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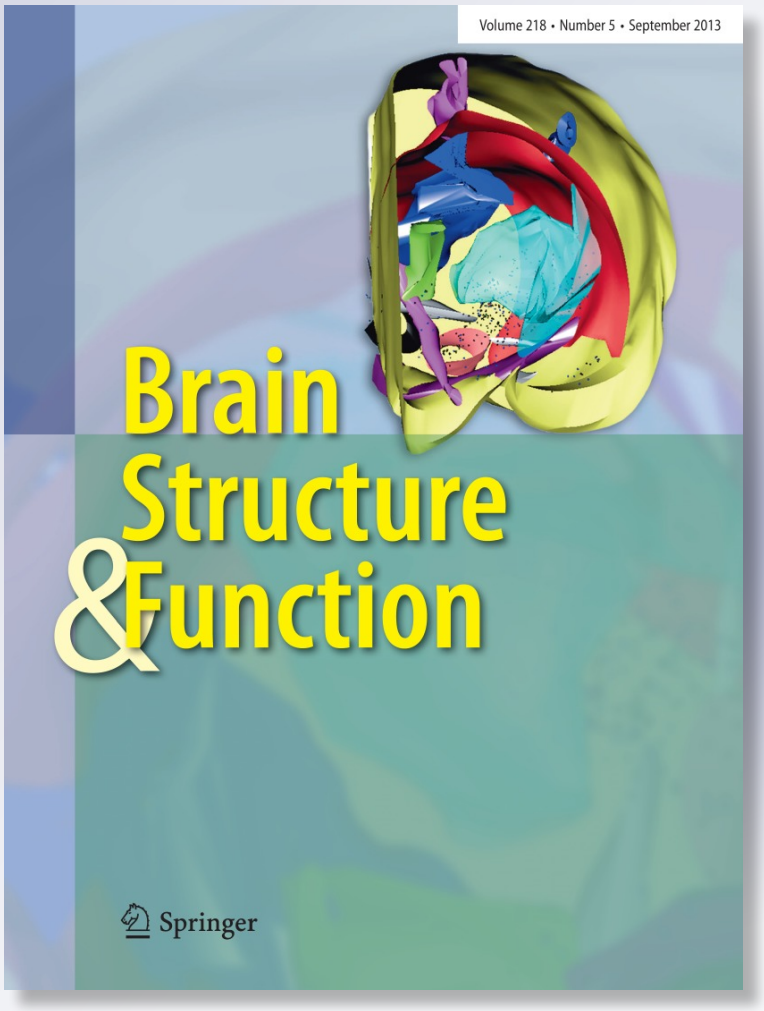

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ORIGINAL ARTICLE

Infusion of GAT1-saporin into the medial septum/vertical limb of the diagonal band disrupts self-movement cue processing and spares mnemonic function

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Abstract Degeneration of the septohippocampal system is associated with the progression of Dementia of the Alzheimer's type (DAT). Impairments in mnemonic function and spatial orientation become more severe as DAT progresses. Although evidence supports a role for cholinergic function in these impairments, relatively few studies have examined the contribution of the septohippocampal GABAergic component to mnemonic function or spatial orientation. The current study uses the rat food-hoarding paradigm and water maze tasks to characterize the mnemonic and spatial impairments associated with infusing GAT1-Saporin into the medial septum/vertical limb of the diagonal band (MS/VDB). Although infusion of GAT1-Saporin significantly reduced parvalbumin-positive cells in the MS/VDB, no reductions in markers of cholinergic function were observed in the hippocampus. In general, performance was spared during spatial tasks that provided access to environmental cues. In contrast, GAT1-Saporin rats did not accurately carry the food pellet to the refuge during the dark probe. These observations are consistent with infusion of GAT1-Saporin into the MS/VDB resulting in spared mnemonic function and use of environmental cues; however, self-movement cue processing was compromised. This interpretation is consistent with a growing literature demonstrating a role for the septohippocampal system in self-movement cue processing.

Keywords Path integration translational neuroscience · Spatial orientation · Limbic system · Medial septum · *Rattus norvegicus*

Abbreviations

AChE	Acetylcholinesterase
ChAT	Choline acetyltransferase
DAT	Dementia of the Alzheimer's type
GAD	Glutamic acid decarboxylase
MS/VDB	Medial septum ventral limb of diagonal band of Broca
PBS	Phosphate buffered saline

Introduction

Dementia of the Alzheimer's type (DAT) is characterized by a progressive decline in cognitive function including impaired mnemonic and spatial processing (Mesulam 2000). This cognitive decline has been associated with episodes of wandering (Rabins et al. 1982; Logsdon et al. 1998) and degeneration of the basal forebrain structures (Davies and Maloney 1976; Perry et al. 1977; Rossor et al. 1984; Whitehouse et al. 1985). Multiple techniques have been used to investigate the relationship between the septohippocampal system and cognitive function (for a review see Parent and Baxter 2004). Initial work employed non-selective lesions of the medial septum and attributed disruptions in performance on spatial tasks to impaired mnemonic function (Hagan et al. 1988; Connor et al.

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1991). Development of immunotoxic lesion techniques that target cells expressing specific proteins (Wiley et al. 1991) has been critical in characterizing the role of the cholinergic component of the septohippocampal system in spatial orientation. Infusion of 192 IgG-Saporin into the medial septum/vertical limb of the diagonal band (MS/VDB) spared performance on spatial tasks that provide access to environmental cues (Baxter and Gallagher 1996; Cahill and Baxter 2001; Kirby and Rawlins 2003; Vuckovich et al. 2004; Martin and Wallace 2007); however, disruptions in performance were observed on spatial tasks in which rats were restricted to using self-movement cues (Martin and Wallace 2007). This work is consistent with a growing literature demonstrating a role for the septohippocampal system in self-movement cue processing (Maaswinkel et al. 1999; Wallace and Whishaw 2003; Philbeck et al. 2004; Wolbers et al. 2007).

Research has traditionally focused on the role of septohippocampal cholinergic projections in cognitive function; however, several lines of evidence support a role for MS/VDB GABAergic neurons in hippocampal function. First, a population of GABAergic neurons in the MS/VDB has been identified that sends projections to the hippocampus, forming synapses on inhibitory interneurons (Köhler et al. 1984; Freund and Antal 1988). Activation of these GABAergic projections results in disinhibition of hippocampal pyramidal neurons. These GABAergic projections from the MS/VDB may mediate the effects of muscarinic agonists and antagonists on hippocampal theta (Wu et al. 2000; Alreja et al. 2000). In addition, infusion of kainic acid into the MS/VDB reduces, somewhat selectively, GABAergic neurons and attenuates movement-elicited hippocampal theta activity (Yoder and Pang 2005). Finally, impaired performance on spatial tasks associated with lesions that target GABAergic neurons in the MS/VDB has been attributed to impaired mnemonic function (Dwyer et al. 2007; Pang et al. 2011; Lecourtier et al. 2011). However, the behavioral tasks used to assess the function of GABAergic projections originating in the MS/VDB provide rats' access to both environmental and self-movement cues. Therefore, it remains to be determined whether disruptions in performance associated with GABAergic deafferentation of the hippocampus reflect deficits in mnemonic or self-movement cue processing or potentially both.

The current study examined the effect of infusing GAT1-saporin into the MS/VDB on spatial orientation and mnemonic function. GAT1-saporin is a conjugate of an antibody to the GABA-1 transporter and the ribosome-inactivating protein saporin. GAT1-saporin lesions are intended to eliminate GABAergic neurons while sparing cholinergic neurons (Radley et al. 2009; Pang et al. 2011). The food-hoarding paradigm and water maze tasks will be used to examine the effects of infusing GAT1-saporin into the MS/VDB on spatial orientation and mnemonic function, respectively. The food-hoarding paradigm assesses environmental and self-movement cue processing individually (Maaswinkel et al. 1999; Wallace et al. 2002; Martin and Wallace 2007; Winter et al. 2011). First, rats are trained to leave a refuge, find a randomly located food pellet, and carry it to the refuge for consumption. Next, access to environmental cues is varied across several probes. During the Cued-probe, rats may use proximal or distal environmental cues and self-movement cues to guide movement back to the refuge. During the Uncued-probe, rats may use distal environmental or self-movement cues to guide movement back to the refuge. During the Dark-probe, rats are limited to self-movement cues to guide movement back to the refuge. Finally, during the New-probe, the location of the refuge is shifted in the room; thereby placing distal environmental cues associated with the refuge location during previous food-hoarding sessions in conflict with self-movement cues generated as the rat exits the refuge. Water maze procedures assess mnemonic function (Morris et al. 1982; Sutherland et al. 1982a, b). First, place training characterizes a rat's ability to encode the location of a hidden escape platform relative to environmental cues. Next, the shift probe assesses the nature of the representation mediating performance during the place training, whether rats encode a map-based (Tolman 1948; O'Keefe and Nadel 1978) or directional vector-based (Blodgett et al. 1949; Skinner et al. 2003; Hamilton et al. 2007, 2008, 2009a, b) representation. Finally, matching-to-place testing assesses the rat's ability to rapidly modify the representation used to guide movement (Sutherland et al. 1982a, b). The combination of these behavioral techniques provides a robust assessment of the effect of infusing GAT1-saporin into the MS/VDB on spatial orientation and mnemonic function.

Materials and methods

Animals

Twelve female (90 days old) Long-Evans rats obtained from the Northern Illinois University vivarium served as subjects for the current study. All rats had previous experience carrying food to a refuge. Rats were pair housed in plastic cages with the colony room temperature maintained at ~21 °C and a 12 h light/dark cycle. Access to rat chow (5L42 Rodent Breeder Diet food pellets; PMI Nutritional International, Brentwood, MO, USA) was only restricted during food-hoarding training to maintain them at 85 % of their free feeding weight; otherwise, rats had ad-lib access to food and water. The Institutional Animal Care and Use

Committee at Northern Illinois University, which follows the guidelines set by the Office of Laboratory Animal Welfare, approved all of the procedures described in this experiment.

Surgery

During surgery, rats were deeply anesthetized with a mixture of isoflurane and oxygen. Rats either received GAT1-saporin ($n = 6$) or vehicle ($n = 6$) infused into the medial septum-diagonal band of Broca. There were two infusion locations per hemisphere, with coordinates relative to bregma and the surface of the dura: AP: +1.30, ML: ± 0.20, DV1: −6.9 [0.40 μL each site], DV2: −5.9 [0.30 μL each site]. Either GAT1-saporin (Advanced Targeting Systems; 0.5 μg/μL) or vehicle was infused at 0.20 μL/min per site. After each infusion, the cannula was left in place for 3 min to minimize the diffusion of the solution up the needle tract.

Apparatus

Food-hoarding table

The apparatus was a large circular table (200 cm in diameter) positioned 75 cm above the floor. The table was located in a lightproof room with multiple visual cues: posters on the walls, wooden door, chair, and experimenter. The night-vision camera attached to the ceiling fed video to a DVD recorder located in an adjacent room. During dark testing, the experimenter used night-vision goggles to handle and observe each rat's behavior.

A small opaque box (20 cm × 29 cm × 22 cm) located at the periphery of the table served as the refuge. The cued refuge was positioned on the top of the table with only a circular hole (11.5 cm) on one side that provided direct access to the surface of the table. The hidden refuge was positioned below the surface of the table with an open top and a short ramp that could be climbed on to access the surface of the table.

Water maze

The apparatus for the water maze was a large circular pool (1.73 m diameter × 0.58 m height) half filled with water (~19 °C) made opaque by the addition of white non-toxic paint. The water maze was located in a rectangular room with multiple visual cues: posters on the walls, wooden door, sink, cabinet and the experimenter. The hidden circular escape platform (15 cm diameter) was submerged 2 cm below the surface of the water. A ceiling mounted camera connected to a DVD recorder provided a record of the rats' behavior for subsequent analysis.

Procedure

Food hoarding

Rats received one food-hoarding session per day. During a session, rats were transported from the colony room to the testing room via an opaque cage with a wire mesh top. During transportation, lights were turned off, the cage was rotated, and the experimenter walked in a circuitous path that varied across days. This transportation procedure minimized the ability of the rat to learn the location of the testing room relative to the colony. After entering the testing room, a rat was gently placed in the refuge. In general, a food-hoarding trial involved the rat: (1) exiting the refuge and searching for a randomly positioned 1.0-g banana-flavored food pellet (BioServ, Frenchtown, NJ, USA), (2) carrying the food pellet to the refuge, and (3) eating the food pellet. While the rat was eating the food pellet, the table was baited with another food pellet. The food-hoarding session continued until the rat recovered five food pellets (five trials) during Cued-probe, Uncued-probe, Dark-probe sessions. The food-hoarding session during the New-probe continued until the rat recovered two food pellets (two trials). After the rat completed a food-hoarding session, the rat was transported to the colony, the table was wiped down with Windex, and the table was rotated 30 degrees. During a training session, rats were shaped to carry food pellets to a cued refuge with the light turn on in the testing room. Rats were required to carry five food pellets to the cued refuge on three consecutive sessions prior to experiencing probe sessions.

Only one probe session was given per day and the sequence of probe sessions occurred on consecutive days was as follows: Cued-probe, Uncued-probe, Cued-probe, Dark-probe, Cued-probe, Uncued-probe, Cued-probe, Dark-probe, Cued-probe, and New-probe. The Cued-probe session involved placing the opaque refuge on the surface of the table with the lights on in the testing room. During Uncued-probe, Dark-probe, and New-probe sessions, the hidden refuge was positioned below the surface of the table. The testing room lights remained on during the Uncued-probe and New-probe sessions and were turned off during the Dark-probe sessions. During the Cued-probe, Uncued-probe, and Dark-probe sessions the refuge remained in the same position, whereas the hidden refuge was shifted 180° around the perimeter of the table during the New-probe session. After the last food-hoarding session, rats were taken off food deprivation and given a week to gain weight prior to water maze testing.

Water maze

Rats were transported between the colony and testing rooms via opaque cages with wire mesh tops. During a

place training trial, a rat was placed in the water maze at one of the four-cardinal compass directions facing the apparatus wall. If the rat did not find the platform before 60 s, the researcher guided the rat to the hidden platform. The rat remained on the platform for 30 s prior to being dried off and returned to the transport cage. All rats received one trial prior to any rat receiving its second trial, resulting in an approximate 20-min interval between trials. After a rat completed a trial, the water was stirred and strained to limit the rat's ability to use odor cues to guide performance (Means et al. 1992). Rats received four trials per day for 5 days.

The shift probe was given the following day. The water maze was shifted half the diameter of the pool, and the hidden escape platform was removed. This shift of the water maze provides the rat with the opportunity to exhibit either a place (swim to the absolute position associated within the reference frame of the room) or directional (swim to the relative direction anchored to the apparatus) response. During the shift probe, the hidden escape platform was removed, and the rats swam for 60 s prior to being removed from the water maze.

Matching-to-place testing began the day after the shift probe and continued for 3 days. Rats received two trials per day with the hidden escape platform remaining in a fixed position. The position of the hidden escape platform changed across days.

Data analysis

Food-hoarding

The Peak Performance (Vicon, Denver, CO, USA) motion capture system was used to quantify movement characteristics of rats in the food-hoarding paradigm. Rat movement was tracked by selecting one pixel every fifth frame that corresponded to the midline of the body at the level of the forelimbs. The resulting *x*- and *y*-coordinates were scaled to real world units and used to calculate each measure of performance. The first two Cued-probe sessions, both Uncued-probe sessions, and both Dark-probe sessions were digitized using the Peak Performance system. Food-hoarding trips were divided into searching and homeward segments. The searching segment began when the rat left the refuge and included all movements until the food pellet was located. The homeward segment began when the rat located the food pellet and included all movement until the rat returned to the refuge.

Several measures were used to characterize rat performance during food-hoarding sessions. First, path circuity or complexity of the path was calculated for outward and homeward segments by dividing the distance between the start and end of a segment by the total distance travelled on the segment. Path circuity ranged from near 0.0 (i.e., circuitous path) to near 1.0 (i.e., straight path). In addition, homeward segment heading error was calculating as the angle subtended by the following points: starting point of the outward segment, food pellet location, and location of the peak speed on the homeward segment. Heading error ranged from 0 degrees (no error) to 180 degrees (maximum error). Both homeward segment measures provide an index of a rat's ability to accurately return to the refuge under conditions with varied access to environmental cues. Mixed design ANOVAs with Lesion (Sham vs. GAT1-saporin) and Probe session (Cued-probe vs. Uncued-probe vs. Dark-probe) as factors were conducted on each measure.

One measure was used to characterize performance observed after rats found the food pellet during both trials of the New-probe session. Specifically, the number of stops made within a body length of the former refuge location was calculated for the homeward segment of each trial. A stop was defined as at least two consecutive points in which speed did not exceed 0.1 m/s. This measure provided an index of a rat's tendency to perseverate to the former refuge location. Mixed design ANOVAs with Lesion (Sham vs. GAT1-saporin) and Trial (Trial 1 vs. Trial 2) as factors were conducted on this measure.

Water maze

The EthoVision (Noldus, Leesburg, VA, USA) motion caption system was used to quantify movement characteristics of rats in the water maze. First, latency to reach the platform and path circuity was calculated for each trial during place training. Both measures were averaged across each day. Mixed design ANOVAs with Lesion (Sham vs. GAT1-saporin) and Day (Day 1 vs. Day 2 vs. Day 3 vs. Day 4 vs. Day 5) as factors were conducted on each measure. Second, percent time spent swimming on the relative side of the water maze was calculated during the shift probe. Independent and single sample *T* tests were used to evaluate group differences. Finally, latency to reach the platform and path circuity was calculated for each trial during matching-to-place testing. Performance on the first (Block 1) and second (Block 2) trial was averaged across all 3 days of matching-to-place testing for both measures. Mixed design ANOVAs with Lesion (Sham vs. GAT1-saporin) and Block (Block 1 vs. Block 2) as factors were conducted on each measure.

Histology

Following completion of behavioral testing, all rats were deeply anesthetized and perfused through the heart with phosphate buffered saline (PBS) followed by 4.0 %

paraformaldehyde (PFA). The brain was removed from the skull and placed in 4.0 % PFA for 24 h and then cryoprotected by sinking in a 30 % sucrose solution. Brains were frozen and cut into two spaced series of 50 μm coronal sections for histological analyses.

Acetylcholinesterase

One set of coronal sections was processed for acetylcholinesterase, a reliable marker of dorsal hippocampal cholinergic function (Hoover et al. 1978; Satoh et al. 1983). First, sections were stained for acetylcholinesterase as published previously (Karnovsky and Roots 1964; Martin and Wallace 2007; Martin et al. 2008). Next, photomicrographs of tissue sections were captured using an Olympus BH-2 microscope equipped with an Olympus DP72 camera connected to a computer running cellSens Dimension 1.3 (Olympus America Inc., Center Valley, PA, USA). The digital photomicrographs were converted to grayscale, and optical density for a square area (52 × 52 pixels or approximately 200 × 200 μm) within the dentate gyrus, CA1, CA3, retrosplenial cortex, and motor cortex was measured using Scion Image (Alpha 4.0.3.2., Scion Corp.). Optical density values ranged from 0.0 (white) to 255.0 (black). Previous work has shown that this characterization of hippocampal and cortical cholinergic tone has been successful in dissociating the effect of electrolytic lesion of the MS/VDB (Martin et al. 2007) and infusion of 192-IgG-saporin into the MS/VDB (Martin and Wallace 2007; Martin et al. 2008) or NBM (Martin et al. 2008). Independent samples *T* tests were used to evaluate group differences in the average optical density within the hippocampal subfields and overlying cortex.

Parvalbumin

The second set of coronal sections was processed for cells at the level of medial septum using an antibody against parvalbumin, which is selectively expressed by GABAergic neurons. For this, sections were first treated with 0.3 % H_2O_2 for 15 min to quench the endogenous peroxidases. Sections were then washed three times with phosphate buffered saline prior to placement into a blocking solution composed of 10 % normal goat serum (NGS) and 0.1 % Triton-X 100 in phosphate buffered saline (PBS) for 1 h at room temperature. After the sections were removed from the blocking solution, they were incubated in a primary antibody solution containing 5 % NGS, 0.1 % Triton-X 100, and a mouse monoclonal anti-parvalbumin antibody (1:1,000; P3088, Sigma) in PBS overnight at 4 °C. The following day, sections were washed three times in PBS prior to placement in a secondary antibody solution containing 5 % NGS, 0.1 % Triton-X, and a biotinylated goat anti-mouse antibody (1:80; B6398, Sigma) in PBS for one hour. Sections were then washed three times in PBS and placed in PBS containing Extravadin peroxidase (1:40, Sigma) for 1 h. Sections were washed three times and placed in PBS containing 0.05 % diaminobenzidine (DAB), 0.1 % nickel ammonium sulfate (NAS) and 0.003 % H_2O_2 for approximately 10 min. Sections were again washed three times in PBS and then mounted on chrom alum–gelatin subbed slides and coverslipped with Permount (Fisher). An Olympus BH-2 microscope was used to conduct cell counts. Parvalbumin-positive cell bodies were identified based on the shape and size of the cell. The total number of cell bodies in the MS/VDB was tabulated for each of six evenly spaced coronal brain section that ranged from AP +1.44 to AP +0.12. The Abercrombie correction (Abercrombie 1946) was applied to the total number of cell bodies in each coronal section to provide more accurate assessment parvalbumin-positive cells in the MS/VDB. The correct number of parvalbumin-positive cell bodies was averaged across the six coronal brain sections.

Results

Histology

Photomicrographs of parvalbumin staining in the MS/VDB are presented for a rat that received infusion of saline (see Fig. 1a, d), a rat that received infusion of GAT1-saporin with minimal damage (see Fig. 1b, e), and a rat that received infusion of GAT1-saporin with maximal damage (see Fig. 1c, f). Although MS/VDB damage varied across lesion rats, reductions in parvalbumin positive cells was consistently observed in rats that received infusions of GAT1-saporin. The average number of parvalbumin-positive cell bodies in the MS/VDB was reduced by approximately 85 % in the GAT1-saporin group (M: 2.5; SEM: 0.6), relative to the Sham group (M: 16.8; SEM: 0.6). The independent samples *T* test conducted on the average number of parvalbumin-positive cell bodies per section [$T\ (10) = 17.73$, $P < 0.001$, $d = -10.2$] revealed a significant difference between groups.

Photomicrographs of acetylcholinesterase staining at the level of the dorsal hippocampus are presented for representative rats that received infusion of saline (see Fig. 2a) or GAT1-Saporin (see Fig. 2b) into the MS/VDB. No evidence of a reduction of cholinergic function was observed between groups. The independent samples *T* tests conducted on the average optical density within the hippocampal subfields and overlying cortex failed to reveal any significant differences between groups (see Table 1).

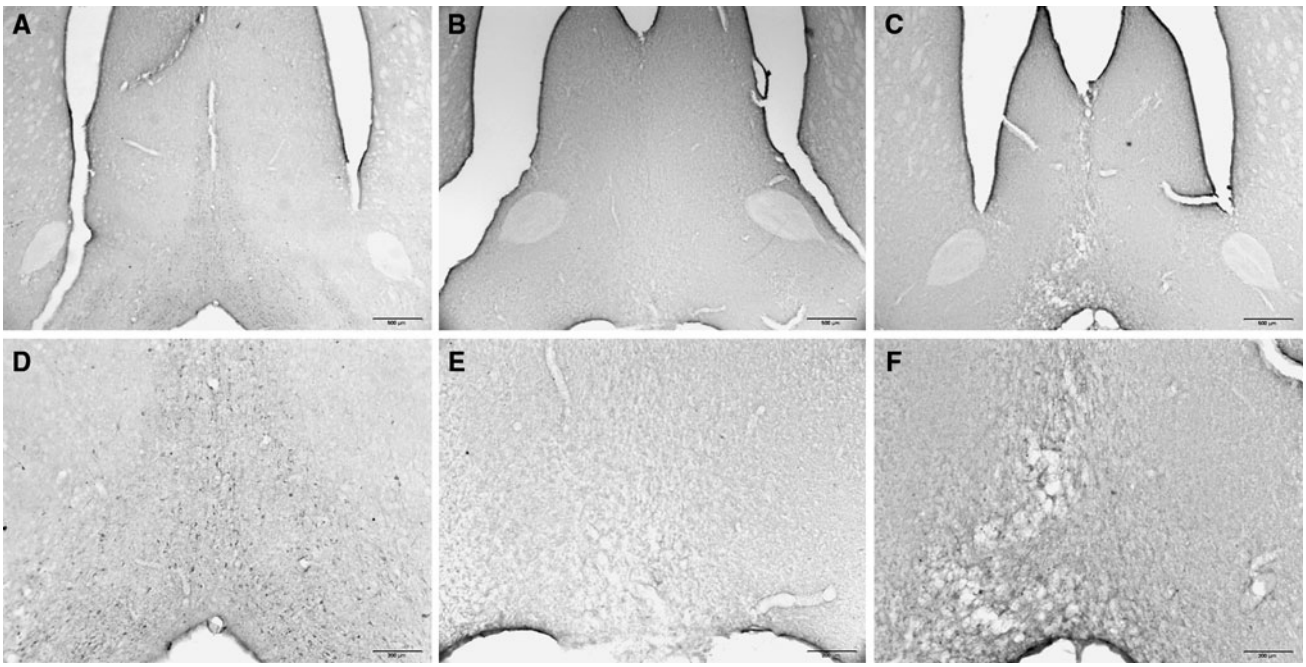

Fig. 1 Photomicrographs are presented from a representative Sham rat (**a**, **d**), a GAT1-saporin rat with minimal MS/VDB damage (**b**, **e**), and a GAT1-saporin rat with maximal MS/VDB damage (**c**, **f**). Parvalbumin positive sections at ×40 (**a–c** *scale bar*: 500 μm) and ×100 (**d–f** *scale bar*: 200 μm) are presented at the level of MS/VDB

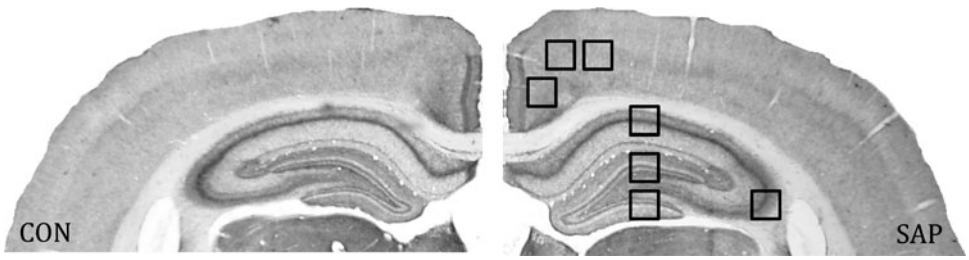

Fig. 2 Photomicrographs are presented from a representative rat in the Sham (*left panel*) and GAT1-Saporin (*right panel*) groups. Acetylcholinesterase stained sections are presented at the level of the dorsal hippocampus. *Boxes* represent the approximate regions of the brain optical densities were sampled from

Table 1 Average hippocampal and cortical optical density values

	Sham	GAT1
Hippocampus (AP: −3.0 mm)		
dDG	119.0 (5.8)	120.9 (1.2)
vDG	112.0 (4.3)	111.4 (3.9)
CA1	120.2 (4.4)	121.7 (5.0)
CA3	140.2 (5.8)	152.1 (3.7)
Cortex (AP: −3.0 mm)		
RSg	133.4 (4.3)	128.6 (5.4)
RSd	118.8 (2.5)	114.0 (5.2)
Motor	117.6 (2.0)	114.1 (4.5)

Group standard errors are provided in parentheses; anterior–posterior measurements are relative to bregma

dDG dorsal dentate gyrus, *vDG* ventral dentate gyrus, *RSg* retrospleinal granaular cortex, *RSd* retrospleinal disgranualar

Food-hoarding paradigm

Infusion of GAT1-saporin into the MS/VDB was associated with selective impairments in returning to the refuge during the food-hoarding paradigm. Food-hoarding trips during Cued-probe (see Fig. 3a, b), Uncued-probe (see Fig. 3c, d), and Dark-probe (see Fig. 3e, f) are plotted for representative Sham and GAT1-saporin rats. Although outward segment paths were more circuitous during the Dark-probe, these differences did not vary as a function of group (see Fig. 4a). The ANOVA conducted on average outward segment path circuity revealed a significant main effect of probe [F (2,20) = 35.221, $P < 0.001$, $\eta_p^2 = 0.779$]; however, neither the main effect of group [F (1,10) = 1.267, $P = 0.287$, $\eta_p^2 = 0.112$] nor the Group × Probe interaction [F (2,20) = 0.492, $P = 0.619$,

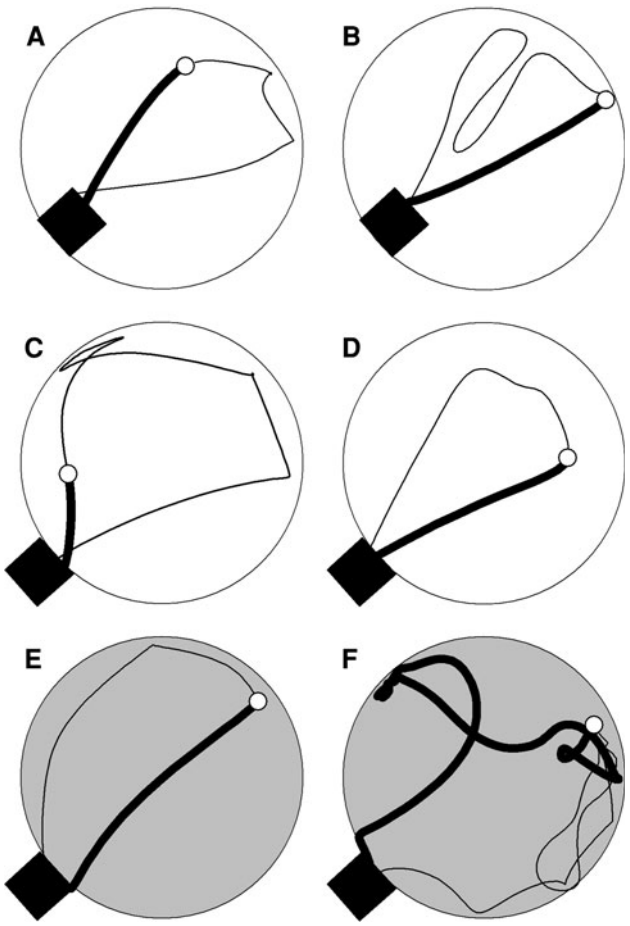

Fig. 3 A single food-hoarding trip is plotted for a representative rat from the Sham (**a**, **c**, **e**) and GAT1-Saporin (**b**, **d**, **f**) groups during the Cued-probe (**a**, **b**), Uncued-probe (**c**, **d**), and Dark-probe (**e**, **f**). The outward segment (*thin line*), food pellet (*white circle*), and homeward segment (*heavy line*) are indicated for each food-hoarding trip

$\eta_p^2 = 0.047$] were significant. Post hoc tests revealed that the outward segments were significantly more circuitous during the Dark-probe, relative to the Cued-probe and Uncued-probe ($P < 0.05$). Rats followed more circuitous paths in searching for the food pellet during the Dark-probe.

Group differences in homeward segment path circuity were only observed during the Dark-probe (see Fig. 4b). The ANOVA conducted on average homeward segment path circuity revealed a significant main effect of probe [F (2,20) = 76.139, $P < 0.001$, $\eta_p^2 = 0.884$], group [F (1,10) = 19.628, $P = 0.001$, $\eta_p^2 = 0.662$], and Group × Probe interaction [F (2,20) = 13.573, $P < 0.001$, $\eta_p^2 = 0.576$]. Post hoc tests revealed that group differences in homeward segment path circuity were only observed during the Dark-probe ($P < 0.05$). When restricted to self-movement cues, GAT1-saporin rats had significantly more circuitous homeward segments relative to Sham rats.

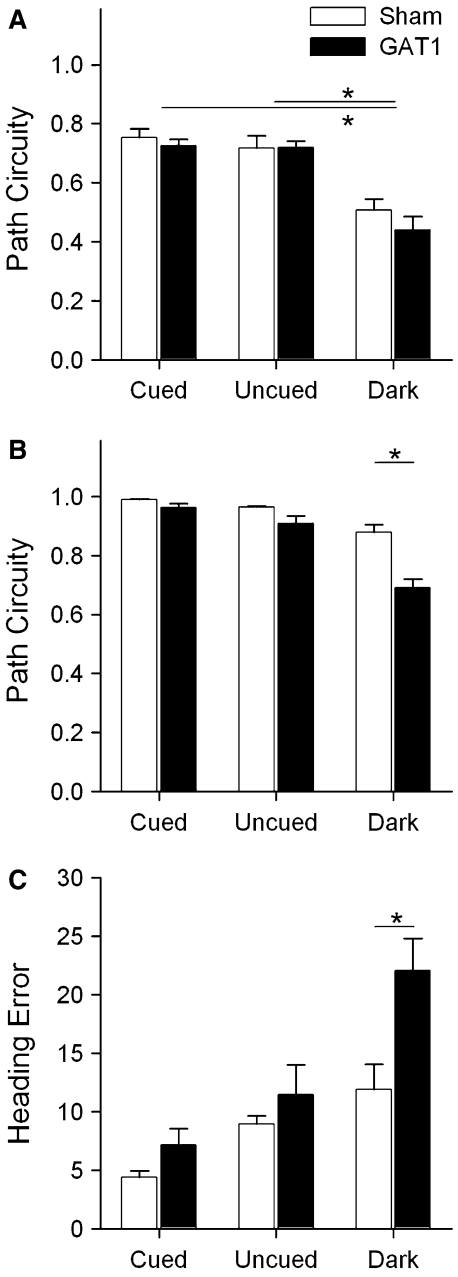

Fig. 4 Average outward (**a**) and homeward (**b**) segment path circuity is plotted for both groups during the Cued-probe, Uncued-probe, and Dark-probe. Average head error on the homeward segment (**c**) is plotted for both groups during the Cued-probe, Uncued-probe, and Dark-probe (* <0.05)

Homeward segment heading error was only observed to differ between groups during the Dark-probe (see Fig. 4c). The ANOVA conducted on average homeward segment heading error revealed a significant main effect of probe [F (2,20) = 28.969, $P < 0.001$, $\eta_p^2 = 0.743$], group [F (1,10) = 6.439, $P = 0.029$, $\eta_p^2 = 0.392$], and Group × Probe interaction [F (2,20) = 4.285, $P = 0.028$, $\eta_p^2 = 0.300$]. Post hoc tests revealed that group differences in

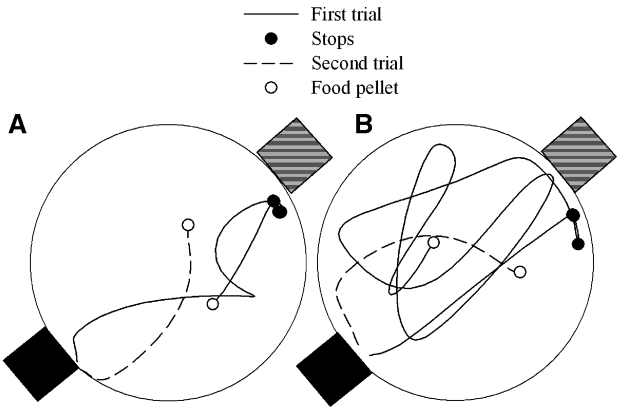

Fig. 5 The homeward segment for the first (*solid line*) and second (*dotted line*) trial of the New-probe is plotted for a representative rat from the Sham (**a**) and GAT1-Saporin (**b**) groups. The current (*black box*) and former (*gray box*) refuge location are presented in each panel

average heading error were only observed during the Dark-probe ($P < 0.05$). When restricted to self-movement cues, GAT1-saporin rats had significantly more circuitous homeward segments relative to Sham rats.

During the first trial of the New-probe, both groups made a number of stops at the former refuge location prior to returning to the new location of the refuge (see solid line plotted in Fig. 5a, b). In addition, both groups displayed an equivalent decrease in the tendency to return to the former refuge location on the second trial (see dotted line plotted on Fig. 5a, b). The ANOVA conducted on the average number of stops at the former refuge location revealed a significant effect of trial [F (1,10) = 13.852, $P = 0.004$, $\eta_p^2 = 0.581$]; however, neither the main effect of group [F (1,10) = 0.723, $P = 0.415$, $\eta_p^2 = 0.067$] nor the Group by Trial interaction [F (1,10) = 0.738, $P = 0.411$, $\eta_p^2 = 0.069$] were found to be significant. Groups exhibited a similar tendency to return to the previous refuge location that was restricted to the first trial.

Water maze

Infusion of GAT1-saporin into the MS/VDB spared performance in water maze tasks. During place training, both groups exhibited a similar decrease in latency to find the hidden platform (see Fig. 6a). The ANOVA conducted on average latency revealed a significant main effect of day [F (4,40) = 30.284, $P < 0.001$, $\eta_p^2 = 0.752$]; however, neither the effect of group [F (1,10) = 0.042, $P = 0.843$, $\eta_p^2 = 0.004$] nor the Group × Day interaction [F (4,40) = 0.212, $P = 0.930$, $\eta_p^2 = 0.021$] were found to be significant. Post hoc analysis revealed a significant linear trend in latency to find the platform across days [F (1,10) = 91.228, $P < 0.001$, $\eta_p^2 = 0.901$]. In addition, as place training progressed, both groups' swim paths became more

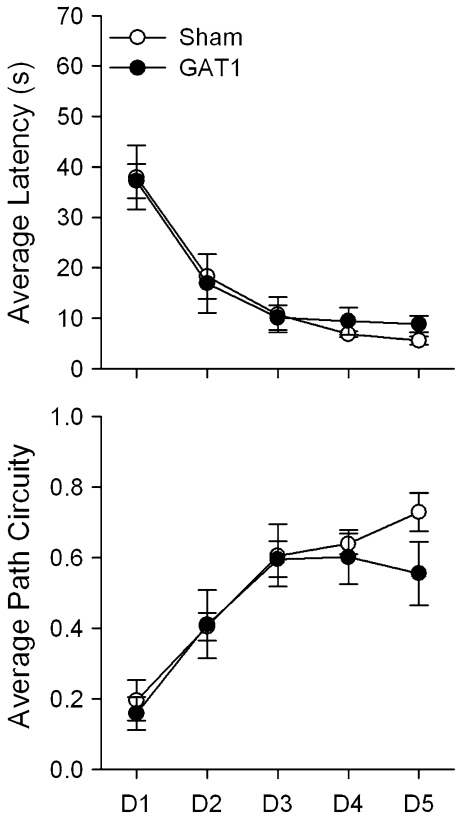

Fig. 6 Average latency (*top panel*) and path circuity (*bottom panel*) are plotted for both groups across all 5 days of place training in the water maze

direct (see Fig. 6b). The ANOVA conducted on average path circuity during place training revealed a significant effect of day [F (4,40) = 23.567, $P < 0.001$, $\eta_p^2 = 0.702$]; however, neither the effect of group [F (1,10) = 0.716, $P = 0.417$, $\eta_p^2 = 0.067$] nor the Group × Day interaction [F (4,40) = 0.762, $P = 0.556$, $\eta_p^2 = 0.071$] were found to be significant. Post hoc analysis revealed a significant linear trend in path circuity observed across days [F (1,10) = 53.678, $P < 0.001$, $\eta_p^2 = 0.843$]. Both groups displayed a similar improvement in locating the hidden platform across days.

During the shift probe, both groups exhibited a similar tendency to search the relative location for the hidden escape platform (see Fig. 7). The T-test conducted on average percent time spent swimming on the relative half of the water maze [$T(10) = 0.184$, $P = 0.857$, $d = 0.108$] failed to reveal a significant effect of group. The absence of significant group differences in the average amount of time spent searching the relative half of the water maze prompted collapsing across groups. The single sample T-test [$T(11) = 5.472$, $P < 0.001$, $d = 1.58$] conducted on percent time revealed that all rats spent significantly more time (mean 74.23, SD 12.34) on the relative side than expected by chance (test value = 50 %). Both groups

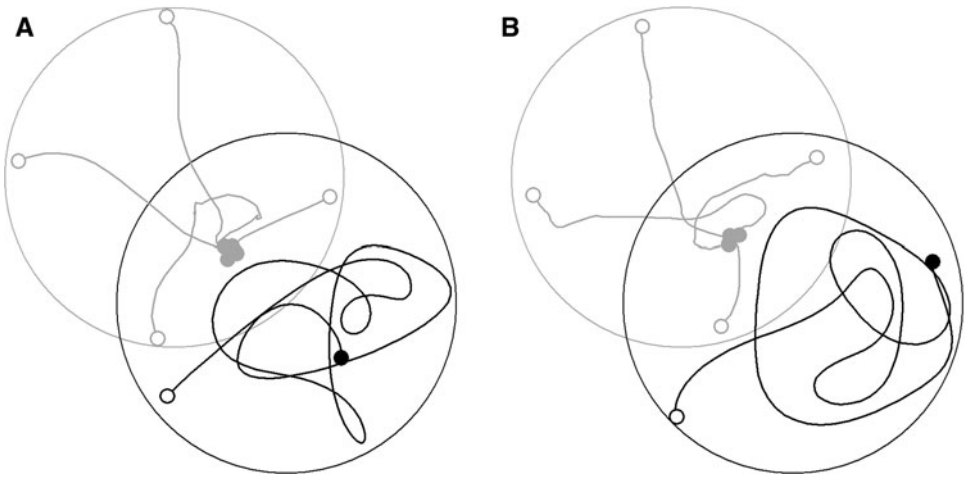

Fig. 7 The four swim paths and pool position (*gray lines*) are plotted for the final day of place training from a representative rat from the Sham (**a**) and GAT1-Saporin (**b**) groups. The start position (*open circle*), swim path, end position (*filled circle*), and location of the pool in the room are plotted for the Sham and GAT1-Saporin rats during the last day of place training (*gray lines*) and the shift probe (*black lines*)

displayed a similar tendency to exhibit a directional response.

Performance during the first or second trial of matching-to-place testing did not significantly differ between groups (see Fig. 8a, b). Both groups exhibited a decrease in the latency to find the platform from trial one to trial two (see Fig. 8c). The ANOVA conducted on average latency revealed a significant effect of trial [F (1,10) = 35.162, $P < 0.001$, $\eta_p^2 = 0.779$]; however, neither the main effect of group [F (1,10) = 0.026, $P = 0.875$, $\eta_p^2 = 0.003$] nor the Group × Trial interaction [F (1,10) = 0.155, $P = 0.702$, $\eta_p^2 = 0.015$] were found to be significant. In addition, both groups' swim paths became more direct from trial one to trial two (see Fig. 8d). The ANOVA conducted on average path circuity revealed a significant effect of trial [F (1,10) = 12.848, $P = 0.005$, $\eta_p^2 = 0.562$]; however, neither the effect of group [F (1,10) = 2.027, $P = 0.185$, $\eta_p^2 = 0.169$] nor the Group × Trial interaction [F (1,10) = 0.653, $P = 438$, $\eta_p^2 = 0.061$] were found to be significant. Although both groups had to search for the hidden escape platform on the first trial, they accurately found the new position on the second trial.

Discussion

The current study investigated the effects of infusing GAT1-saporin into the MS/VDB on performance in behavioral tasks that assess spatial orientation and mnemonic function. Infusing GAT1-saporin in the MS/VDB significantly reduced the number of parvalbumin cells into the MS/VDB while sparing markers of cholinergic function in the hippocampus and cortex. In addition, disruption in performance associated with infusion of GAT1-saporin was limited to the homeward segment during the Dark-probe of the food-hoarding task. The following sections discuss the implications of these observations in light of the role of the septohippocampal system in organizing behavior and the neurobiology of self-movement cue processing.

Functional role of the septohippocampal system

Advances in behavioral assessment and surgical techniques have shaped the current view of septohippocampal involvement in behavior. Discovering that hippocampal neurons have firing characteristics tuned to specific locations and independent of direction (O'Keefe and Dostrovsky 1971) led to the development of the theory that the hippocampus mediates encoding symbolic relationships between environmental cues (O'Keefe and Nadel 1978). This prompted studies examining the effects of damaging specific components of the septohippocampal system on performance during tasks thought to depend on cognitive mapping. For example, hippocampal (Morris et al. 1982; Sutherland et al. 1982a, b), medial septum (Kelsey and Landry 1988; Hagan et al. 1988), and fimbria-fornix (Sutherland and Rodriguez 1989) lesions were observed to disrupt place learning in the water maze. In contrast, alternative behavioral assessments (e.g., radial-arm maze) of septohippocampal damage provided evidence that was consistent with spared use of environmental cues and impaired spatial working memory (Olton et al. 1979; Walker and Olton 1984; Hepler et al. 1985). Further refinements in behavioral assessment provided additional evidence that septohippocampal lesions spared encoding relationships among environmental cues and provided a novel mechanism for explaining disruptions in performance attributed to impaired spatial working memory (Whishaw et al. 1995; Whishaw and Tomie 1997a, b). Specifically, the septohippocampal system contributes to processing cues generated because of movement in the corresponding temporal context to estimate direction and distance to the point where movement originated

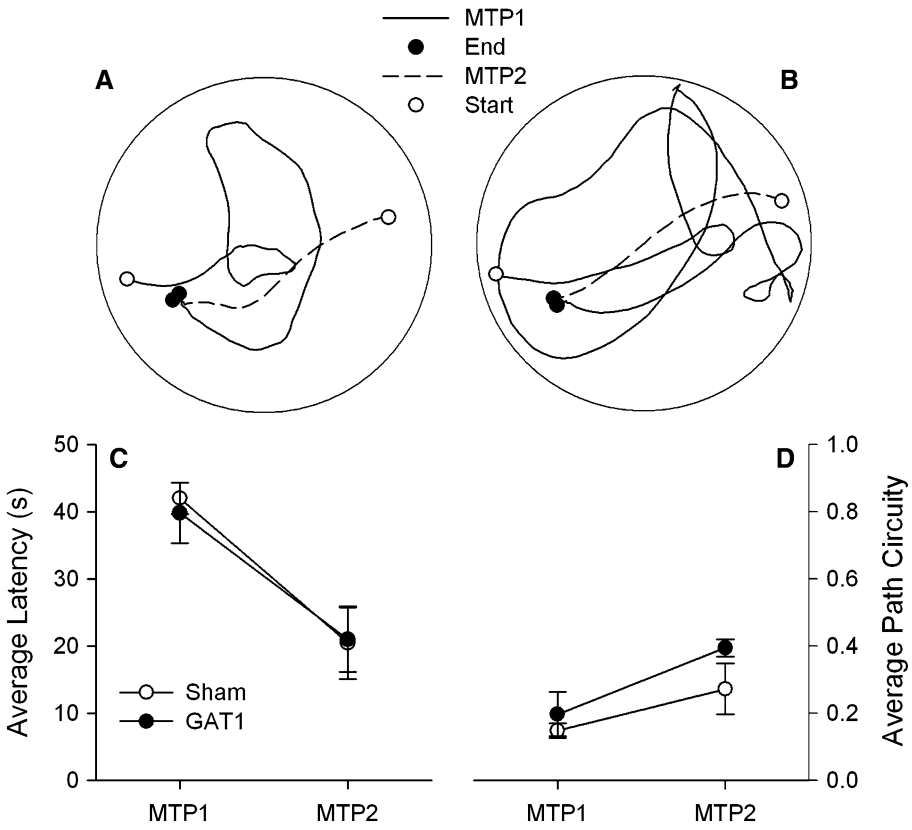

Fig. 8 The swim path of the first (*solid line*) and second (*dotted line*) trial of matching-to-place testing is plotted for a representative rat from the Sham (**a**) and GAT1-Saporin (**b**) groups. Average latency (**c**) and path circuity (**d**) are plotted for both groups across the first and second trials of matching-to-place testing in the water maze

(Whishaw and Maaswinkel 1998; Maaswinkel et al. 1999; Wallace and Whishaw 2003; Martin et al. 2007). Therefore, performance associated with disruptions in septohippocampal function reflects impaired self-movement cue processing and spared environmental cue processing (Whishaw 1998). Conceptualizing performance on behavioral tasks as dependent on parallel processing of self-movement or environmental cues to maintain spatial orientation has provided a framework to understand the effects of more selective lesion techniques.

Advances in lesion techniques have also provided insight to the function of the septohippocampal system. Development of 192-IgG-saporin immunotoxic lesion techniques enabled the selective destruction of septohippocampal cholinergic function (Wiley et al. 1991). Initial behavioral assessment of infusing 192-IgG-saporin in the MS/VDB failed to yield significant disruptions in performance on spatial tasks (Baxter and Gallagher 1996; Jonasson et al. 2004; Frielingsdorf et al. 2006; Kirby and Rawlins 2003; Vuckovich et al. 2004); however, both environmental and self-movement cues were available to guide movement in those tasks. Therefore, it is possible that the spared performance may actually reflect rats' use of compensatory navigational strategies to guide movement on those particular tasks. The food-hoarding paradigm, which includes probes that can manipulate cue access, has been used to investigate the effects of infusing 192-IgG-saproin into the MS/VDB on spatial orientation (Martin and Wallace 2007). Martin and Wallace (2007) showed that performance was spared when rats were provided access to environmental cues (i.e., Cued-probe and Uncued-probe), whereas performance was impaired when rats did not have access to environmental cues (i.e., Dark-probe) or the environmental cues conflicted with self-movement cues (i.e., New-probe). These observations are consistent with a role for the cholinergic component of the septohippocampal system in processing self-movement cues.

Recent work has used various toxins to characterize the function of the GABAergic component of the septohippocampal system. These studies have reported mixed results in disrupting performance on various behavioral tasks. For example, infusion of kainic acid into the MS/VDB failed to disrupt performance on standard versions of the radial-arm maze and water maze (Pang et al. 2001); however, disruption in performance was observed on a repeated acquisition task in the radial-arm maze (Dwyer et al. 2007). Although infusion of orexin–saporin has been shown to impair performance in the water maze and plus maze (Smith and Pang 2005; Lecourtier et al. 2011), this lesion technique appears to be less selective than the kainic acid lesion technique. Finally, infusion of GAT1-saporin into the MS/VDB has been shown to spare performance during place learning and impairs performance during delayed

matching-to-place testing in the water maze (Pang et al. 2011). In contrast, infusion of GAT1-saporin into the MS/VDB was observed to spare performance during matching-to-place testing in the water maze in the current study. Procedural differences between the matching-to-place testing in the Pang et al. (2011) study and the current study may have contributed to the varied results observed between the two experiments. Specifically, Pang et al. (2011) provided four trials prior to shifting the location of the hidden platform; whereas, the current study only provided two trials. Increasing the number of trials prior to shifting the location of the hidden platform may bias a tendency to perseverate to the former location of the hidden platform. Providing only two trials during matching-to-place testing might not have been sufficient to elicit strong enough response perseveration, thereby limiting the ability to detect group differences. The range of performance disruptions associated with a specific lesion technique has traditionally been attributed to varying levels of impaired mnemonic function (i.e., spatial working memory, proactive interference, or spatial memory consolidation); however, a growing literature has demonstrated the utility of behavioral tasks that can dissociate environmental and self-movement cue processing.

Results from the current study provide additional support for the role of the septohippocampal system in self-movement cue processing. Specifically, selectivity of lesion technique is related to the magnitude of the self-movement cue-processing deficit. Non-selective lesion techniques eliminate cholinergic and GABAergic components of the septohippocampal system and completely disrupt self-movement cue processing. Previous work has shown that accuracy of self-movement cue processing facilitates encoding relationships among environmental cues (Semenov and Bures 1989; Biegler and Morris 1996). Therefore, the near complete loss of self-movement cue processing associated with non-selective lesion techniques would be expected to attenuate, but not prevent, learning a response in spatial tasks. Previous work has demonstrated that non-selective lesion techniques result in self-movement cue processing deficits that were associated with attenuated acquisition of a place response in the water maze (Martin et al. 2007). Although both components of the septohippocampal system vary in anatomical connections and physiology, the cholinergic (i.e., excitatory) and GABAergic (i.e., inhibiting hippocampal interneurons) components have a similar net effect on hippocampal pyramidal neurons. Therefore, damage restricted to one component is likely to result in an intermediate level of impairment of self-movement cue processing. This level of self-movement cue processing deficit spares initial response learning in spatial tasks; however, disruptions in performance would be expected on tasks that eliminated environmental cues (i.e., Dark-probe) or place environmental and self-movement cues in conflict (i.e., New-probe). Previous work has shown that infusion of 192-IgG-saporin into the MS/VDB spares place learning in the water maze (Baxter and Gallagher 1996; Jonasson et al. 2004; Vuckovich et al. 2004; Frielingsdorf et al. 2006) and impairs homing accuracy during the Dark- and New-probes of the food-hoarding paradigm (Martin and Wallace 2007). The results of the current study are consistent with damage restricted to the GABAergic component producing a milder level of self-movement cue processing deficit, relative to damage restricted to the cholinergic component. Specifically, infusion of GAT1-saporin into the MS/VDB spared performance during the New-probe and impaired performance during the Dark-probe. This level of impairment may reflect an accelerated accumulation of error (i.e., changes in gain/leak of integration) during dead reckoning that can be corrected for when provided access to environmental cues. A similar mechanism has been advanced to explain the effects of aging on dead reckoning based navigation in humans (Harris and Wolbers 2012). Together these results demonstrate that the extent of septohippocampal system loss is related to the magnitude of self-movement cue processing deficit.

Neurobiology of self-movement cue processing

The septohippocampal system is part of larger network of structures that contribute to self-movement cue processing to maintain spatial orientation (Wallace et al. 2008). One component of this network is the set of structures that process information about direction (i.e., compass). Changes in heading are detected by the vestibular system (Goldberg and Fernández 1975) and communicated as a head direction signal through a series of structures to maintain a current representation of directional heading (for a review, see Taube 2007). Damage to structures within this system (i.e., vestibular system, dorsal tegmental nucleus, mammillothalamic tract, anterior dorsal thalamus, retrosplenial cortex, and entorhinal cortex) has been shown to impair rats' ability to use self-movement cues to guide movement or dead reckoning based navigation (Wallace et al. 2002; Zheng et al. 2006, 2009; Frohardt et al. 2006; Winter et al. 2011; Whishaw et al. 2001; Parron and Save 2004). In general, GABAergic projections that originate in the MS/VDB do not converge on neural structures that have been shown to be critical in the generation of the head direction signal. It is possible that infusion of GAT1-saporin into the MS/VDB spared lower level generation of the head direction cell signal, and this signal was sufficient to guide performance during conditions in which environmental cues were available. In the absence of

environmental cues, infusion of GAT1-saporin into the MS/VDB may have impaired higher level processing of the HD signal. An impaired ability to integrate this head direction cell signal within the temporal context in which it was generated (i.e., dead reckoning based navigation) produced the disruption in performance observed during the Dark-probe. Further research is needed to evaluate the contribution of the septohippocampal GABAergic system to head direction cell signal stability under conditions with varied access to environmental cues.

Another component of the self-movement cue network is the set of structures involved in detecting changes in position or rectilinear motion (i.e., odometer). Several electrophysiological signals have been discovered that may mediate processing of distance information. Although hippocampal field activity or theta rhythm has been implicated in a variety of processes (for a review, see Buzsáki 2005), characteristics of hippocampal theta have been consistently shown to vary in response to movement extent (Vanderwolf 1969; Morris and Hagen 1983; Whishaw and Vanderwolf 1971; Oddie et al. 1997). Neurons in the MS/VDB have been shown to contribute to hippocampal theta rhythm and performance on tasks that depend on distance estimation (Whishaw 1993; Bland and Oddie 1998; Yoder and Pang 2005). Another potential signal for rectilinear motion is the activity of medial entorhinal cortex grid cells as the rat moves through an environment (Fyhn et al. 2004; Hafting et al. 2005). Interestingly, some grid cells also display firing characteristics similar to head direction cells and conjunctive grid × direction cells (Sargolini et al. 2006). Recent work has demonstrated that infusion of the GABAergic agonist muscimol into the MS/VDB eliminated the grid like firing characteristics and spared directional firing characteristics in conjunctive grid × direction cells (Brandon et al. 2011). These observations demonstrate a role for MS/VDB neurons in generating hippocampal theta and maintaining firing characteristics of entorhinal cortex grid cells. Therefore, it is possible that infusion of GAT1-saporin into the MS/VDB impaired processing of self-movement cues related to rectilinear movement resulting in the performance deficits observed during the Dark-probe.

Finally, the hippocampus has been posited to be the component of the self-movement cue network that integrates information about changes in direction and distance within the appropriate temporal context to estimate the direction and distance to the point where movement originated, or dead reckoning based navigation (Wallace et al. 2008). A growing number of studies have implicated a role for the hippocampus in dead reckoning-based navigation. First, excitotoxic lesions of the hippocampus have been shown to spare use of environmental cues and impair dead reckoning (Maaswinkel et al. 1999; Wallace and Whishaw 2003; however, see Alyan and McNaughton 1999). Similar patterns of results have been observed in human patients with damage restricted to the medial temporal lobe (Philbeck et al. 2004; however, see, Shrager et al. 2008). In addition, selective hippocampal activation has been observed during virtual dead reckoning tasks (Wolbers et al. 2007). The processing involved in dead reckoning has been compared to the processing posited to mediate episodic memory (Whishaw and Wallace 2003; Hasselmo and Brandon 2008). In fact, recent theories have characterized episodic memory as encoding and retrieving specific spatiotemporal trajectories (Hasselmo et al. 2010; for a review see Hasselmo 2011). Computational modeling of the biophysical properties of septohippocampal neurons has identified MS/VDB GABAergic neurons as essential for encoding and retrieval of spatiotemporal trajectories (Cutsuridis et al. 2010; Cutsuridis and Hasselmo 2012). The current study supports a role for the hippocampus in dead reckoning-based navigation and provides empirical evidence consistent with MS/VDB GABAergic neurons' involvement in episodic memory.

Selectivity of GAT1-saporin lesion technique

Infusion of GAT1-saporin into the MS/VDB was intended to produce a selective GABAergic deafferentation of the hippocampus. Several lines of evidence support the selectivity of the GAT1-saproin lesion technique. First, markers of GABAergic neurons (i.e., glutamic acid decarboxylase [GAD] and parvalbumin) have been shown to decrease by approximately 85 % with the infusion of GAT1-saporin into the MS/VDB (Pang et al. 2011). Similar results were observed in the current study; infusion of GAT1-saporin into the MS/VDB resulted in an 85 % reduction in parvalbumin-positive neurons. This represents a significant loss of hippocampal GABAergic afferents; however, these results must be evaluated relative to damage to non-GABAergic MS/VDB neurons.

Cholinergic neurons represent another subset of cells in the MS/VDB that may be influenced by the GAT1-saporin lesion technique. Previous work has demonstrated that GAT1-saporin has a limited effect (28 % reduction) on cholinergic neurons within the MS/VDB (Pang et al. 2011). This is in contrast to the near total loss of Choline acetyltransferase (ChAT) positive neurons and the 80 % reduction in Acetylcholinesterase (AChE) positive staining in the hippocampus associated with infusing 192-IgG-saporin into the MS/VDB (Lehmann et al. 2003). Results from the current study demonstrate that infusion of GAT1-saporin into the MS/VDB did not significantly reduce optical density of AChE staining in the hippocampal or cortical areas sampled. Although failure to find reductions

in AChE staining in the hippocampus does not preclude that the lesion had limited effects on ChAT positive neurons in the MS/VDB, multiple studies have demonstrated a direct relationship between these markers of cholinergic function (Naumann et al. 1997; Gu et al. 1998; Birthelmer et al. 2003; Vuckovich et al. 2004; Aisa et al. 2009; Leung et al. 2011). Provided that infusion to GAT1-Saporin into the MS/VDB selectively reduced the number of parvalbumin cells, it is still possible that the cell loss may have influenced the function MS/VDB cholinergic cells. For example, the loss of GABAergic neurons within the MS/VDB may have attenuated the inhibitory control over MS/VDB cholinergic neurons resulting in an increased cholinergic tone in projection areas. Considering that previous work has shown that infusion of 192-IgG-saporin into the MS/VDB reduces release of acetylcholine in the hippocampus (Chang and Gold 2004), combining infusion of GAT1-saporin into the MS/VDB with hippocampal microdialysis may provide further insight to role of the cholinergic and GABAergic components of the septohippocampal system.

Several other types of neurons have been identified in the MS/VDB that may have been sensitive to the infusion of GAT1-saporin. Although colocalization of calcium binding proteins (i.e., parvalbumin, calbindin, calretinin) and GAD has been used to identify GABAergic cells in the hippocampus (Freund and Buzsáki 1996; Shetty and Turner 1998) and cortex (DeFelipe 1997; Hof et al. 1999), a different pattern of colocalization has been observed in the MS/VDB. GAD has been shown to be colocalized in a majority of parvalbumin positive neurons (90 %); whereas, GAD has been shown to be colocalized in relatively few calbindin (5 %) and calretinin (10 %) positive neurons (Gritti et al. 2003). Calbindin and calretinin positive neurons were observed to exhibit moderate levels of phosphate-activated glutaminase (i.e., an enzyme involved in the synthesis of glutamate) colocalization. Therefore, it remains to be determined whether reduction in either class (i.e., calbindin or calretinin) of potential glutamatergic neurons may have contributed to the behavioral impairments associated with infusing GAT1-saporin into the MS/VDB. Further work is needed to characterize the selectivity of infusing GAT1-saporin into the MS/VDB.

Conclusion

Infusion of GAT1-saporin into the MS/VDB significantly reduced the number of parvalbumin positive neurons in the MS/VDB while sparing markers of cholinergic function in the hippocampus and cortex. The reduction in the number of parvalbumin-positive neurons in the MS/VDB was associated with impaired self-movement cue processing but did not affect environmental cue use or mnemonic function. These results provide additional evidence for the role of the septohippocampal system in self-movement cue processing and add to a growing literature characterizing the neurobiology of spatial orientation. Observing a relationship between the function of the septohippocampal system and self-movement cue processing in rats provides a translational model to investigate the factors that contribute to wandering behavior observed during the progression of DAT. For example, recent work has demonstrated deficits in self-movement cue processing observed during the progression of DAT that was related to disruptions in spatial orientation (Tetewsky and Duffy 1999; Kavcic et al. 2006; Mapstone and Duffy 2010). The combination of selective lesion techniques and behavioral tasks that can dissociate the type of cues used to guide movement and mnemonic function provides the foundation necessary to model disruptions in spatial orientation associated with neurodegenerative disorders.

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