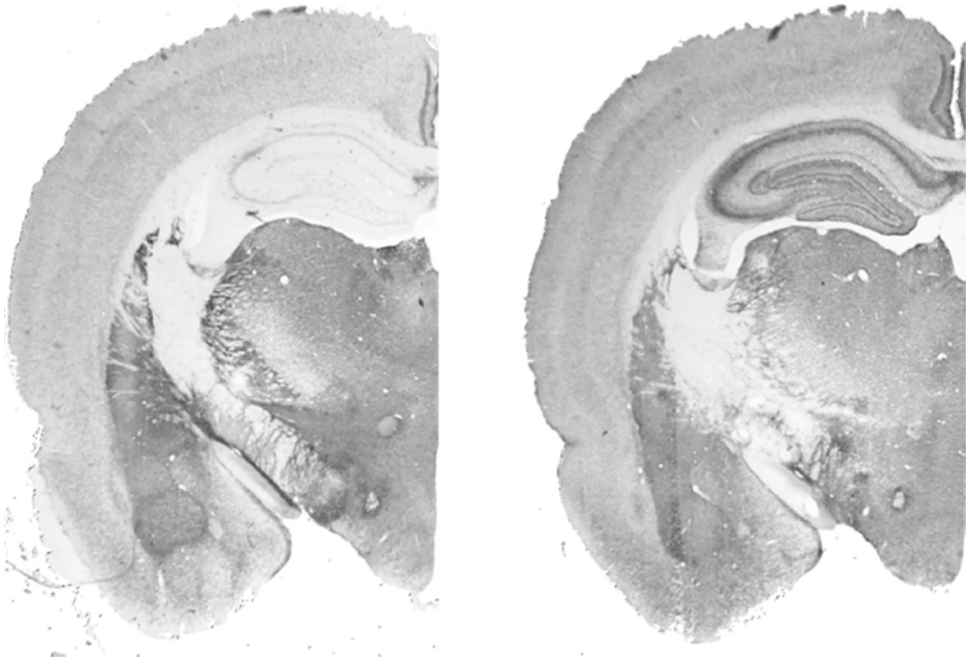

Fig. 1. Photomicrographs of coronal-sliced brains stained for acetylcholinesterase are presented at the level of the dorsal hippocampus. Shown are whole brain sections (4×) for a SAP (left) and sham (right) rat.

Table 2
Optical density scores from hippocampus, cortex, and thalamus.

	Sham	SAP	*t*-Value	Probability
Hippocampus				
dDG	161.76	86.51	24.09	<0.001
vDG	151.40	72.22	24.33	<0.001
CA1	175.08	103.97	31.37	<0.001
CA3	193.90	114.45	24.54	<0.001
Cortex				
RSg	150.29	93.11	17.48	<0.001
RSd	136.17	94.70	15.05	<0.001
Motor	134.02	93.62	10.21	<0.001
Thalamus				
LatThal	171.53	176.22	−1.46	0.151

Abbreviations: dDg is the dorsal dentate gyrus; vDG is the ventral dentate gyrus; CA1 is the CA1 field of the hippocampus; CA3 is the CA3 field of the hippocampus; RSg is the retrosplenial granual cortex; RSd is the retrosplenial dysgranular cortex; Motor is the secondary motor cortex; LatThal is the lateral posterior thalamic nucleus.

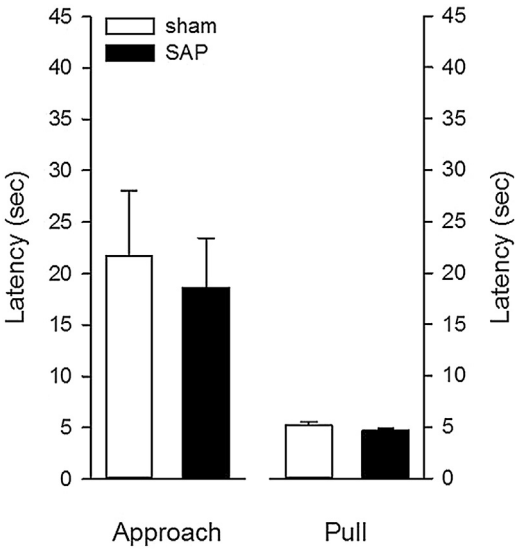

Fig. 2. Latency to approach the string (left) and latency to pull up the string (right) are plotted for the acquisition phase in sham and SAP groups.

for acquisition to assess if the discrimination was learned. An ANOVA was conducted with lesion and delay as between-subjects variables. It took all rats approximately 6 days to learn the discrimination [remote: sham (6.0 days), SAP (5.2 days); recent: sham (6.0 days), SAP (6.0 days)]. There was no significant main effect for either variable [lesion: $F(1, 32) = 1.153$, $p = 0.291$, $d = 0.035$; delay: $F(1, 32) = 1.153$, $p = 0.291$, $d = 0.035$], and in addition, there was no significant lesion × delay interaction [$F(1, 32) = 1.153$, $p = 0.291$, $d = 0.035$]. As expected, groups did not differ in performance prior to surgery, such that they were all able to learn the discrimination in the acquisition phase.

Due to the fact that the numbers of correct versus incorrect responses were unequal across days, data for both latency to approach the string and latency to pull up the string were collapsed across the first four days of acquisition. A univariate ANOVA was conducted for each latency measure (see Fig. 2). It took a similar amount of time for all rats to approach the string before surgery [remote: sham ($M = 32.98$ s) and SAP ($M = 31.04$ s); recent: sham ($M = 34.58$ s) and SAP ($M = 36.99$ s)]. In addition, it took a similar amount of time for all rats to pull up the string before surgery [remote: sham ($M = 5.26$ s) and SAP ($M = 5.10$ s); recent: sham ($M = 5.51$ s) and SAP ($M = 4.86$ s)]. There were no significant differences between groups for both latency to approach the string [lesion: $F(1, 32) = 0.001$, $p = 0.979$, $d = 0.000$; delay: $F(1, 32) = 0.179$, $p = 0.675$, $d = 0.006$; lesion × delay interaction: $F(1, 32) = 0.060$, $p = 0.809$, $d = 0.002$] and latency to pull up the string [lesion: $F(1, 32) = 0.828$, $p = 0.370$, $d = 0.025$; delay: $F(1, 32) = 0.000$, $p = 0.990$, $d = 0.000$; lesion × delay interaction: $F(1, 32) = 0.293$, $p = 0.592$, $d = 0.009$]. The data analysis of latency demonstrated that there were no differences in the motivation to approach the string or motor coordination to pull up the string before surgery. Overall,

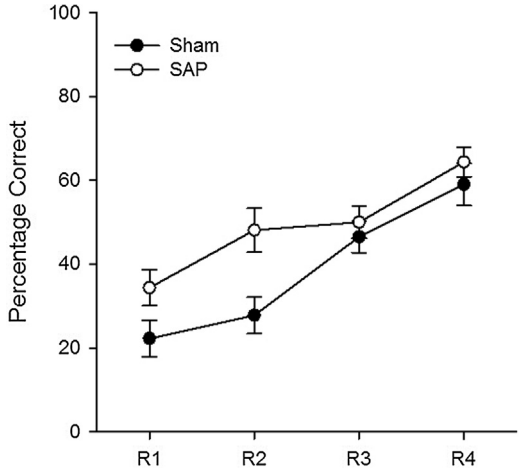

Fig. 3. Percentage of correct trials is plotted across days for sham and SAP groups during the reversal phase.

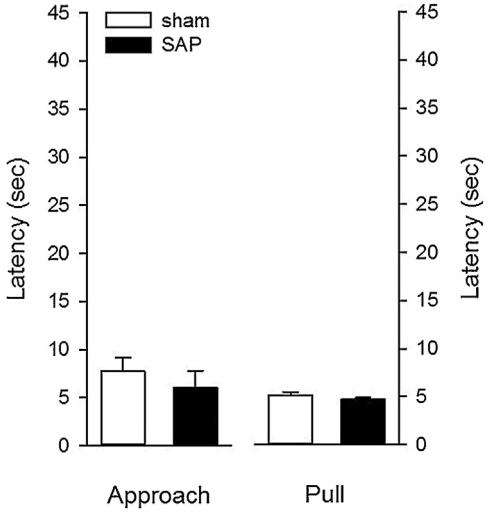

Fig. 4. Latency to approach the string (left) and latency to pull up the string (right) are plotted for the reversal phase in sham and SAP groups.

the analysis of performance suggests all groups were performing at the same level prior to surgery.

3.3. Reversal phase

Percent correct, latency to approach the string, and latency to pull up the string were analyzed for all trials in the reversal phase. Percent correct was calculated for each of the four days. There were no significant effects of delay when analyzing latency to approach the string [$F(1, 32) = 0.952$, $p = 0.336$, $d = 0.029$], pull the string [$F(1, 32) = 0.614$, $p = 0.439$, $d = 0.019$] and percent correct [$F(1, 32) = 0.237$, $p = 0.630$, $d = 0.007$]; therefore, the groups were collapsed across remote and recent delays for subsequent analyses on all measures. For percent correct during the reversal, a repeated-measures ANOVA was conducted with day as the within-subjects variable and lesion as the between-subjects variable (see Fig. 3). There was a significant main effect of day [$F(3, 102) = 25.870$, $p < 0.001$, $d = 0.432$] and significant main effect of lesion [$F(1, 34) = 5.492$, $p = 0.025$, $d = 0.139$]. There was not a significant day × lesion interaction [$F(3, 102) = 1.865$, $p = 0.140$, $d = 0.052$]. The data analysis for percent correct during reversal revealed that SAP rats had a higher percent correct than sham rats.

Again, due to the fact that the numbers of correct versus incorrect responses were unequal across days, data for both latency to approach the string and latency to pull up the string were collapsed across all four days of reversal. Two rats were discarded from the latency to reach the string analysis because their latencies were more than two standard deviations away from the mean. An independent-samples t test was conducted for each latency measure (see Fig. 4). It took a similar amount of time for all rats to approach the string after surgery [sham ($M = 7.72$ s) and SAP ($M = 6.52$ s)]. In addition, it took a similar amount of time for all rats to pull up the string before surgery [sham ($M = 4.22$ s) and SAP ($M = 3.83$)]. There were no significant differences between groups for both latency to approach the string [$t(32) = 1.239$, $p = 0.224$] and latency to pull up the string [$t(32) = 1.711$, $p = 0.096$]. The data analysis of latency demonstrated that there were no group differences to approach and pull up the string after surgery. Further analyses were conducted to determine if there was a change between latencies from acquisition to reversal, demonstrating a "practice effect". A mixed design ANOVA was conducted for each latency measure, with lesion (sham vs. SAP) as the between-subjects variable, and phase (acquisition vs. reversal) as the within-subjects variable. There was not a main effect of lesion [$F(1, 32) = 0.432$, $p = 0.516$, $d = 0.013$], nor a phase × lesion interaction [$F(1, 32) = 1.061$, $p = 0.311$, $d = 0.032$] to reach the string. There was, however, a significant main effect for phase [$F(1, 32) = 36.63$, $p < 0.001$, $d = 0.534$]. This suggests that during the progression of the task, all rats approached the string faster. For latency to pull up the string, there was not a main effect of lesion [$F(1, 32) = 1.814$, $p = 0.188$, $d = 0.054$], nor a phase × lesion interaction [$F(1, 32) = 0.023$, $p = 0.880$, $d = 0.001$] to reach the string. There was, however, a significant main effect for phase [$F(1, 32) = 23.333$, $p < 0.001$, $d = 0.586$]. This suggests that as the task progressed, all rats pulled up the string faster. These analyses do demonstrate a "practice effect" for both approach and pulling up the string; however, this is not unexpected, such that as the rats experienced the task, they became more proficient at it. Furthermore, these effects are across all rats, and do not reflect lesion, which suggests that infusion of 192-IgG-Saporin into the medial septum did not influence performance across time in the task.

Analysis of the reversal data showed that there were no differences in motivation or motor function, and although there was no evidence for a temporal gradient, the results revealed that SAP rats were able to learn the reversal discrimination at a quicker rate than sham rats, suggesting a lack of memory for the learned discrimination.

3.4. Novel pair training

Percent correct was analyzed for all trials in the novel pair training phase. One SAP rat would not perform; therefore, the data from that rat is missing from this analysis. Data for the percent correct was analyzed across the four days of the novel pair training. A repeated-measures ANOVA was conducted with day as the within-subjects variable and lesion as the between-subjects variable (see Fig. 5). For the percent correct during the novel pair training, there was a significant main effect of day [$F(3, 99) = 48.127$, $p < 0.001$, $d = 0.593$]. There was no significant effect of lesion [$F(1, 33) = 2.232$, $p = 0.145$, $d = 0.063$] nor a day × lesion interaction [$F(3, 99) = 0.532$, $p = 0.661$, $d = 0.016$]. Therefore, all groups were making similar correct choices by the end of the novel pair training. Overall, analysis of performance during the novel pair training suggests all groups were able to learn the new discrimination.

4. Discussion

The current study evaluated the role of cholinergic projections from the medial septum in mnemonic function across two time

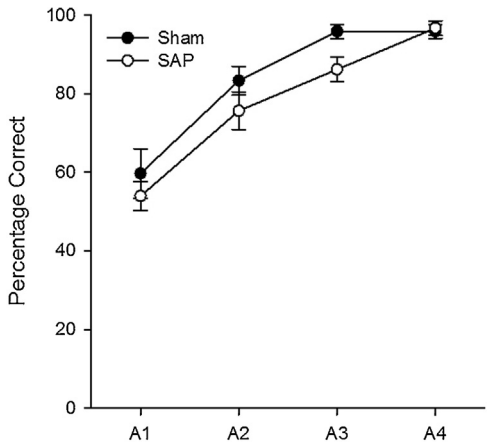

Fig. 5. Percentage of correct trials is plotted across days for sham and SAP groups during the novel odor training.

points. Histological analyses indicated a reduced amount of acetylcholinesterase activity in the dorsal hippocampus and cortex. It has been previously shown that 192-IgG-Saporin infusion is effective in significantly reducing the number of cholinergic neurons in the medial septum, while leaving the majority of GABAergic neurons intact [15,20,27,28]. Therefore, it can be concluded that functioning of the cholinergic projection originating in the medial septum was successfully reduced in SAP rats in the current study.

Prior to surgery, there were not any differences between groups in the ability to learn the discrimination in the acquisition phase. After surgery, differences emerged during the reversal phase, in which SAP rats displayed a higher percent correct relative to shams. For sham rats, these results suggest that the memory from acquisition interfered with learning the reversed discrimination. In contrast, infusion of 192-IgG-Saporin into the medial septum disrupted retrieval of the previously learned discrimination, thereby reducing the amount of interference experienced during reversal testing. Even though retrograde amnesia was apparent, there was no difference between the remote and recent groups. This is consistent with a flat gradient, such that all SAP rats had a higher percent correct relative to shams in the reversal phase, regardless of time between criterion and surgery. Furthermore, there were no group differences during the novel pair training, thus providing evidence that infusion of 192-IgG-Saporin into the medial septum did not produce anterograde amnesia. Last, there were no differences observed in latency to reach the string or latency to pull up the string before and after surgery. This suggests that disruptions in performance reflected impaired mnemonic function rather than causing impairment in motivation or motor function. The current results are consistent with infusion of 192-IgG-Saporin into the medial septum producing non-temporally graded retrograde amnesia. This supports a role for the cholinergic projections originating in the medial septum in mnemonic function.

Much of the literature evaluating mnemonic function suggests that retrograde amnesia occurs as a result of failed memory consolidation or reconsolidation [29,30]. The memories are improperly encoded (or re-encoded), and therefore, the memory that is accessed during recall is not the same memory as before. Studies have provided evidence for this reconsolidation failure by using a protein synthesis inhibitor, anisomycin, to prevent the ability to recall a learned association [29]. This theory assumes that memories are dynamic and change as time goes on [31]. This change is thought to reflect a shift in the recruitment of the hippocampus as the time interval increases from initial consolidation. A temporal gradient, where recent memories are more susceptible to memory loss than remote memories, has been found when damage occurs to the entorhinal cortex [10] and fornix [11]. This is consistent with a change in hippocampal involvement, such that the hippocampus plays a role in recently encoded memory retention, but as the time interval increases, the hippocampal involvement diminishes. Although there are many studies that support the effects of retrograde amnesia being caused by errors in memory consolidation, not all studies have found a temporal gradient [9,12–14]. Therefore, it could be suggested that hippocampal function may remain unchanged throughout a time course. There is similar hippocampal involvement as long as the memory persists, and the memory, itself, may remain unchanged. Thus, when the hippocampus is compromised, retrograde amnesia is constant across different time points. Other theories have been developed to explain the lack of a temporal gradient seen in some studies, which reflect the persistent role of the hippocampus in mnemonic function.

The multiple traces theory has been used to explain non-temporally graded retrograde amnesia seen in many studies. In particular, this theory supports data showing that spatial tasks (such as the Morris water maze) have flat gradients [13,32]. The explanation for a flat gradient is dependent on the type of information learned. Less detailed, more semantic information is thought to first involve the hippocampus, and then shift to other structures. In contrast, context dependent, episodic memories recruit long-term involvement of the hippocampus. Therefore, spatial tasks (which are believed to fall into the latter category) result in a flat gradient, such that remote and recent memories are equally affected when the hippocampus becomes compromised. This theory does not explain the results from the current study because of the information that was learned did not depend on a spatial representation. The string pulling task, which required the rats to learn an association between an odor and a reward, falls into the "semantic" category. If this theory were to hold true, there should have been a temporal gradient observed, because the information would have been transferred from the hippocampus to other regions. In summary, both the consolidation and multiple trace theories of memory are not supported from the evidence found in the current study.

Memory retrieval has been described as being the activation of latent information or associations, facilitated by a cue [33]. Therefore, memory retrieval failure refers to the inability to retrieve consolidated memories from long-term storage [34]. It was hypothesized that the memories are intact, just difficult to access. Previous research has provided empirical evidence that offers retrieval failure as an explanation for retrograde amnesia. For example, hypothermia has been shown to produce retrograde amnesia, and the use of a "reactivation cue" can result in successful retrieval of the memory [35]. Lesion studies have been shown to function in a similar fashion. Rats with hippocampal lesions that received a "reactivation cue" did not display retrograde amnesia compared to those that received the lesion but no "reactivation cue" [36], providing further evidence for the retrieval failure theory of retrograde amnesia.

Evidence from the current study is consistent with the hippocampus being involved in long-term memory retrieval. Although retrograde amnesia was demonstrated after infusion of 192-IgG-Saporin into the medial septum, the current study did not find evidence for a temporal gradient, such that there was no difference in rats that had a two-week delay relative to a six-week delay. This is consistent with other studies that also did not observe a temporal gradient [9,12–14], and also demonstrates that even selective, cholinergic lesions can produce a flat gradient, although it would be expected that a selective lesion would be more likely to show a temporal gradient. This suggests that the string pulling task used, which required much less training, may be more "episodic" in nature. If this is the case, then the multiple trace theory would apply; however, this is an assumption, and further studies need to be

conducted to classify the information learned in the string pulling task as "episodic". The current study has demonstrated that it is the cholinergic projection to the hippocampus, in particular, that is crucial for mnemonic function. Removing this input alone is enough to produce retrograde amnesia, regardless of time. Therefore, the hippocampus may play a role in retrieval of memories acquired prior to the lesions. Furthermore, in both experiments, rats with infusion of 192-IgG-Saporin into the medial septum were able to learn new discriminations, suggesting that they still had the ability to consolidate memories properly. Therefore, the current study corroborates other literature that suggests retrograde amnesia is due to an inability to access the memory and that the memory itself remains intact.

Even though consolidation and retrieval failure seem to be competing theories, they may not be mutually exclusive. There may be a role for both processes. Consolidation is important for memory retention and retrieval functions to recall those memories. There may be instances that one or both processes are compromised, which can lead to retrograde amnesia. Future research would benefit from trying to unite these theories in order to further understand mnemonic function.

Several procedural aspects of the current study may have contributed to the observed effects of infusing 192-IgG-Saporin into the medial septum on performance during the string pulling task. One factor that could have resulted in non-temporally graded retrograde amnesia may have been the use of a lesion technique that has a more protracted window of cell death. Previous studies have suggested that neurotoxic lesions (e.g., using immunotoxins) may not induce temporal gradients [37,38]. This may be due to the longer amount of time that the neurotoxins need to produce the lesion (e.g., one to two weeks). Electrolytic lesions are fast and produce immediate damage. This is unlikely, however, because other studies using electrolytic lesions have failed to find a temporal gradient [12,14]. In addition, a retrograde amnesia study observing the social transmission of food preference behavior used 192-IgG-Saporin and did find a temporal gradient [39]. Therefore, lesion type is an unlikely candidate for the cause of the non-temporally graded retrograde amnesia observed in the current study. Another factor contributing to a flat gradient may have been the length of the delay. This is unlikely because it has been shown that a shorter delay (~28–30 days) produces a temporal gradient [39]. Studies have also shown a temporal gradient at 42 days [10,11], which was the rationale behind the delay used in the current study. Even still, studies have used a much longer delay (80 days), and have failed to demonstrate a temporal gradient [14]. Therefore, the inability of the current study to find a temporal gradient may not reflect the length of the delay. The observation of a temporal gradient remains inconsistent within the retrograde amnesia literature; however, the current study provides evidence in support of a role for medial septum cholinergic projections to the hippocampus in mnemonic function, regardless of time.

The current study used the string-pulling task to evaluate the role of the hippocampal cholinergic projection from the medial septum in mnemonic function. These results demonstrated that infusion of 192-IgG-Saporin into the medial septum impaired performance when presented with the reversed pairing of two learned discriminations; however, no temporal gradient was observed. In addition, this lesion did not affect the ability to learn novel discriminations. These results provide evidence supporting a role for the cholinergic projection originating in the medial septum in long-term memory retrieval. In conclusion, the current study adds to the growing literature that supports a role for the cholinergic projection originating in the medial septum in multiple stages of memory. The current study uses this task to detect impaired performance reflecting retrograde amnesia, and it may be adapted to evaluate the effectiveness of novel therapeutic interventions. This may allow for the development of a translational model to assess mnemonic function deficits in those with neurodegenerative disorders.

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