RESEARCH ARTICLE

Differential impacts on multiple forms of spatial and contextual memory in diazepam binding inhibitor knockout mice

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Abstract

Learning and memory are fundamental processes that are disrupted in many neurological disorders including Alzheimer's disease and epilepsy. The hippocampus plays an integral role in these functions, and modulation of synaptic transmission mediated by γ-aminobutyric acid (GABA) type-A receptors (GABA$_A$Rs) impacts hippocampus-dependent learning and memory. The protein diazepam binding inhibitor (DBI) differentially modulates GABA$_A$Rs in various brain regions, including hippocampus, and changes in DBI levels may be linked to altered learning and memory. The effects of genetic loss of DBI signaling on these processes, however, have not been determined. In these studies, we examined male and female constitutive DBI knockout mice and wild-type littermates to investigate the role of DBI signaling in modulating multiple forms of hippocampus-dependent spatial learning and memory. DBI knockout mice did not show impaired discrimination of objects in familiar and novel locations in an object location memory test, but did exhibit reduced time spent exploring the objects. Multiple parameters of Barnes maze performance, testing the capability to utilize spatial reference cues, were disrupted in DBI knockout mice. Furthermore, whereas most wild-type mice adopted a direct search strategy upon learning the location of the target hole, knockout mice showed higher rates of using an inefficient random strategy. In addition, DBI knockout mice displayed typical levels of contextual fear conditioning, but lacked a sex difference observed in wild-type mice. Together, these data suggest that DBI selectively influences certain forms of spatial learning and memory, indicating novel roles for DBI signaling in modulating hippocampus-dependent behavior in a task-specific manner.

KEYWORDS
Barnes maze, contextual fear conditioning, diazepam binding inhibitor, hippocampus, knockout mouse, object location memory, sex differences

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Significance

The diazepam binding inhibitor (DBI) peptide is implicated in both normal and pathological functions in the central nervous system. In the hippocampus, DBI modulates GABA$_A$ receptor-mediated transmission and may thus be involved in shaping hippocampus-dependent behavioral tasks. However, the impacts of the genetic loss of DBI signaling on hippocampal learning and memory in mice have not been investigated. In these studies, we demonstrate that DBI knockout mice display task-specific impairments in different assays of spatial and contextual learning and memory. These data offer further support for a critical role of DBI in proper learning and memory functions.

1 | INTRODUCTION

The processes of learning and memory are indispensable to proper cognitive function and are impaired in a variety of neurological disorders, including Alzheimer's disease, other forms of dementia, and epilepsy. Decades of research has established that the hippocampus plays imperative roles in these functions (Burgess, Maguire, & O'Keefe, 2002; Ergorul & Eichenbaum, 2004; Scoville & Milner, 1957; Zola et al., 2000). Although learning and memory have been rigorously studied over the years, elucidation of the myriad molecular players involved in hippocampus-dependent learning and memory remains incomplete.

The γ-aminobutyric acid (GABA) neurotransmitter system is critically involved in hippocampal function, and manipulations of GABA transmission have been linked to alterations in learning and memory. For example, injections of muscimol, a GABA$_A$ receptor (GABA$_A$R) agonist, into the dorsal hippocampus blocks long-term memory of object location (Haettig et al., 2011), and mice with a genetic deletion of the GABA$_A$R α5 subunit display enhanced performance in the Morris water maze task of spatial memory (Collinson, Atack, Laughton, Dawson, & Stephens, 2006). Treatment with benzodiazepines, which exert effects via allosteric modulation of GABA$_A$Rs, impacts multiple measures of spatial memory (Hogan et al., 2005; Joksimović et al., 2013; Kant, Wylie, Vasilakis, & Ghosh, 1996; Timić et al., 2013). Furthermore, mice with a point mutation that renders α5-containing GABA$_A$Rs insensitive to benzodiazepines show improved performance in trace fear conditioning (Crestani et al., 2002). These findings suggest that modulatory actions on GABA$_A$Rs, and specifically at the benzodiazepine binding site, can have profound impacts on hippocampal learning and memory.

Diazepam binding inhibitor (DBI), also known as acyl-CoA binding protein, is a 10 kDa protein that was first identified for its ability to displace diazepam from GABA$_A$R benzodiazepine binding sites (Guidotti et al., 1983). DBI protein immunoreactivity is present in several brain areas, including the hippocampus (Ball, Ghatei, Sekiya, Krausz, & Bloom, 1989; Ferrarese et al., 1989). Importantly, DBI can modulate GABA$_A$Rs in various brain regions. Negative allosteric modulation of GABA$_A$Rs by DBI was seen in cultured spinal neurons (Bormann, 1991) and in transit-amplifying cells of the subventricular zone (Alfonso, Magueresse, Zuccotti, Khodosevich, & Monyer, 2012). In the thalamic reticular nucleus, however, DBI acts as a positive allosteric GABA$_A$R modulator (Christian et al., 2013; Christian & Huguenard, 2013), indicating that the modulatory effects of DBI are region-specific. In this regard, our lab recently demonstrated hippocampal subregion-specific alterations in GABA$_A$R-mediated transmission in DBI knockout (DBI$^{-/-}$) mice (Courtney & Christian, 2018), suggesting that DBI may play a role in hippocampus-dependent learning and memory processes. DBI may also modulate GABA transmission via mechanisms independent of actions at GABA$_A$R benzodiazepine binding sites. For example, DBI may upregulate the biosynthesis of neurosteroids, another class of GABA$_A$R allosteric modulators, by acting as an endogenous ligand of the mitochondrial benzodiazepine receptor (also known as 18 kDa translocator protein, TSPO) (Korneyev et al., 1993; Papadopoulos, Berkovich, Krueger, Costa, & Guidotti, 1991). Furthermore, DBI could modulate neuronal and/or glial function through effects on lipid metabolism and other intracellular actions (Bouyakdan et al., 2015; Neess, Bek, Engelsby, Gallego, & Faergeman, 2015), and the *Dbi* gene also has ubiquitous housekeeping functions (Mandrup et al., 1992). DBI is thus capable of exerting multiple biological actions that have impacts on neural cellular and synaptic physiology and thus modulate complex behaviors.

Interactions between DBI and GABA$_A$Rs may influence many behavioral abnormalities (Costa & Guidotti, 1991). Notably, cerebrospinal fluid levels of DBI are elevated in patients with depression, anxiety, and hepatic encephalopathy (Barbaccia et al., 1986; Rothstein et al., 1989). Furthermore, studies of animal behavior suggest a role for DBI in various hippocampus-dependent tasks. For example, a DBI-overexpressing transgenic mouse line displays impaired learning performance on the Morris water maze (Siiskonen et al., 2007). Additionally, intracerebroventricular injection of DBI reverses a corticotropin-releasing hormone receptor-mediated enhancement in contextual fear conditioning (Sherrin et al., 2009). However, investigations into the role of DBI in hippocampus-dependent cognitive tasks using mice with genetic removal of DBI are lacking. Therefore, the primary purpose of the present studies was to identify the behavioral phenotypes of a constitutive DBI knockout mouse (Neess et al., 2011) to determine roles for DBI signaling in a variety of hippocampus-dependent behaviors. We hypothesized that mice with a genetic knockout of DBI would display impaired performance compared with wild-type mice with functional DBI signaling. We employed three well-validated assays of hippocampal learning and memory: object location memory (OLM) (Sharma, Rakoczy, & Brown-Borg, 2010; Vogel-Ciernia & Wood, 2014); Barnes maze (Barnes, 1979; Holmes, Wrenn, Harris, Thayer, & Crawley, 2002); and contextual fear conditioning (Curzon, Rustay,

& Browman, 2009). These tasks were selected to assess multiple forms of hippocampal-dependent spatial and contextual learning and memory. Our results indicate that mice with a genetic loss of DBI display differential performance across the tasks compared with control mice, providing novel evidence of a role for DBI in modulating specific forms of hippocampus-dependent learning and memory, most notably spatial navigation memory.

2 | MATERIALS AND METHODS

2.1 | Mice

All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Illinois at Urbana-Champaign. DBI heterozygous (DBI$^{+/-}$) and homozygous knockout (DBI$^{-/-}$) founder mice on the C57BL/6BomTac background (Neess et al., 2011) were acquired from Dr. Susanne Mandrup (University of Southern Denmark). Re-derivation of the colony and back-crossing onto the C57BL/6J background were described previously (Ujjainwala, Courtney, Rhoads, Rhodes, & Christian, 2018). Breeding pairs of DBI$^{+/-}$ males and females yielded DBI$^{+/+}$, DBI$^{+/-}$, and DBI$^{-/-}$ pups, and genotypic identities were confirmed via PCR as previously described (Neess et al., 2011). Mice used in the present experiments were produced in the fifth and sixth filial generations of this colony after backcrossing was completed. Mice were bred and housed on a 14:10 hr light:dark cycle with food and water available ad libitum. At weaning, mice were group housed (up to 5 mice per cage) with littermates of the same sex. A total of 34 mice were used for all 3 behavioral tests, consisting of 16 males (DBI$^{+/+}$ n = 9, DBI$^{-/-}$ n = 7) and 18 females (DBI$^{+/+}$ n = 9, DBI$^{-/-}$ n = 9). An additional 12 mice (males: DBI$^{+/+}$ n = 3, DBI$^{-/-}$ n = 3; females DBI$^{+/+}$ n = 3, DBI$^{-/-}$ n = 3) were used as unshocked controls in fear conditioning. For female mice, the estrous cycle stage was recorded by examining their vaginal cytology (Li et al., 2017) on each first day of testing for OLM, Barnes maze, and contextual fear conditioning. A Fisher's exact test demonstrated that the proportions of the estrous cycle stages were not significantly different between DBI$^{+/+}$ and DBI$^{-/-}$ mice on any of the three tests (OLM: odds ratio = 4, p = 0.33; Barnes maze: odds ratio = 0.64, p = 1; contextual fear conditioning: odds ratio = 1, p = 1), so all females tested were included in all the analyses. Mice were postnatal days (P) 50-250 old at the time of testing. OLM testing was performed when mice were P57-199, Barnes maze testing was performed when mice were P85-230, and contextual fear conditioning was performed when mice were P92-248 (Table 1). Two-way analysis of variance (ANOVA) of age with genotype and sex as factors showed a main effect of sex on all three tests (p < 0.025 for all tests), in which female mice were older than male mice by an average of 40.5 days.

2.2 | Behavioral testing

Experiments were performed between 10 a.m. and 2 p.m., relative to 7 p.m. lights off, in accordance with other studies testing

TABLE 1 Age of mice on individual behavioral tasks

Age at testing			
Group	Object location memory	Barnes maze	Fear conditioning
DBI$^{+/+}$ males			
666 L	P199	P214	P229
690 R	P193	P224	P243
708 R	P189	P219	P232
780 N	P113	P149	P155
780 L	P113	P149	P155
780 R	P113	P149	P155
808 N	P92	P134	P164
808 L	P92	P134	P164
820 2R	P126	P140	P148
DBI$^{+/+}$ females			
645 L	P189	P196	P234
642 R	P187	P216	P231
657 L	P190	P215	P230
676 N	P182	P197	P212
688 N	P187	P218	P244
706 N	P189	P219	P232
706 L + R	P189	P219	P232
809 N	P92	P140	P164
862 N	P84	P86	P100
DBI$^{-/-}$ males			
647 R	P187	P216	P231
654 N	P190	P221	P229
654 R	P190	P221	P229
690 L + R	P193	P224	P243
855 L	P73	P85	P108
855 R	P73	P85	P108
861 N	P57	P87	P95
DBI$^{-/-}$ females			
645 N	P189	P196	P234
657 R	P190	P222	P230
655 N	P191	P229	P249
655 L	P191	P229	P249
665 L	P192	P214	P228
665 R	P192	P208	P228
688 R	P187	P218	P244
688 L + R	P187	P218	P244
688 2L+R	P187	P225	P244

Note. Postnatal age (days) at which each individual mouse started each behavioral task, broken down by genotype and sex. Mouse identities indicate cage number and ear tag.

hippocampus-dependent behavior (Kleschevnikov et al., 2012; Patil, Sunyer, Höger, & Lubec, 2009; Paylor, Tracy, Wehner, & Rudy, 1994). For all tasks, mice were tested in arenas with ambient light levels

between 0.1 and 0.2 mW. Animals were tested in order of litter date of birth. Although the mice were not intentionally randomized for testing, the heterozygote breeding strategy created a nearly random mixture of genotype and sex combinations. All mice were tested on all behavioral tests described (excluding the unshocked control animals for contextual fear conditioning), performed in the following order: (1) OLM; (2) Barnes maze; and (3) contextual fear conditioning, with 1 to 30 days in between tests (Table 1). This order was chosen so that the task with the least complexity was performed first (OLM), followed by the more complex Barnes maze task. Contextual fear conditioning was performed last to prevent the possibility of retained fear behavior influencing performance on the other tasks. This serial testing paradigm significantly diminishes the inter-individual variation that would likely arise if the order of testing had been counterbalanced.

2.3 | OLM

The following procedure was adapted from previous reports (Barrett et al., 2011; Bevins & Besheer, 2006; Bui et al., 2018; Kleschevnikov et al., 2012; McQuown et al., 2011; Roozendaal et al., 2010; Vogel-Ciernia et al., 2013). On the day of testing, mice were brought to the testing room, weighed, and placed individually into clean cages with fresh bedding to habituate for 30 min. A clear, glass-walled 25 cm × 25 cm terrarium without bedding was used as the testing chamber. Prior to the start of each phase and experiment, the testing chamber and objects were cleaned with 70% ethanol. Each test consisted of two phases: the acquisition phase and the testing phase. The testing area was an enclosed space isolated from the rest of the laboratory room by opaque curtains hanging from the ceiling. Each mouse was tested individually, and mice were left undisturbed for the duration of the testing. Prior to the acquisition phase, two identical plastic objects were placed diagonally from each other in opposite corners, approximately 6 cm away from each wall, and secured into place using Velcro. The objects used were triangular in shape, with each side 4.5 cm in length.

Following acclimation to the testing room, mice began the acquisition phase. Each mouse was individually placed in the center of the testing chamber with the two objects present and allowed to explore freely for 10 min. The activity of the mouse was recorded using a video camera. Mice were then returned to the home cage and colony. The testing phase occurred 24 hr following the acquisition phase. Prior to beginning the testing phase, one object was moved to the corner vertically opposite from its initial location, while the other object remained in its former location. The moved object was counterbalanced across subjects. During the testing phase, mice were placed into the center of the testing chamber and allowed to explore the chamber for 3 min. The percentage of time spent exploring either object was recorded and scored from the video. Interaction with an object was defined as nose sniffing and head orientation within <1.0 cm of an object, without any further interaction with the object (e.g., biting or climbing). The discrimination index (DI) was computed as $DI(\%) = ((T_{new} - T_{old})/$

$(T_{new} + T_{old})) \times 100$, T_{new} representing the amount of time spent interacting with the moved object and T_{old} representing the time spent interacting with the unmoved object. Side preference for the acquisition phase was calculated using the same DI equation as above, replacing T_{new} with interaction with the object on the left and T_{old} with interaction with the object on the right. For analyses, all video identities were coded and randomized, and scorers were blinded to genotype and sex.

2.4 | Barnes maze

The following procedure was adapted from previous reports (Harrison, Reiserer, Tomarken, & McDonald, 2006; Patil et al., 2009; Rosenfeld & Ferguson, 2014). In the Barnes maze test, mice use extra-maze cues located around the testing room to learn how to escape from the open maze to a dark "escape box" located underneath the platform. The custom-built Barnes maze consisted of a circular grey, type 1 PVC sheet 91.44 cm in diameter and 91.44 cm from the ground, with 20 equally spaced holes (each 5.08 cm in diameter) located 2.85 cm from the edge of the maze. Each hole was left open during each trial with the exception of the target hole, under which was a black cast acrylic escape box (5.08 × 10.16 × 4.76 cm) to which mice had direct access. Because the escape box was located directly under the maze, mice could not visually identify the location of the escape hole from most points on the maze. Prior to each trial, the escape box was cleaned with 70% ethanol and filled with approximately 2.5 cm of fresh bedding. Three extra-maze cues were placed approximately 38.1 cm away from the edges of the maze in cardinal directions surrounding the maze. Extra-maze cues were similar in size yet distinctive from one another, and included a patterned throw pillow, a stuffed animal toy, and a colorful printed image. The experimenter acted as the fourth cue during the trials by sitting in a chair in a consistent place.

Prior to testing, each mouse was brought to the testing room, weighed, and individually placed in a clean cage with fresh bedding to habituate for 30 min. Directly before the start of each trial, mice that were not immediately undergoing testing were moved from the testing room to a different laboratory room ("waiting room") to prevent them from being prematurely exposed to the buzzer (see below). At the conclusion of the four trials, the test mouse was returned to the waiting room, and the next mouse to undergo testing was moved from the waiting room into the testing room. Mice were transported in covered cages at all times.

For the pretraining trial (day 0), the mouse was placed in the center of the maze in a black opaque cylindrical start chamber (10.2 cm in diameter), and a buzzer tone (85 dB) and light (positioned directly above the maze) were turned on. The buzzer was emitted via two speakers positioned near the maze. After 10 s elapsed, the start chamber was lifted and the mouse was gently guided into the escape box. After the mouse entered the escape box, the buzzer and light were turned off and a square lid was placed over the hole. The mouse remained in the hole for 2 min and was returned to the habituation cage following the trial.

On days 1–4, training trials began with the mouse in the center of the maze under the same start chamber that was used during the pre-training trial. Ten seconds following the onset of the light and buzzer, the chamber was lifted and the mouse was allowed to freely explore the maze. The trial ended after the mouse entered the escape box, or if 3 min elapsed. After all four paws of the mouse entered the escape box, the light and the buzzer were immediately turned off and the mouse was allowed to stay in the box for 1 min. If the mouse left the escape box, it was gently guided back in. Mice underwent four trials per day for 4 days; each trial was separated by a 15-min inter-trial interval. During the inter-trial interval of one mouse, up to two more mice would be run. The maze was cleaned with 70% ethanol following each trial, and the maze was rotated after each mouse had undergone a trial in order to eliminate any odor cues still present on the maze. The target box was subsequently moved to compensate for the maze rotation.

On day 5, mice underwent one probe trial. The purpose of this trial was to evaluate the short-term retention memory the subjects had for the target hole. During the probe trial, the escape box under the target hole was removed. The probe trial began similarly to the pretraining and training trials and the mouse was allowed to explore the maze for 90 s under the buzzer- and light-aversive stimuli.

Trials were recorded using TopScan video tracking software (CleverSys, Reston, VA). During the analysis of the videos, scorers were blinded to mouse genotype and sex. From the training trials, latency to enter the escape tunnel, primary latency, distance travelled, search strategy, and number of total errors were analyzed. Errors were identified as nose pokes over any hole that was not defined as the target hole. The definition for primary latency, the first uninterrupted 3-s interaction with the target hole, was adapted from a previous report (Harrison et al., 2006). The search strategy was divided into three categories: (a) direct, navigating directly to the target or adjacent hole with three or fewer errors without crossing the center of the maze more than once; (b) serial, searching consecutive holes in a clockwise or counterclockwise manner starting at least two holes away from the target hole; (c) random, unorganized search that involves crossing through the center of the maze. Each mouse was classified into a single strategy for each trial. For analysis of the probe trial, the number of nose pokes into each hole for the duration of the trial was calculated.

2.5 | Contextual fear conditioning

The following procedure was adapted from a previous report (Clark et al., 2008). Prior to testing, each mouse was brought to the testing room, weighed, and individually placed in a clean cage with fresh bedding to habituate to the room for 30 min. Mice that were not being tested were separated from the testing area by an opaque curtain, and were placed on a different table than that holding the fear conditioning apparatus. The contextual fear conditioning experiment took place over the span of 2 days: the training phase (day 1) and the testing phase (day 2). On day 1, mice were individually placed in a fear conditioning chamber with a metal grid floor that

delivers shock stimuli under the control of a digital timer (Med Associates, St. Albans, VT) (Clark et al., 2008; Kohman et al., 2012). Mice were allowed to explore the chamber for 3 min. During the training phase, mice received one footshock (0.5 mA, 2-s duration) (Clark et al., 2008; Curzon et al., 2009) at 120 s and a second shock at 150 s. Thirty seconds after the final shock, mice were returned to the habituation cage, then returned to the home cage and colony. Twenty-four hours later, mice were placed in the same chamber as above for a 3-min test in the absence of a shock. Control animals were not shocked on either day. Between days and test subjects, the chamber, grid, and the area under the grid were cleaned with 70% ethanol.

Trials were recorded using TopScan video tracking software. The total percentage of time spent freezing and distance traveled were recorded for each day. Recorders were blinded to genotype and sex of the test subjects for analysis.

2.6 | Statistical analysis

In the OLM task, both the percentage of time spent sniffing and the DI were analyzed using a three-way repeated measures ANOVA, with day as the repeated within-subjects factor and sex and genotype as between-subjects factors. For situations in which a significant interaction involving day was detected, the analysis was further broken down for each individual day using two-way ANOVA with sex and genotype as between-subjects factors.

In the Barnes maze task, latency, primary latency, distance traveled, and number of errors (averaged across 4 trials per day) over four consecutive days were analyzed using three-way repeated measures ANOVA with day as the repeated within-subjects factor and sex and genotype as between-subjects factors. In cases where sex or the interaction between sex and other variables were insignificant, two-way repeated measures ANOVAs (with day and genotype as factors) were applied. Search strategy was assessed using a logistic regression with sex and genotype as between-subjects factors and day as a within-subjects factor. The probabilities of using the direct, serial, or random strategy were analyzed separately. For these analyses, the deviance is reported instead of the F statistic. Comparisons of the probability of using each search strategy between genotypes within days, or within genotypes between day 1 to day 4, were made using Fisher's exact tests. For the probe trial, a three-way repeated measures ANOVA was performed with hole as the repeated within-subjects factor and sex and genotype as between-subjects factors. When required, all pairwise comparisons were made using Fisher's least significant difference (LSD) *post hoc* tests for all Barnes maze parameters.

For the contextual fear conditioning task, freezing behavior and total distance traveled were analyzed using three-way repeated measures ANOVA with day as the repeated measure and sex and genotype as between-subjects factors. Due to significant interactions involving day, both freezing behavior and total distance were subsequently analyzed independently by day using two-way ANOVA with sex and genotype as between-subjects factors. Fisher's LSD *post hoc* tests were used for pairwise comparisons of means.

Age was included as a covariate in all statistical analyses due to the large age range. In all parametric analyses, if skewness was outside the range of −1 to 1, the data were square root- or log-transformed, depending on which method produced skewness nearest zero, to improve the normality assumption. Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC), OriginPro2016 (OriginLab, Northampton, MA), or R software. $p < 0.05$ was considered statistically significant.

3 | RESULTS

3.1 | Genetic loss of DBI does not impact contextual OLM

DBI$^{+/+}$ and DBI$^{-/-}$ mice were first tested on the OLM test. Animals were evaluated for the total percentage of time spent interacting with both objects, as well as the DI between the two objects. No effect of age was detected for either variable. For the percentage of time spent interacting with both objects, a three-way repeated measures ANOVA showed a main effect of genotype ($F_{1,29} = 19.0$, $p < 0.0002$) (Figure 1a), in which DBI$^{-/-}$ mice spent less time interacting with the objects than did DBI$^{+/+}$ mice. Additionally, a main effect of sex was detected ($F_{1,29} = 6.3$, $p = 0.02$) (Figure 1b), in which male mice spent more time investigating the objects than did females, independent of genotype. No main effect of day and no interactions were significant, indicating that the effects were similar on both days and that the main effects of genotype and sex were independent of each other.

We next sought to determine if DBI$^{+/+}$ and DBI$^{-/-}$ mice displayed differences in the ability to identify an object in a novel location on day 2 versus day 1. To see if either genotype displayed an inherent side preference on day 1, paired t tests were performed to see if the DI of each mouse was statistically different from 0 (with 0 indicating no side preference). Neither DBI$^{+/+}$ mice ($p = 0.35$) nor DBI$^{-/-}$ mice ($p = 0.91$) displayed a side preference on day 1. Next, three-way repeated measures ANOVA of the DI yielded a significant main effect of day ($F_{1,30} = 8.5$, $p = 0.007$); no other significant main effects or interactions were present (Figure 1c). These results indicate that the animals learned the task and that no differences were observed between genotypes or sex in the magnitude of the DI. Overall, these data suggest that the lack of DBI signaling may lead to a decrease in the overall object investigation levels, but does not lead to a deficit in OLM.

3.2 | Genetic loss of DBI impairs long-term spatial navigation memory in the Barnes maze

The mice were next tested on the Barnes maze to determine if the loss of DBI signaling impairs spatial navigation memory. Although the Morris water maze is more commonly used to test spatial relational memory, the Barnes maze assay was chosen because it is less stressful than the Morris water maze and recapitulates ethological features
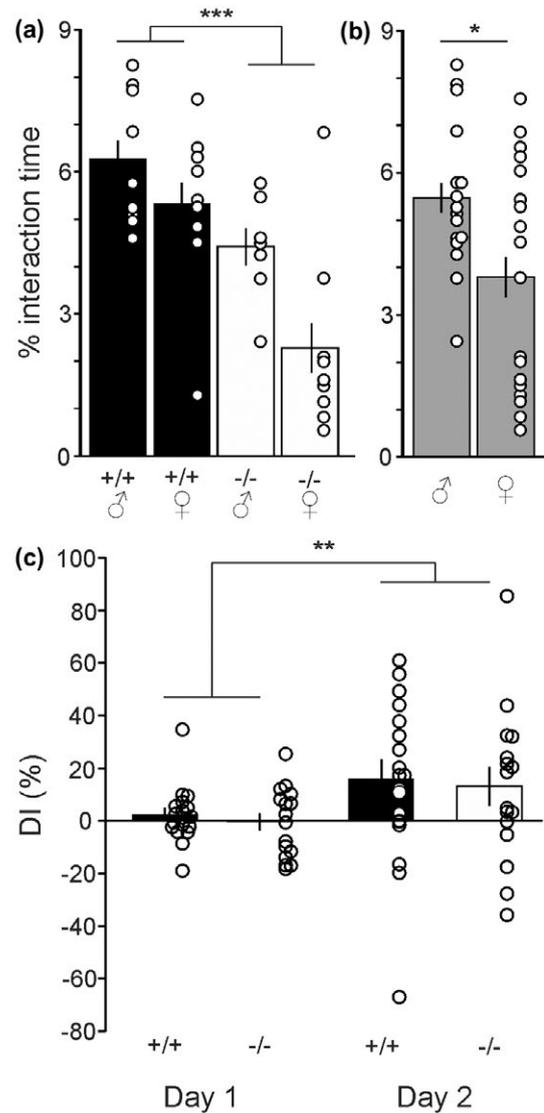

FIGURE 1 DBI$^{-/-}$ mice show diminished investigation of objects, but no alterations in OLM. (a) Mean ± *SEM* of percentage time sniffing both objects on days 1 and 2 in male and female DBI$^{+/+}$ and DBI$^{-/-}$ mice. Black bars: DBI$^{+/+}$ mice; open bars, DBI$^{-/-}$ mice. Open circles represent individual data values. (b) Percentage time sniffing both objects on days 1 and 2, with data for DBI$^{+/+}$ and DBI$^{-/-}$ mice combined. (c) Discrimination index (DI) on days 1 and 2, with one object moved to a novel location on day 2. *, **, *** ($p < 0.05$, $p < 0.01$, $p < 0.001$, respectively)

relevant to terrestrial rodents (Harrison, Hosseini, & McDonald, 2009). Furthermore, we observed that DBI$^{-/-}$ mice have difficulty swimming and are unable to reliably complete the Morris water maze (unpublished observations), prompting our use of a land-based maze. For all Barnes maze outcome variables, no main effects of sex or interactions involving sex were found. Therefore, all analyses were collapsed across sex to examine the effects of genotype. For latency to reach the target hole, two-way repeated measures ANOVA showed main effects of day ($F_{3,96} = 46.43$, $p < 0.0001$), genotype ($F_{1,31} = 8.3$, $p = 0.007$), and a day-by-genotype interaction ($F_{3,96} = 5.77$, $p = 0.001$) (Figure 2a). Pairwise *post hoc* comparisons using Fisher's LSD showed
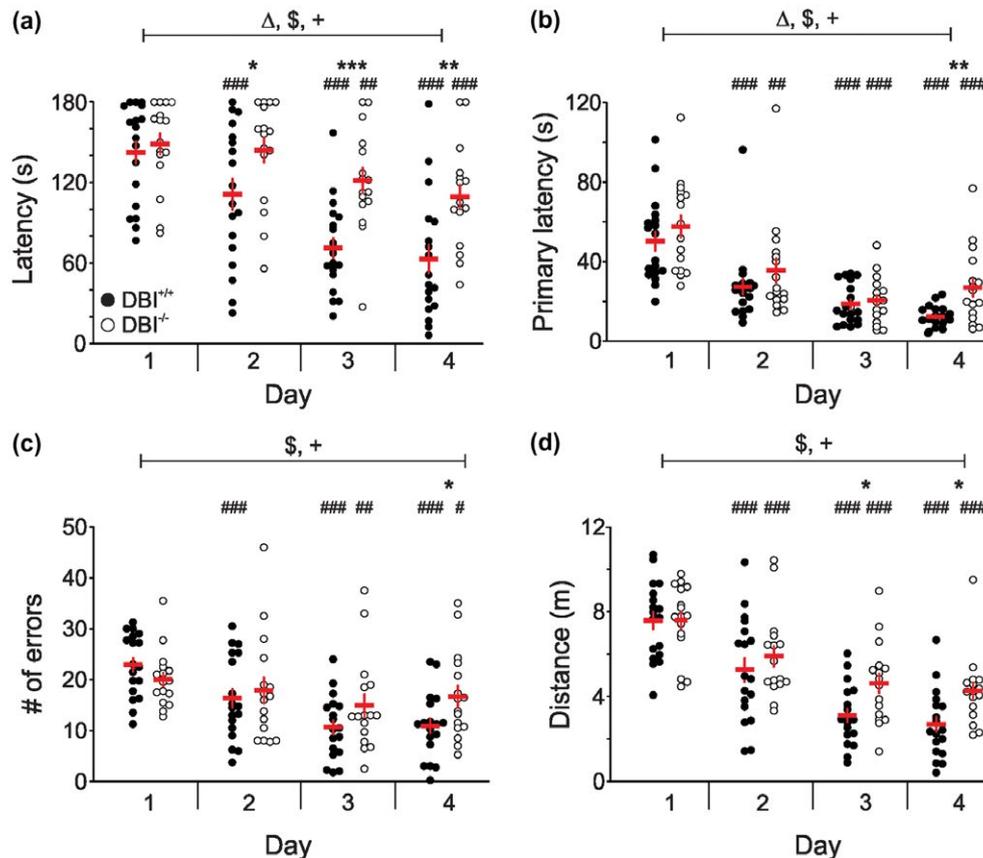
FIGURE 2 DBI$^{-/-}$ mice display impaired performance on multiple parameters of the Barnes maze. Analysis of values from DBI$^{+/+}$ (closed circles) and DBI$^{-/-}$ mice (open circles) over the course of 4 days (each circle represents the average of four trials for an individual mouse). Red lines represent Mean ± *SEM*. (a) Latency to enter escape box. (b) Primary latency to interact with target hole. The data were log-transformed to improve normality for statistical analysis; the raw data are presented here. (c) Number of errors made. The data were square root-transformed to improve normality for statistical analysis; the raw data are presented here. (d) Total distance traveled during duration of test. Δ, significant effect of genotype; $, significant effect of day; +, significant day-by-genotype interaction; see Results for exact *p* values for each parameter. *,**,***, significant difference between DBI$^{+/+}$ and DBI$^{-/-}$ mice on given days ($p < 0.05$, $p < 0.01$, $p < 0.001$, respectively); #, ##, ###, significant difference compared with day 1 within the same group ($p < 0.05$, $p < 0.01$, $p < 0.001$, respectively) [Color figure can be viewed at wileyonlinelibrary.com]

significantly increased latency for DBI$^{-/-}$ mice on days 2 ($p < 0.02$), 3 ($p < 0.001$), and 4 ($p = 0.001$) compared with DBI$^{+/+}$ mice (Figure 2a). Notably, some mice would reach the target hole as expected, but would not physically enter the hole, a phenomenon previously observed by other groups (Harrison et al., 2006; Patil et al., 2009). Therefore, in addition to latency, we also analyzed the primary latency, defined as the time needed to first reach the target hole and remain at the target for at least 3 s. The primary latency data displayed a positively skewed residual distribution, so a log transformation was used to improve normality. As with latency, two-way repeated measures ANOVA of primary latency showed main effects of day ($F_{3,96} = 45.87$, $p < 0.0001$) and genotype ($F_{1,31} = 4.18$, $p = 0.049$), as well as a day-by-genotype interaction ($F_{3,96} = 2.76$, $p = 0.047$) (Figure 2b). Fisher's LSD comparisons showed that DBI$^{-/-}$ mice displayed increased latency on day 4 ($p = 0.008$) compared with DBI$^{+/+}$ mice. No effect of age was seen for either latency or primary latency. Together, the latency and primary latency data revealed that DBI$^{-/-}$ mice took significantly longer to reach the target hole than did DBI$^{+/+}$ mice across the four days, suggesting impaired spatial memory.

We next investigated whether DBI$^{+/+}$ and DBI$^{-/-}$ mice differed in the number of errors made during the task, defined as the number of visits to holes other than the target hole. The residual distribution was slightly positively skewed, so a square root transformation was used to improve normality. An age effect was detected, in which older animals displayed significantly fewer errors than younger animals ($F_{1,31} = 7.4$, $p = 0.01$). After correcting for age, two-way repeated measures ANOVA of the transformed data revealed a main effect of day ($F_{3,96} = 26.14$, $p < 0.0001$) and a day-by-genotype interaction ($F_{3,96} = 5.24$, $p < 0.003$), indicating that DBI$^{-/-}$ mice had a higher rate of errors as the days progressed than did DBI$^{+/+}$ mice. Fisher's LSD comparisons revealed that DBI$^{-/-}$ mice made significantly more errors on day 4 than did DBI$^{+/+}$ mice ($p < 0.02$) (Figure 2c). We also analyzed the total distance that the mice traveled on the maze. Two-way repeated measures ANOVA showed a significant effect of day ($F_{3,96} = 92.55$, $p < 0.0001$) and a day-by-genotype interaction ($F_{3,96} = 3.56$, $p < 0.02$), demonstrating that DBI$^{+/+}$ and DBI$^{-/-}$ mice traveled different distances in the maze as the days progressed (Figure 2d). Fisher's LSD comparisons showed that

DBI$^{-/-}$ mice traveled a greater distance than DBI$^{+/+}$ mice did on days 3 ($p < 0.02$) and 4 ($p < 0.02$). No effect of age was detected. These data are in agreement with the increased latency, primary latency, and number of errors displayed by the DBI$^{-/-}$ mice. Altogether, the phenotypic differences observed in these four parameters of Barnes maze testing provide evidence for impaired spatial navigation memory in mice with a genetic loss of DBI signaling.

We next investigated whether the genetic loss of DBI signaling impacted the search strategy used by the mice to exit the maze (Figure 3). Search strategy is an important indicator of the ability of mice to use the external visual cues to solve the task, thereby signaling intact long-term spatial memory (Patil et al., 2009). All individual trials for each mouse were analyzed *post hoc* and scored for one of three possible search strategies: direct, serial, or random (see Materials and Methods for a complete description of strategy criteria). No effect of age was detected for any of the three strategies. Logistic regression analysis yielded main effects of day for the direct and random search strategies (Deviance$_3$ = 62.3, $p < 0.0001$ and Deviance$_3$ = 105.4, $p < 0.0001$, respectively) (Figure 3), indicating that the mice shifted their search strategy as the days progressed independent of genotype. Specifically, the probability of using the direct strategy increased and the probability of using the random

strategy decreased across days. To confirm this, Fisher's exact tests were run on days 1 and 4 for both direct and random search strategies. For the direct search strategy, both DBI$^{+/+}$ ($p < 0.0001$) and DBI$^{-/-}$ mice ($p = 0.0002$) displayed an increase in the probability of using this strategy on day 4 compared with day 1. Concomitantly, both DBI$^{+/+}$ ($p < 0.0001$) and DBI$^{-/-}$ mice ($p < 0.0001$) displayed a decrease in the probability of using the random search strategy across days, suggesting that both genotypes learned and used the external visual cues by switching from a random to direct strategy. However, logistic regression analysis also yielded a main effect of genotype (Deviance$_1$ = 4.65, $p = 0.04$) for the direct strategy and a day-by-genotype interaction for the random strategy (Deviance$_3$ = 8.2, $p = 0.04$) (Figure 3), indicating that differences were present between DBI$^{+/+}$ and DBI$^{-/-}$ mice in the use of these search strategies over the course of the task. In confirmation of this effect, a Fisher's exact test revealed that DBI$^{-/-}$ mice used the direct search strategy significantly less than DBI$^{+/+}$ mice did on day 4 ($p < 0.03$). No significant effects were observed for the serial strategy. Taken together, both genotypes displayed the ability to learn the task, as evidenced by the shift from random to direct search strategies. In comparison to DBI$^{+/+}$ mice, however, DBI$^{-/-}$ mice displayed a reduced rate of using the visual cues necessary for the direct strategy, providing further support of a role for DBI signaling in spatial navigation memory.

Finally, to investigate long-term spatial memory retention, DBI$^{+/+}$ and DBI$^{-/-}$ mice were analyzed on a probe trial in which the escape box was removed from the target hole. Three-way repeated measures ANOVA on the number of visits made to each hole showed a significant effect of hole ($F_{19,570}$ = 32.83, $p < 0.0001$) and a significant genotype-by-hole interaction ($F_{19,570}$ = 1.82, $p = 0.018$). A Fisher's LSD *post hoc* test revealed that DBI$^{-/-}$ mice made significantly fewer visits to the target hole during the probe trial than did DBI$^{+/+}$ mice ($p < 0.0001$) (Figure 4). No effect of age was detected, and no significant differences between genotypes were seen in the number of visits to any of the other 19 holes, further suggesting that DBI$^{-/-}$ mice displayed a specific deficit in long-term spatial memory.

In summary, mice with a genetic lack of DBI signaling on average took longer to escape the maze, made more performance errors, traveled a greater distance, displayed decreased aptitude in learning external visual cues, and displayed impaired long-term retention of the target hole location. These data thus provide evidence for a role of DBI signaling in long-term spatial navigation learning and memory.
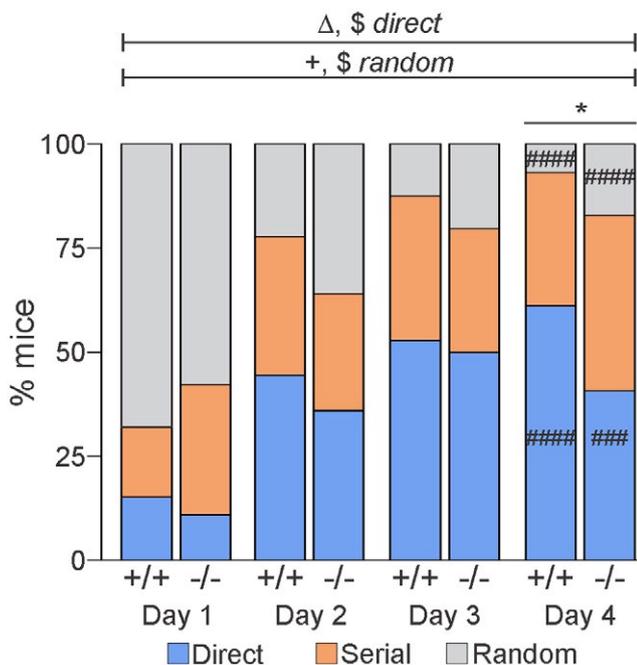

FIGURE 3 DBI$^{-/-}$ mice show curtailed adoption of direct search strategy by day 4. Percentage of DBI$^{+/+}$ and DBI$^{-/-}$ mice using each search strategy (direct: blue bars; serial: orange bars; random: gray bars) over the course of 4 days (each day represents the average of 4 trials). Δ, significant effect of genotype ($p < 0.05$); $, significant effect of day ($p < 0.0001$); +, significant day-by-genotype interaction ($p < 0.05$) for the indicated search strategies; *, significant difference in the use of direct search strategy between DBI$^{+/+}$ and DBI$^{-/-}$ mice on day 4 ($p < 0.05$); ###, ####, significant difference comparing days 1 and 4 for each search strategy within genotype ($p < 0.001$, $p < 0.0001$, respectively) [Color figure can be viewed at wileyonlinelibrary.com]

3.3 | Genetic loss of DBI abolishes sex differences in contextual fear conditioning

DBI$^{+/+}$ and DBI$^{-/-}$ mice were also assessed on a third hippocampus-dependent assay of learning and memory, contextual fear conditioning. To determine if mice associated the environmental context with a subsequent foot shock, mice were placed in a testing chamber on day 1 and footshocked at two separate intervals. On day 2, mice were placed within the same testing chamber and not shocked. Two outcome variables were evaluated: (a) percentage of time spent freezing; and (b) total distance traveled. No effect of age was detected for either variable.
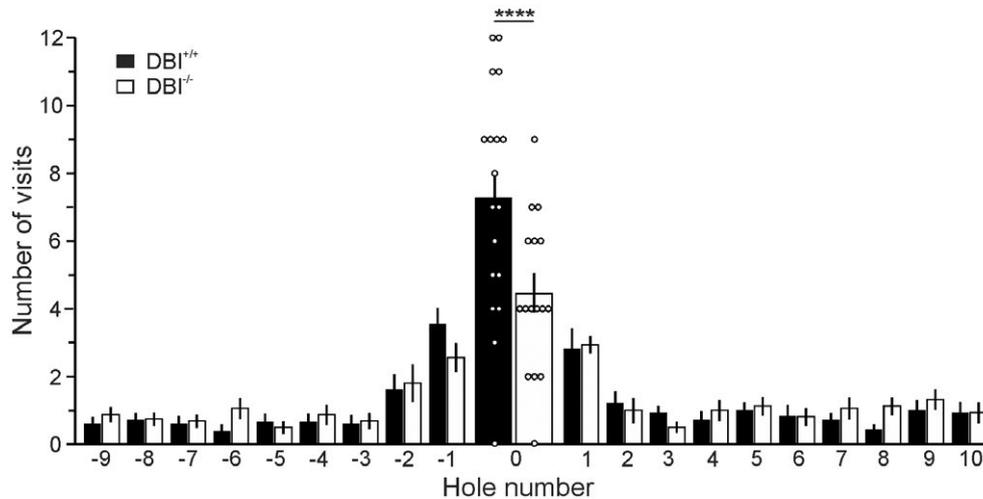
FIGURE 4 DBI$^{-/-}$ mice display impaired performance in the probe trial of the Barnes maze test. Mean ± *SEM* of number of nose pokes (visits) into each hole during probe trial. Hole 0 is the target hole, with positive values progressing clockwise from the target hole. Open circles represent individual data values for number of visits to the target hole by each mouse. ****Significant difference between DBI$^{+/+}$ and DBI$^{-/-}$ mice ($p < 0.0001$)

For the time spent freezing (Figure 5a), an overall three-way repeated measures ANOVA found a significant effect of day ($F_{1,30} = 118.9$, $p < 0.0001$), a significant interaction between sex and genotype ($F_{1,29} = 5.3$ $p = 0.029$), and a day-by-sex-by-genotype interaction ($F_{1,30} = 6.0$, $p = 0.02$); therefore, the data were analyzed separately for each day. On day 1, two-way ANOVA of freezing behavior detected no significant main effects or interactions. On day 2, two-way ANOVA of freezing behavior found no main effect of sex ($F_{1,29} = 1.42$, $p = 0.07$) nor genotype ($F_{1,29} = 0.00$, $p = 0.96$), but there was a significant sex-by-genotype interaction ($F_{1,29} = 5.3$, $p = 0.03$). Fisher's LSD *post hoc* tests revealed that DBI$^{+/+}$ males displayed more robust freezing behavior than did DBI$^{+/+}$ females ($p < 0.05$), but no differences between sexes were observed in the DBI$^{-/-}$ mice. This finding suggests that DBI$^{+/+}$ males formed stronger associations between the context and the footshock than did females, confirming previous results in both mice (Villasana, Rosenberg, & Raber, 2010; Wiltgen, Sanders, Behne, & Fanselow, 2001) and rats (Kudo, Qiao, Kanba, & Arita, 2004; Maren, De Oca, & Fanselow, 1994). This effect of sex was lost in DBI$^{-/-}$ mice (Fisher's LSD: $p > 0.2$) (Figure 5a). In addition, although there was not a significant difference in freezing between DBI$^{+/+}$ and DBI$^{-/-}$ males ($p > 0.1$), the effect between genotypes was borderline significant for female mice ($p = 0.056$), with DBI$^{-/-}$ females showing a trend toward increased freezing.

It is conceivable that DBI$^{-/-}$ mice may display altered baseline freezing behavior compared with DBI$^{+/+}$ mice. To investigate this possibility, we tested three additional mice of each genotype and sex combination that did not receive any footshocks on either day. A three-way repeated measures ANOVA of the data from unshocked controls showed no main effects of day, genotype, sex, or any interactions (Figure 5b), indicating that the phenotypic differences seen in the shocked mice on day 2 were not due to a characteristic disparity in baseline freezing behavior.

For the total distance traveled in the chamber, an overall three-way repeated measures ANOVA of the data from shocked mice revealed a significant effect of day ($F_{1,29} = 322.6$, $p < 0.0001$), a

significant genotype-by-sex interaction ($F_{1,30} = 7.0$, $p = 0.013$), and a significant day-by-genotype interaction ($F_{1,30} = 6.5$, $p = 0.016$) (Figure 5c). Therefore, the data were analyzed separately for each day. Two-way ANOVA of distance traveled on day 1 did not yield a main effect of sex ($F_{1,29} = 3.17$, $p = 0.09$) nor a sex-by-genotype interaction ($F_{1,29} = 1.4$, $p = 0.24$), but there was a significant main effect of genotype ($F_{1,29} = 8.8$, $p = 0.006$), in which DBI$^{-/-}$ mice traveled a shorter distance than did DBI$^{+/+}$ mice. On day 2, two-way ANOVA found no main effects of sex ($F_{1,29} = 0.51$, $p = 0.48$) nor genotype ($F_{1,29} = 0.68$, $p > 0.42$), but there was a significant sex-by-genotype interaction ($F_{1,29} = 7.5$, $p = 0.01$). A Fisher's LSD *post hoc* test demonstrated greater distance traveled by females than males in the DBI$^{+/+}$ genotype ($p < 0.04$). However, although the sex difference observed in DBI$^{+/+}$ mice was lost in DBI$^{-/-}$ mice, it was nearly reversed (Fisher's LSD: $p = 0.054$), with DBI$^{-/-}$ females showing a trend toward shorter distances traveled compared to males. In addition, within males, the DBI$^{-/-}$ mice traveled a greater distance than DBI$^{+/+}$ mice ($p < 0.02$), but no differences between genotypes were observed for females, and the trend was in the opposite direction. The data for distance traveled by the shocked mice on both days support the effects seen in freezing behavior. For the unshocked controls, an overall three-way repeated measures ANOVA of distance traveled showed no main effects of day, genotype, sex, or any interactions (Figure 5d), suggesting that DBI$^{-/-}$ mice do not display inherent locomotor impairment. Overall, these results indicate a potential role for DBI in regulating a sexually dimorphic circuit mediating contextual fear conditioning.

4 | DISCUSSION

In these studies, we sought to determine the effects of genetic loss of DBI signaling in mice on performance in multiple hippocampus-dependent tasks of spatial and contextual learning and memory. Our data show that the loss of DBI plays a selective role in
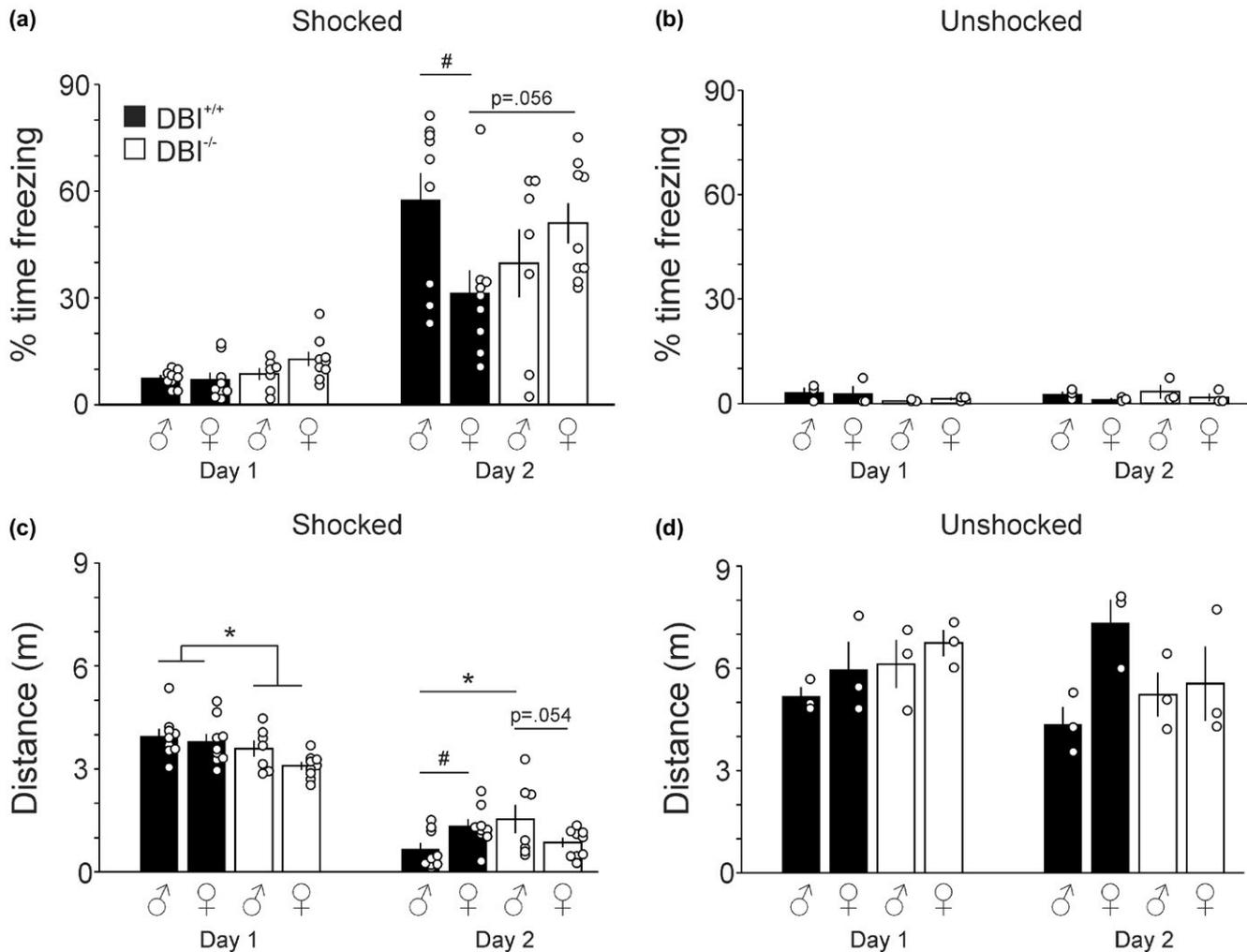
FIGURE 5 DBI$^{-/-}$ mice lack sex effect seen in DBI$^{+/+}$ mice in contextual fear conditioning. (a) Mean ± *SEM* of percentage time that shocked DBI$^{+/+}$ (black bars) and DBI$^{-/-}$ mice (open bars) spent freezing on both days of the fear conditioning task. Open circles represent individual data values. (b) Percentage time that unshocked DBI$^{+/+}$ and DBI$^{-/-}$ mice spent freezing on both days of the task. (c) Distance traveled by the shocked mice on both days of the fear conditioning task. (d) Distance traveled by the unshocked mice on both days of the task. *Significant difference between DBI$^{+/+}$ and DBI$^{-/-}$ mice ($p < 0.05$); #significant difference between sexes within respective genotype ($p < 0.05$)

hippocampus-dependent behaviors, modulating performance in a task-specific manner. We found no significant differences between DBI$^{+/+}$ and DBI$^{-/-}$ mice in the DI of OLM, although DBI$^{-/-}$ mice showed lower degrees of total object exploration. In the Barnes maze task, we found that DBI$^{-/-}$ mice showed inferior learning of spatial reference cues necessary for the memory of the escape hole location. Furthermore, our results indicate that DBI$^{-/-}$ mice did not simply fail to associate entering the escape box with completion of the trial, as analysis of search strategy demonstrated that these mice showed lower rates of relying on extra-maze cues to locate the escape box. Analysis of the contextual fear conditioning task revealed an intriguing sex-by-genotype interaction on day 2, with a sex effect existing in DBI$^{+/+}$ mice that was not observed in DBI$^{-/-}$ mice. These results potentially suggest a role for DBI in maintaining and modulating sexually divergent fear behavior in mice. Taken together, these results provide evidence of a role for DBI in modulating various hippocampus-dependent assays of learning and

memory. These effects are task specific, showing genotype- and sex-dependent deficiencies in some, but not all, behavioral paradigms.

The hippocampus has long been associated with multiple forms of spatial knowledge (Gray & McNaughton, 1983; O'Keefe & Nadel, 1978). Several assays of spatial memory in rodents have been invented with varying degrees of complexity and differential requirements of hippocampal engagement. Each of the paradigms examined in this study require the animal to use the learned information in a distinctive way. For example, spatial relational memory is a hippocampus-demanding form of spatial knowledge that relies on the animal to encode geometric relationships of intra- and extra-maze cues, and to apply this knowledge to locate the target from multiple viewpoints (Eichenbaum, 2000). With a specific goal in place, tasks such as the Barnes maze and the Morris water maze force the animal to use various strategies employing the given cues to solve the maze. Conversely, tasks such as OLM and contextual fear conditioning rely on the subject forming a cognitive map of the environment

by investigating cues and forming associations. These representations do not necessarily require a flexible use of the learned information regarding the setting (Moses & Ryan, 2006; Sutherland & Rudy, 1989). Furthermore, tasks involving the detection of novelty, such as OLM, are fairly simple and useful in investigating spatial cognitive mapping rather than spatial reference memory (Sharma et al., 2010). In addition, multiple studies have implicated the basolateral amygdala and the retrosplenial cortex in influencing contextual fear conditioning (Phillips & LeDoux, 1992; Sparta et al., 2014), and inactivation of the dorsal hippocampus was shown to lead to only a ~ 35% impairment in contextual fear conditioning (Desmedt, Marighetto, Garcia, & Jaffard, 2003), suggesting that other brain regions play critical roles influencing this task. Therefore, it is reasonable to expect that impairments in some hippocampus-dependent tasks do not necessarily correlate with deficits in others.

Recent evidence supports a role for DBI in the regulation of neurogenesis in the dentate gyrus of the hippocampus (Dumitru, Neitz, Alfonso, & Monyer, 2017). Specifically, DBI appears to control the balance between preserving the neural stem cell population and facilitating the development of new granule cells. Importantly, disruption of neurogenesis in the dentate gyrus selectively impairs performance in the Morris water maze. This effect, however, was not seen in either contextual fear conditioning or tasks involving identification of a novel environment (Drapeau et al., 2003; Dupret et al., 2008). Therefore, it is possible that the lack of DBI signaling may disrupt hippocampal neurogenesis, thus specifically impacting behavioral tasks that require spatial reference memory over other neurogenesis-independent forms of memory. Our results, demonstrating a specific impairment in mice lacking DBI on performance in the Barnes maze, but not in OLM nor contextual fear conditioning, support this conclusion. In addition, it is noteworthy that the impairment of spatial memory seen here upon genetic removal of DBI signaling is similar to the effect seen previously with transgenic overexpression of DBI (Siiskonen et al., 2007). These results suggest that DBI levels may need to be strictly regulated to ensure proper neural function, and that a disruption of this balance in either direction can lead to cognitive deficits, particularly in spatial memory. Further work is needed to uncover the complex role of DBI in the hippocampus, with a potential focus on how learning and memory may depend on the regulation of hippocampal DBI levels and its subsequent impact on neurogenesis.

We recently reported that DBI$^{-/-}$ mice lack the typical sex difference in social interest seen in DBI$^{+/+}$ mice, in which males display higher levels of social interest than females, suggesting that DBI may play a role in developing and/or maintaining sexually dimorphic circuits underlying social behavior (Ujjainwala et al., 2018). In the present studies, DBI$^{+/+}$ male mice exhibited greater freezing levels compared with females in contextual fear conditioning, and this sex effect was diminished and nearly reversed in DBI$^{-/-}$ mice. The current study thus provides further evidence that DBI may play a role in mediating behaviors that show established sex differences. These results may be explained by, for example, DBI impacting the formation and maintenance of critical sexually dimorphic circuits and/or the sex-specific production of certain GABA$_A$R-modulating

neurosteroids. In the latter regard, DBI can potentially upregulate steroid hormone biosynthesis through actions at the mitochondrial benzodiazepine receptor TSPO (Korneyev et al., 1993; Papadopoulos et al., 1991), although the role of TSPO in this process has recently come into question (Morohaku et al., 2014). Furthermore, within the hippocampus, testosterone and/or its metabolites generated by 5α-reduction, dihydrotestosterone and 3α-androstanediol, can improve performance in spatial tasks and contextual fear conditioning in rats and modulate hippocampal synaptic spine density (Edinger, Lee, & Frye, 2004; Frye & Lacey, 2001; Isgor & Sengelaub, 2003; Leranth, Petnehazy, & MacLusky, 2003). *Dbi* mRNA expression in at least some areas of the mouse brain is androgen-sensitive (Compère et al., 2006), and seminal vesicle weights, a proxy of circulating testosterone levels, are not different in DBI$^{-/-}$ male mice (Ujjainwala et al., 2018). Therefore, it is also possible that some of the observed effects of genetic loss of DBI represent impaired mediation of certain effects of testosterone in the hippocampus and associated brain structures.

The OLM task relies on the testing groups displaying similar levels of interaction with the objects on both days of the test. In our studies, DBI$^{+/+}$ and DBI$^{-/-}$ mice did not show similar levels of investigation on either day 1 or day 2. Therefore, it is difficult to ascertain whether the lack of a genotype effect in the DI is a secondary consequence of differences in object interest. It may be useful in future studies to utilize a behavioral assay that does not depend on innate object interest. The reduced levels of interest of DBI$^{-/-}$ mice in the OLM task recapitulate findings we recently reported in an odor discrimination task (Ujjainwala et al., 2018), in which DBI$^{-/-}$ mice could discriminate between social and nonsocial odors, but showed reduced investigation of cotton swabs containing only water. The reduced level of investigation displayed in these behavioral tests could potentially reflect an overall disinterest in various novel tasks and objects. Mouse models of anhedonia have been shown to exhibit decreased interest across multiple parameters (Dedic, Walser, & Deussing, 2011; Nestler & Hyman, 2010), and it is possible that the genetic lack of DBI modulates pathways or substrates associated with anhedonia. Interestingly, although a sex difference was present for the percentage of time interacting with the objects during the OLM task, this effect appears to be primarily driven by the low interaction time of the DBI$^{-/-}$ females rather than DBI$^{+/+}$ females. DBI$^{+/+}$ females do not differ from DBI$^{+/+}$ males and display higher interaction times than DBI$^{-/-}$ males, suggesting that the lack of DBI signaling is likely a more critical factor than sex in the overall investigation levels in the OLM task.

Several potential alternative explanations for our results exist. Although extensive backcrossing onto the original C57BL/6BomTac background was performed when this knockout line was first developed (Neess et al., 2011), it is possible that genetic material linked to the knockout region may still exist in DBI$^{-/-}$ mice. However, since heterozygote breeding was utilized during backcrossing onto the C57BL/6J strain in our colony, it is unlikely that the behavioral differences seen between DBI$^{+/+}$ and DBI$^{-/-}$ mice are due to off-target genetic variations related to the creation of the knockout. It is also possible that the results seen in these tasks may be explained by

impaired visual acuity or locomotor impairment in DBI$^{-/-}$ mice. As mentioned previously, DBI$^{-/-}$ mice display difficulty swimming and have demonstrated an inability to consistently complete four consecutive daily trials in the Morris water maze. It should be noted that DBI$^{-/-}$ mice are phenotypically distinguishable by varying degrees of alopecia and distinctly oily fur (Bloksgaard et al., 2012), which may be debilitating to swimming ability, for example by decreasing the ability of the mice to regulate body temperature in an aquatic environment. In previous studies, DBI$^{-/-}$ mice did not display large degrees of locomotor impairment on a rotarod task (Ujjainwala et al., 2018) or in open field tests (Budry et al., 2016), and in the present studies, DBI$^{-/-}$ mice traveled the same distance as DBI$^{+/+}$ mice did on day 1 of the Barnes maze task, and greater distances on subsequent days. In addition, the overall distance traveled by the unshocked control DBI$^{-/-}$ mice in the contextual fear conditioning task was not statistically different from the values of the unshocked DBI$^{+/+}$ mice (see Figure 5d). Therefore, these mice do not appear to have a gross locomotor impairment, and it appears more likely that the swimming impairment is related to the distinct skin condition. However, the visual acuity of DBI$^{-/-}$ mice compared to their DBI$^{+/+}$ counterparts remains untested and may be a target of future experimentation. In the contextual fear conditioning task, in which each mouse received shocks of the same intensity, one possible explanation for the differences seen between groups could be attributed to the varying degrees of shock sensitivity. Although we did not test whether DBI$^{-/-}$ mice show altered shock sensitivity, it is a variable to consider when evaluating data in fear conditioning assays. In addition, it should be noted that the unshocked control mice were tested as a separate cohort, and these animals did not previously undergo the OLM or Barnes maze tasks. Although the unshocked mice performed as expected and clearly displayed phenotypic differences compared to the shocked mice, the cohort difference should be considered when making direct comparisons between the two groups.

In summary, the goal of these studies was to determine the effects of genetic loss of DBI in mice on performance in multiple hippocampus-dependent behavioral tasks of spatial memory correlates. Our data indicate that DBI$^{-/-}$ mice show an impairment in Barnes maze learning, but perform similarly to DBI$^{+/+}$ mice in OLM, suggesting a role for DBI in specific forms of spatial navigation memory. In addition, we observed that the loss of DBI appears to negate an established sex difference in contextual fear conditioning response, providing further evidence of a potential role of DBI in mediating sex differences in certain behaviors. Overall, this work provides novel evidence that DBI is a modulator of hippocampus-dependent learning and memory, and supports further investigation into the roles that DBI plays in cognition.

DECLARATION OF TRANSPARENCY

The authors, reviewers and editors affirm that in accordance to the policies set by the Journal of Neuroscience Research, this manuscript presents an accurate and transparent account of the study being reported and that all critical details describing the methods and results are present.

CONFLICT OF INTEREST

The authors declare no competing interests.

AUTHOR CONTRIBUTIONS

All authors had full access to the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. *Conceptualization*: C.A.C.; *Investigation*, A.L.U., C.D.C., and N.M.W.; *Formal Analysis*, A.L.U, C.D.C., N.M.W., J.S.R., and C.A.C.; *Writing – Original Draft*, A.L.U., C.D.C., and C.A.C.; *Writing – Review & Editing*, A.L.U., C.D.C., J.S.R., and C.A.C.; *Visualization*, A.L.U., C.D.C., and C.A.C.; *Supervision*, C.A.C.; *Funding Acquisition*, C.A.C.

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