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1 Title: Transection of the ventral hippocampal commissure impairs spatial but not contextual
2 memory in split-brain mice
3 Abbreviated Title: Learning and memory in split-brain mice
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24 **Abstract**

25 The left and right rodent hippocampi are functionally lateralized with respect to memory.
26 Though theories of bilateral hippocampal function are beginning to incorporate hemispheric
27 lateralization, there is a lack of data concerning how interhippocampal communication
28 contributes to memory. One hypothesis suggests that synaptic plasticity in left CA3 facilitates
29 acquisition and storage of new information, while synaptic stability in right CA3 is ideal for
30 rapidly producing spatial representations. Convergence of these inputs in bilateral CA1 may bind
31 memories to locations. Loss of interhippocampal communication would then spare the ability to
32 acquire, store and retrieve memories, but would impair the binding of information and events to
33 particular locations. To test this hypothesis in male and female mice, we performed split-brain
34 surgery to transect the ventral hippocampal commissure, which contains direct interhippocampal
35 projections. Mice underwent surgery to section the hippocampal commissure and overlying
36 corpus callosum (HC+CC), just the corpus callosum (CC), or neither (SHAM) and then
37 underwent a battery of hippocampus-dependent behavioral paradigms. We found deficits
38 indicative of impaired binding of events and information to particular locations in HC+CC mice
39 only. Despite these deficits, hippocampus-dependent contextual fear memory was unaffected by
40 HC+CC surgery. Moreover, CC mice did not show any deficits in these tasks. These data suggest
41 that interhippocampal communication may be needed for the memory of events at certain
42 locations, but not for contextual associative memory. We propose that consideration of
43 hemispheric lateralization and interhemispheric communication is necessary to formulate a more
44 comprehensive understanding of hippocampal memory processes.

45

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46 **Significance Statement**

47 Hemispheric asymmetries in memory encoding and retrieval are well established in humans and
48 rodents. However, less is known about the role of inter-hemispheric communication *per se* in
49 memory function. Here, we studied "split-brain" mice in which we severed interhemispheric
50 pathways connecting the left and right hippocampi, structures essential for spatial and episodic
51 memory. Mice with transected inter-hippocampal pathways showed deficits in spatial, but not
52 contextual memory, indicating that integration of the left and right hippocampus is required for
53 spatial memory, but not hippocampus-dependent associative memory. We compare models of
54 bilateral hippocampal function and propose that hemispheric asymmetry in function and
55 interhemispheric communication may specifically endow the hippocampus with the ability both
56 to store memories and to guide navigation.
57

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58 **Introduction**

59 Damage to the hippocampus bilaterally produces profound amnesia for episodic, spatial and
60 contextual memories in mammals (Scoville & Milner, 1957; Morris et al., 1982; Kim &
61 Fanselow, 1992). Like many brain regions underlying complex cognitive processing, the human
62 hippocampus is functionally lateralized with task-dependent hemispheric specializations.
63 Interestingly, an understanding of rodent hippocampal asymmetries has lagged behind that in
64 humans.Increasingly, it appears that that some, but not all, hippocampus-dependent memory
65 tasks are functionally lateralized (Klur et al., 2009; Shinohara et al., 2012; Shipton et al., 2014).,
66 However, it is not clear how the interaction between the left and right hippocampi play a role in
67 memory.

68 The rodent hippocampus was long considered to be functionally symmetric due to an
69 absence of evidence for hemispheric lateralization. For example, lesions of the left and right
70 hippocampi in rats produced similar behavioral deficits on the Morris Water Maze (MWM)
71 (Fenton & Bures, 1993), a widely used task to assess hippocampus-dependent learning and
72 memory. Interestingly, an elegant study by Klur et al. (2009) reported that while indeed both
73 hippocampi are needed for the MWM, they offer distinct contributions. Inactivation of the left
74 hippocampus during acquisition prevented formation of an engram as evidenced by amnesia on a
75 probe trial, while right inactivation during acquisition produced no such amnesia. Conversely,
76 following intact acquisition, inactivation of the left hippocampus during a probe trial had no
77 effect, while right inactivation prevented accurate searching. These data suggest complementary
78 roles for the left and right hippocampi in, possibly being involved in different stages of engram
79 formation, storage, and retrieval, necessary for spatial memory and navigation.

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80 Behavioral lateralization may be reflected in anatomical asymmetries. Axons originating
81 from left or right CA3 neurons project bilaterally to form synapses with CA1 neurons that have
82 different properties (Kawakami et al., 2003; Shinohara et al., 2008; Kohl et al., 2011; Shipton et
83 al., 2014; see El-Gaby et al. (2015) for review). CA1 dendritic spines in either hemisphere that
84 receive left CA3 input are small and have a high density of GluN2B (Shinohara et al., 2008), a
85 molecule associated with LTP induction (Lisman et al., 2012). CA1 spines in either hemisphere
86 targeted by right CA3 are large, suggesting saturation of LTP, and have a high density of GluA1
87 (Shinohara et al., 2008). Consistent with these findings, optogenetic stimulation of left CA3
88 fibers produces LTP in left and right CA1, whereas stimulation of right CA3 fibers does not
89 (Kohl et al., 2011; Shipton et al., 2014). It has been proposed that the convergence of left and
90 right CA3 inputs in CA1 may be important for hippocampal function (El-Gaby et al., 2015).
91 Specifically, El-Gaby et al. (2015) proposed that left CA3 alone may be sufficient for the storage
92 of new learned associations, while input from right CA3 allows for the rapid emergence of
93 cognitive maps to which new associations can be rapidly bound. According to this model,
94 eliminating interhemispheric convergence of CA3 input in CA1 would impair performance on
95 tasks that require binding of new memories stored in the left hemisphere to spatial
96 representations of specific locations contributed by the right, while sparing performance on tasks
97 that simply require the storage and retrieval of new memories (El-Gaby et al., 2015).
98 To test the role of inter-hemispheric communication in hippocampal-dependent memory
99 and retrieval, we performed split-brain surgery on mice to sever pathways connecting the left and
100 right hippocampi. These mice were trained on hippocampus-dependent behavioral paradigms,
101 including the Y-Maze short-term memory task and the MWM, which require binding of
102 memories to locations, as well as contextual fear memory and the elevated plus maze, which

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103 require no such binding. We then discuss our results in the context of bilateral hippocampal
104 processing.
105
106 **Methods**
107 *Animals.*
108 We used C57/BL6J mice (Jackson Labs, Bar Harbor, ME) that have been bred in-house for 2-5
109 generations. All mice were adults aged 2.5-7 months at the time of surgery. Mice were housed on
110 a 12 h light/dark cycle, with all behavioral sessions occurring during the light phase.
111
112 *Experimental Design.*
113 The ventral hippocampal commissure (VHC; Fig. 1) contains axons connecting the left and right
114 hippocampi (Amaral & Lavenex, 2007). The term *ventral* hippocampal commissure is meant to
115 distinguish this structure from the *dorsal* hippocampal commissure, which connects extra-
116 hippocampal cortical areas in the left and right hemisphere, and should not be confused with the
117 dorsal/ventral distinction used to describe hippocampal function (Kheirbek et al., 2013). Fibers
118 in the VHC originate and terminate throughout the entire dorsal-ventral extent of the
119 hippocampus. We performed "complete" or "partial" split-brain surgeries in mice. Complete
120 split-brain surgery consisted of transection of both the VHC and the overlying corpus callosum
121 (Fig. 1B, 2C), as the VHC cannot be accessed without transecting the corpus callosum. Partial
122 split-brain surgery consisted of transection of only the corpus callosum located over the VHC
123 (Fig. 2B) to control for possible contributions of the corpus callosum to hippocampus-dependent
124 memory (Zaidel, 1995). Sham surgeries consisted of sectioning cortex overlying the corpus
125 callosum (Fig. 2A). We refer to mice receiving complete split-brain surgery as HC+CC (n = 14;

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126 6 females, 8 males), mice receiving partial split-brain surgery as CC (n = 10; 6 females, 4 males),
127 and sham-operated mice as SHAM (n = 9; 4 females, 5 males). There were no sex differences in
128 any behavioral measure, therefore males and females were combined for all analyses. Sample
129 sizes of mice used were corrected following histological confirmation of surgery and then varied
130 slightly by behavioral test, see individual sections in Results.
131
132 *Surgery.*
133 In order to sever interhemispheric pathways, we modified a method developed by Schalomon &
134 Wahlsten (1995). We used an L-shaped, sharpened piece of tungsten wire (0.25 mm in diameter)
135 as a knife. Temperature of the mice was monitored and maintained via a temperature probe and
136 heating pad. Mice were anesthetized with a ketamine xylazine cocktail (i.p., 90-120 mg/kg and
137 5-10 mg/kg, respectively) and further anesthetized for 1 minute in an isofluorane chamber (4.5%
138 isoflurane). Mice were placed in a stereotaxic apparatus, receiving a constant flow of 1.5%
139 isofluorane and oxygen (1.5 L/minute) and were given an injection of bupivacaine (1.25-2
140 mg/kg) under the scalp for local analgesia. An opening was made by drilling two adjacent 1 mm-
141 wide holes into the skull to access the brain. To avoid the superior sagittal sinus, openings in the
142 skull were made 0.5 mm off the midline and the side of surgery for each animal was randomly
143 chosen (±0.5 ML, -0.8 AP from bregma for the first hole, ±0.5 ML, -1.6 AP from bregma for the
144 second hole). To sever both the HC and CC, the short, sharpened end of the L-knife was placed
145 on the surface of the brain along the medial side of the hole and was then slowly lowered 3.4
146 mm. Once lowered, the knife was translated anteriorly so that the knife moved posterior to
147 anterior to "hook" the HC and CC fibers. The knife was then raised 3.4 mm until the short arm
148 reached the underside of the skull. The knife was then translated back and raised out of the hole.

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149 To sever the CC only, we performed the same procedure as HC+CC transection, however the
150 knife was only lowered 2.2 mm. For sham surgeries, we used the same procedure, but the knife
151 was lowered 1.0 mm. Mice were administered buprenorphine following surgery (0.1 mg/kg SC).
152 Transected and sham mice were indistinguishable by observing their behavior in the homecage.
153
154 *Behavior.*
155 Behavioral testing began about four weeks after surgery. Mice were habituated to handling for
156 one day by the experimenter. On testing days, mice were transported to a designated behavior
157 room and were allowed to acclimate for a minimum of 20 minutes before the start of the task. All
158 behavior was scored by an experimenter blind to surgical condition and sex using the
159 Stopwatch+ program.
160 Short-term spatial memory was measured using the Y-Maze, known to be equally
161 sensitive to inactivation of either the left or the right hippocampus (Shipton et al., 2014). The Y-
162 maze apparatus was constructed of clear acrylic and had three arms (height: 20 cm; length: 30
163 cm; width: 8 cm) 120 degrees apart. The room contained many spatial cues including light
164 fixtures and furniture. In addition, a painting and a movie poster were placed on the walls in line
165 with the axes of the familiar and novel arms, while the experimenter stood along the axis of the
166 start arm during each trial. Each arm was marked with a black line at the entrance for
167 determining whether the mouse was in the arm or not. Mice were considered to be in an arm if
168 all four paws were across the entrance line. The paradigm consisted of a 2-minute encoding trial
169 during which one arm was blocked off, followed by a 1-minute intertrial interval, then a 2-
170 minute retrieval session (Fig. 4A). The start arm remained the same in both the encoding and
171 retrieval trial, while the exposed arm during the encoding trial was considered the familiar arm

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172 and the blocked arm was considered the novel arm. At the start of the encoding trial, mice were
173 placed facing outward in the start arm and were allowed to explore the start and familiar arms for
174 two minutes, beginning when the mouse left the start arm. Mice were removed from the Y-Maze
175 and placed back into their home cage for one minute. While mice were in the home cage, the
176 block was removed to expose the novel arm. To remove any potential confounds from odor cues,
177 the apparatus was wiped with 70% ethanol, rotated 120 degrees, and then wiped with a dry paper
178 towel before the retrieval trial. After the intertrial interval, the mice were again placed facing out
179 in the start arm and were allowed to explore the entire maze for two minutes. At the end of the
180 retrieval session, mice were placed back in their home cages and the maze wiped and dried
181 before the next animal was run. Y-Maze spatial memory was scored as the time spent in the
182 novel and familiar arms during the retrieval paradigm.

183 Long-term spatial learning and memory was measured using the Morris Water Maze
184 (MWM), following the protocol of Vorhees & Williams (2006). The pool was 110 cm in
185 diameter and was filled with opaque water colored with non-toxic white paint maintained at a
186 temperature of 25.3°C $\pm$ 0.5°C. The escape platform was white and was submerged
187 approximately 0.5 cm under the surface of the water. The platform remained in the same location
188 throughout training. Salient room cues were visible from the surface of the pool and included
189 colored and patterned posters, lighting, and furniture. Training consisted of four trials per day for
190 five days with the starting location varying on each trial. Intertrial intervals were 30 seconds,
191 during which the mice remained on the platform before starting the next trial. Mice that did not
192 reach the platform within 60 seconds were placed onto the platform. Twenty-four hours after
193 training, a 60-second probe trial was given during which the escape platform was removed. To
194 measure spatial learning, latencies to the escape platform were recorded for each training trial

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195 and were averaged across trials for each mouse on each of the 5 training days. For the probe
196 trials, spatial memory was scored by time spent in the target quadrant versus the average of the
197 times spent in non-target quadrants, computed by summing the time spent in non-target
198 quadrants divided by three (following Teixeira et al., 2006; Arruda-Carvalho et al., 2011;
199 Cancino et al., 2013).

200 Hippocampus-independent learning was assessed using a version of the MWM in which
201 the escape platform was visible, as described by Vorhees & Williams (2006). Mice were tested
202 in the same pool as in the spatial paradigm. However, the platform was above water level and a
203 red disk was placed on top to contrast the platform with the white pool and water. Training
204 consisted of three trials per day over five days. Mice were placed in the water facing the wall on
205 the opposite side of the pool from the target platform. After finding the platform, mice were left
206 for 15 seconds before being moved to the home cage for the intertrial interval and the platform
207 was moved to a new spatial location. The intertrial interval had no set time and ended when the
208 platform was moved and the water settled (approximately 30 seconds). If mice did not find the
209 platform within 60 seconds, they were placed onto it by the experimenter and remained for 30
210 seconds.

211 Short- and long-term contextual fear memory was tested using a one-shock conditioning
212 paradigm that is particularly sensitive to hippocampal manipulations (Wiltgen et al., 2006).
213 Conditioning took place in a fear conditioning chamber housed in a sound-attenuating cubicle
214 (Med Associates, Fairfax, VT). Before each session, the fear chamber was wiped down with 70%
215 ethanol and dried. On the first day, mice were placed in the fear chamber and allowed to explore
216 freely for 3 minutes, then given a mild foot-shock (2 s, 0.75 mA), and removed 15 seconds later
217 (total conditioning session time = 3 minutes, 17 seconds). Two 3-minute retrieval sessions

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218 occurred 1 and 24 hours after conditioning (Fig. 6A), a protocol previously used to dissociate
219 molecular contributions to short-and long-term contextual fear memory (Schafe et al., 1999).
220 Fear expression was scored as time spent freezing during each minute of the three-minute
221 retrieval trial (absence of all movement, except breathing).
222 Anxiety was measured using the elevated plus maze, a paradigm sensitive to both dorsal
223 and ventral hippocampal manipulations (Kjelstrup et al., 2002; Kheirbek et al., 2013). The
224 elevated plus apparatus consisted of four arms (30.5 cm long, 6.4 cm wide), two of which were
225 enclosed on three sides with walls (20.3 cm high). Mice were placed in the center of the
226 apparatus and were allowed to explore freely for 5 minutes (Fig. 7A) and were then returned to
227 their home cage. The apparatus was wiped with 70% ethanol and dried both before and after
228 each trial. Anxiety was scored as the time with all four paws on an open arm.
229
230 *Tissue Processing and Surgical Verification.* Mice were euthanized with 0.3 mL of euthasol.
231 Brains were extracted and post-fixed in 4% paraformaldehyde. Brains were then cryoprotected in
232 30% sucrose before cryosectioning at 60 μm. To assess transection to the hippocampal
233 commissure, we stained sections with luxol blue and cresyl violet. Slides were dried overnight at
234 37°C. Histology began with a de-fat step in which sections were serially dehydrated and then
235 placed in xylene (twice for 5 minutes each). Sections were then rehydrated and then incubated in
236 70% ethanol for one hour at room temperature. Sections were then incubated in a 0.1% luxol
237 blue solution in 95% ethanol overnight at 56°C. Myelin was differentiated via rinses in deionized
238 water, followed by 0.05% lithium carbonated in deionized water, followed by 70% ethanol (2
239 minutes each; differentiation was repeated as necessary). Sections were then stained in 0.1%

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240 cresyl violet in deionized water, serially dehydrated, cleared in xylene and then coverslipped
241 using Krystalon.
242
243 *Statistical Analysis*
244 To assess short-term memory during the Y-Maze retrieval trial, we planned comparisons for
245 whether each treatment group exhibited a preference for the novel arm over the familiar arm, but
246 not whether treatment groups differed in total time exploring these arms as all retrieval sessions
247 were 2 minutes in duration. Therefore, we compared time spent in the novel arm to time spent in
248 the familiar arm within each group using Student's paired t-tests. To assess cued and spatial
249 learning on the MWM, we performed a two-way mixed model ANOVA (treatment x training
250 day, with training day as the repeating factor) on escape latency, followed by post hoc tests
251 (Tukey's HSD). We used Student's paired t-tests within each treatment group to assess spatial
252 memory on the 60-second MWM probe trial comparing time spent in the target to time spent in
253 an average of the other quadrants (Teixeira et al., 2006; Arruda-Carvalho et al., 2011; Cancino et
254 al., 2013). We used a one-way ANOVA to determine whether minimum swim latencies differed
255 across groups on the final day of visible MWM training. To assess short- and long-term
256 contextual fear memory, we performed a two-way mixed model ANOVA (treatment x minute,
257 with minute as the repeating factor) on levels of freezing during each of the two retrieval trials.
258 To assess anxiety during the elevated plus maze test, we performed a one-way ANOVA on time
259 spent in the open arms across all groups.
260
261 **Results**

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262 *Histology*. Four mice (2 male, 1 female HC+CC; 1 female CC) were excluded from analysis
263 because surgeries missed the HC or CC fibers. Sham surgeries spared interhemispheric pathways
264 (Fig. 2A) in all SHAM mice. Partial and complete split-brain surgeries resulted in severing the
265 corpus callosum (Fig. 2B, C). Complete split-brain surgeries sectioned the hippocampal
266 commissure such that the HC either remained completely severed at the time of confirmation
267 (Fig. 2C) or resulted in a clear scar across the hippocampal commissure (Fig. 3). Due to the
268 position of the VHC (Figure 1), damage to nearby structures, such as the fornix, fimbria, and
269 septal nuclei likely occurred. However, in all cases we verified that these structures were not
270 completely destroyed (Fig. 2C, 3).
271
272 *Y-Maze*. We performed a short-term spatial memory version of the Y-Maze (Fig. 4A) and
273 measured time spent exploring the familiar arm versus the novel arm during the retrieval trial.
274 One male HC+CC mouse was removed from analysis for failure to leave the start arm during the
275 retrieval trial and therefore had scores of zero for both the novel and familiar arms. SHAM and
276 CC mice both exhibited a preference for the novel arm over the familiar arm during the retrieval
277 session (SHAM: $t(8) = 3.502$; $p = 0.008$; CC: $t(8) = 4.641$; $p = 0.002$; paired t-tests) mice,
278 whereas HC+CC mice showed no such preference ($t(9) = 0.205$; $p = 0.842$; paired t-test, Fig.
279 4B).
280
281 *Morris Water Maze*. Mice were trained on a spatial version of the MWM in which the escape
282 platform was hidden below the surface of the water and could be found by the use of distal
283 spatial cues. One female SHAM mouse was removed from analysis as it showed signs of
284 hypothermia following a training session.

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285

286 Spatial acquisition was assessed by measuring the average escape latency on each day
287 over the five days of training (Fig. 5A). We found a main effect of training day ($F(4,25) = 8.304$,
288 $p < 0.0001$) and of treatment group ($F(2,25) = 4.268$, $p = 0.025$) on escape latency during
289 acquisition, with no interaction between these factors ($F(8,25) = 0.513$, $p = 0.845$). Post hoc
290 analyses confirmed that HC+CC mice were significantly slower on training day 4 compared to
291 the other groups (HC+CC vs. SHAM: $p = 0.026$; HC+CC vs. CC: $p = 0.036$). CC mice did not
292 differ from SHAM mice at any point during acquisition ($p > 0.79$ for each day).

293 Twenty-four hours after the end of acquisition, a 60-second probe trial was conducted in
294 which the escape platform was removed and time spent searching in the target quadrant versus
295 time spent searching in others was measured (Fig. 5B). SHAM and CC mice searched
296 selectively, exhibiting a preference for the target quadrant over others (SHAM: $t(7) = 3.924$, $p =$
297 0.003; CC: $t(8) = 2.480$, $p = 0.038$). However, HC+CC mice did not appear to distinguish
298 between the target quadrant and others ($t(10) = 1.320$; $p = 0.216$).

299 To rule out potential effects of split-brain surgery on vision, locomotion, or non-
300 hippocampus-dependent procedural learning, mice were trained on a visible version of the
301 MWM in which mice could see the platform throughout the duration of each trial (Fig. 5C). No
302 mice were removed from this analysis. We found a main effect of training day on escape latency
303 ($F(4,26) = 98.1$, $p < 0.0001$), but no effect of treatment ($F(2,26) = 0.024$, $p = 0.976$), and no
304 interaction between these factors ($F(8,26) = 0.766$, $p = 0.6332$). As an additional test, we
305 compared shortest latencies at the end of training across groups. Minimum escape latency for
306 each mouse on the final day of training revealed no effect of treatment ($F(2,26) = 0.632$, $p =$
307 0.539).

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308

309 *Contextual Fear Memory.* To determine whether surgical treatment affected contextual fear
310 memory, we used a one-shock contextual fear conditioning protocol that is sensitive to
311 hippocampal manipulations (Wiltgen et al., 2006). Specifically, lesions of the dorsal
312 hippocampus produce anterograde amnesia of contextual fear memory when only a single
313 training trial is given (Wilgten et al., 2006). We conducted retrieval sessions 1 and 24 hours after
314 conditioning (Fig. 6A). There appeared to be no impairment of either short- or long-term
315 contextual fear memory in HC+CC or CC mice. There was no effect of treatment ($F(2,26) =$
316 0.69; $p = 0.511$) or of minute ($F(2,26) = 2.29$; $p = 0.111$) on freezing during the 1-hour delay
317 short-term retrieval test (Fig. 6B) and no interaction between these factors ($F(2,26) = 1.15$; $p =$
318 0.344). There was a trend towards an effect of treatment ($F(2,26) = 2.66$; $p = 0.089$) on freezing
319 during the 24-hour delay long-term retrieval test (Fig. 6C), though this difference did not appear
320 to suggest any memory impairment in split-brain groups compared to SHAM mice, but instead
321 indicated greater fear expression in CC mice. There was an effect of minute ($F(2,26) = 3.46$; $p =$
322 0.038) but no interaction between the two factors ($F(2,26) = 0.37$; $p = 0.839$).
323

324 *Elevated Plus Maze.* A previous study suggested that split-brain mice may have higher levels of
325 anxiety which may contribute to impaired performance on a Barnes Maze spatial memory test
326 (Shinohara et al., 2012). To determine whether split-brain surgery affected anxiety, we exposed
327 mice to a single session of exploration of an elevated plus maze (Fig. 7A). One male HC+CC
328 mice was removed from analysis as it fell off the apparatus during the session. Reduced
329 exploration of the open arms of the maze would suggest increased anxiety. Surgical treatment

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330 did not appear to alter levels of anxiety as there was no effect of treatment on exploration of open
331 arms in the elevated plus maze ($F(2,25) = 0.867$, $p = 0.433$; Fig. 7B).
332
333 **Discussion**
334 We found that sectioning the hippocampal commissure resulted in spatial learning and memory
335 deficits, but did not affect contextual fear memory, anxiety or hippocampus-independent
336 learning. HC+CC mice showed impaired short-term memory on a spatial Y-Maze task with a 1-
337 minute interval between encoding and retrieval, impaired acquisition of a hidden platform in the
338 MWM, and a lack of selective searching during a probe trial occurring 24 hours after the end of
339 training. In contrast, CC mice did not show impairments in any task. HC+CC surgery sectioned
340 the VHC, which contains direct projections between the left and right hippocampi, but is also
341 situated near the fornix, fimbria, and septum. Though we cannot rule out damage to these
342 structures as a factor, our histology showed a sparing of fornix and fimbria fibers in our HC+CC
343 mice. Further, complete ablation of the fimbria and fornix produces anterograde amnesia for
344 contextual fear (Maren & Fanselow, 1997) and inactivation of the lateral septum impairs the
345 expression of conditioned contextual fear (Reis et al., 2009), neither of which appeared to occur
346 in HC+CC mice. Therefore, we attribute the effects of HC+CC surgery to a loss of
347 interhippocampal communication. Interestingly, our data do not support a role of the corpus
348 callosum in hippocampal memory, as has been proposed in humans (Zaidel, 1995); however, in
349 our study portions of the corpus callosum both rostral and caudal to the VHC were spared. Our
350 data indicate a potential role of the mouse hippocampal commissure in some forms of memory,
351 similar to data reported in humans (Phelps et al., 1991), though the function of the human

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352 hippocampal commissure has been debated (Wilson et al., 1987; Gloor et al., 1993; Rosenzweig
353 et al., 2011).

354 Shinohara et al. (2012) found slower spatial learning in mice with both the VHC and
355 corpus callosum sectioned. However, these mice were also monocularly deprived, which may
356 have contributed to this finding. Contextual fear was found to be impaired in genetic split-brain
357 mice, however these mice showed impaired hippocampal synaptic transmission (Schimanski et
358 al., 2002) and baseline differences in freezing (MacPherson et al., 2008) that potentially
359 confound the results. Further, it is not clear if genetic split-brain mice have similar hippocampal
360 asymmetries as has been demonstrated in wild-type mice (Shinohara et al., 2008; Kohl et al.,
361 2011; Shipton et al., 2014).

362 The left hippocampus may be a locus of engram formation (Klur et al., 2009; Shipton et
363 al., 2014; El-Gaby et al., 2016), which is not surprising given its capacity for synaptic plasticity
364 (Kohl et al., 2011; Shipton et al., 2014). Reversible inactivation of the left but not the right
365 hippocampus of rats during acquisition of the MWM resulted in non-selective searching during a
366 probe trial with both hippocampi intact. This suggests that the left hippocampus is necessary for
367 the establishment of a long-term memory trace (Klur et al., 2009). Consistent with this idea,
368 optogenetic silencing of left but not right CA3 impaired learning on a long-term Y-Maze test of
369 spatial learning (Shipton et al., 2014; El-Gaby et al., 2016). However, it is not clear whether right
370 CA3 participates in memory storage, or whether it contributes to memory-guided behaviors in a
371 complementary way. Inactivation of the right hippocampus during learning of the MWM did not
372 impair probe retrieval (Klur et al., 2009). However, inactivation of the right hippocampus during
373 a probe trial after learning prevented selective searching in the target quadrant. This was
374 interpreted as a role of the right hippocampus in spatial memory retrieval. In this model, spatial

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375 memories acquired by the left hemisphere are transferred to the right for long-term storage and
376 retrieval. Conversely, it has been suggested that the contribution of the right hippocampus to
377 spatial memory may degrade over time. Spatial working memory on the T-Maze was impaired
378 by inactivation of either CA3 but was impaired to a greater degree by inactivation of right CA3
379 (Shipton et al., 2014). Additionally, Shipton et al., (2014) found that inactivation of both the left
380 and right CA3 impaired performance on a short-term spatial memory version of the Y-Maze (as
381 was used in the present study). The right CA3 was not required for a long-term 11-day Y-Maze
382 spatial learning task. This is reminiscent of an fMRI study in humans that found degradation of
383 right hippocampal activation correlating with the remoteness of an autobiographical memory,
384 while left activation invariantly increased (Maguire & Frith, 2003). The right hippocampus in
385 humans is, however, required for accurate spatial navigation in a learned environment (Spiers et
386 al., 2001). Thus, the function and contribution of the right hippocampus to memory is currently
387 less well understood than that of the left.

388 One model of the bilateral hippocampus suggests that left CA3 acquires and stores new
389 memories via its capacity for synaptic plasticity (Shinohara et., 2008; Kohl et al., 2011; Shipton
390 et al., 2011) and that right CA3 provides spatial representations via stable neural networks that
391 were preconfigured during development (El-Gaby et al., 2015). Rapid emergence of bilateral
392 CA1 cognitive maps in new environments would be contributed from right CA3. These maps
393 could then be modified via spatial learning that engages left CA3, allowing the binding of
394 acquired memories and learned associations to these spatial representations (El-Gaby et al.,
395 2015). Such modifications may establish new place cells that would integrate into networks with
396 left CA3, reducing the contribution of right CA3 spatial representations to memory over time
397 (consistent with data reported by Shipton et al., 2014). Interestingly, in a study that did not

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398 consider hemispheric lateralization, place cells in the left hemisphere accumulated near goal
399 locations after learning, consistent with this model (Hollup et al., 2001). Our data also support
400 this model proposed by El-Gaby et al. (2015), as eliminating the convergence of left and right
401 CA3 input to CA1 preserved hippocampus-dependent associative memory in the contextual fear
402 task, while impairing spatial learning and memory in the Y-Maze and MWM. One limitation of
403 the model, however, is that it is not clear how it would account for the requirement of the right
404 hippocampus when searching during the probe trial of a well-learned MWM, (as seen in Klur, et
405 al., 2009). Klur et al., (2009) explained this result by suggesting that spatial memory engrams are
406 transferred from the left hemisphere to the right. Our data from spatial memory tasks would
407 appear to support the model proposed by Klur et al. (2009), however, the lack of impairment
408 seen in contextual fear memory would indicate a lack of interhemispheric engram transfer at
409 least for this type of engram.

410 Our data are consistent with the proposals of Klur et al. (2009) and El-Gaby et al. (2015)
411 that the left hippocampus specializes in new engram acquisition. However, our findings do not
412 support the idea of interhemispheric transfer of engrams (Klur et al., 2009), unless this process
413 varies depending on memory type. Instead, our work supports a very similar model to that of El-
414 Gaby et al. (2015) in which memories are stored via synaptic modification in the left hemisphere
415 while the right contributes rapidly emerging spatial representations of the environment to which
416 memories can be bound in CA1. The contribution of the right hippocampus to memory storage
417 and retrieval decays rapidly after acquisition (Shipton et al., 2014; Maguire & Frith, 2003). We
418 add to this model that the right hippocampus is always needed during spatial navigation to
419 compute and continually update distance and direction from a goal location as position along the
420 route progresses, consistent with an invariant necessity for bilateral hippocampus in the MWM

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421 (Teixeira et al., 2006), but not in contextual fear memory (Kim & Fanselow, 1992). Data in
422 humans (Howard et al., 2014) and in bats (Sarel et al., 2017) suggest that direction and distance
423 computations may indeed exist in the right hippocampus. Studies of spatial navigation in humans
424 have suggested a principle contribution of the right hippocampus (Maguire et al., 1998; Spiers et
425 al., 2001). Such a component would not be needed when performing the long-term Y-Maze task
426 as the goal arm merely needed to be identified to obtain the goal without any need for continual
427 updating of distance from and direction to the goal location (Shipton et al., 2014; El-Gaby et al.,
428 2016). Thus, we argue that the developmental configuration of synaptically stable networks in
429 the right hippocampus, which El-Gaby et al. (2015) have suggested allows for the rapid
430 emergence of cognitive maps in new environments, also contributes route computation functions
431 required during navigation in learned environments.

432 In summary, our findings support the view of El-Gaby et al. (2015) that CA1 binds
433 memories acquired by left CA3 to spatial representations provided by right CA3 via
434 preconfigured cell assemblies and add the proposition that spatial navigation will always require
435 the right hippocampus. Thus, we suggest that hippocampal lateralization may be a solution to the
436 problem of "knowing where and getting there" (Wishaw et al., 1995; Maguire et al., 1998),
437 whereby memories of events occurring at particular locations are stored via synaptic
438 modification in left CA3 while right CA3 is involved in route computation.

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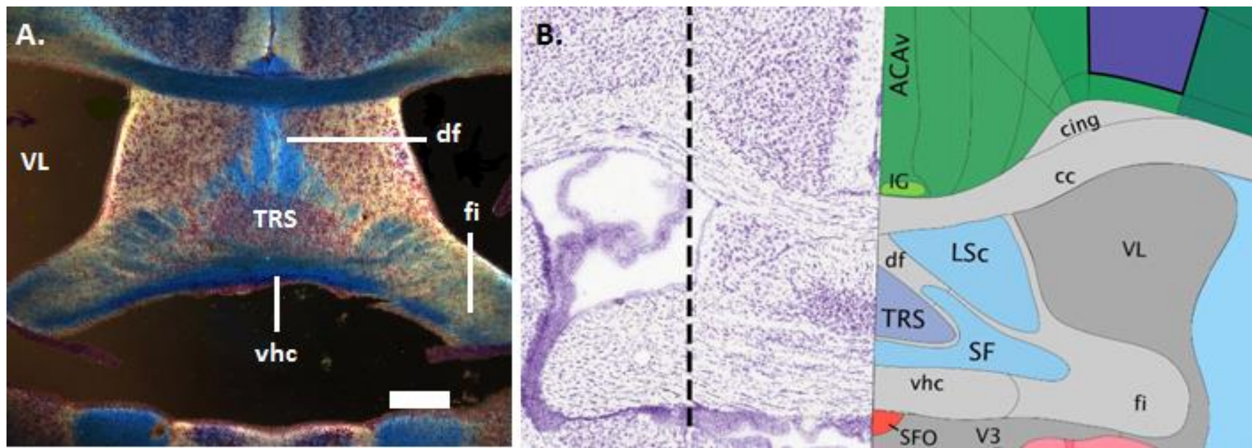
544 

545 **Figure 1.** Anatomy of local myelinated pathways. ***A***, Myelin of interhemispheric
546 pathways stained with luxol fast blue with cell nuclei stained with cresyl violet. Scale bar
547 = 1mm. ***B***, Anatomical illustration of area targeted by HC+CC surgery. Dotted line
548 indicates path of surgical knife. cc, corpus callosum; df, dorsal fornix; fi, fimbria; vhc,
549 ventral hippocampal commissure; VL, lateral ventricle; LSc, TRS, and SF are all nuclei
550 of the septum. Image credit: Allen Institute, © 2004 Allen Institute for Brain Science.
551 Allen Mouse Brain Atlas, available from http://mouse.brain-map.org/

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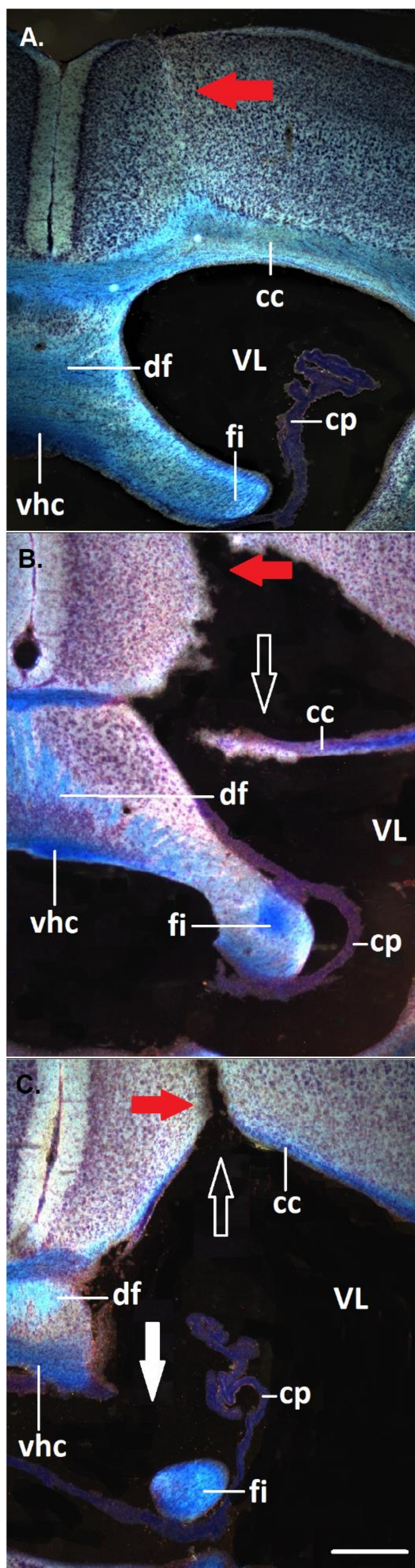

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555 **Figure 2.** Treatment groups. ***A***, SHAM mice underwent surgery that spared
556 interhemispheric pathways. ***B***, CC mice underwent surgery to transect the corpus
557 callosum while sparing the hippocampal commissure. ***C***, HC+CC mice underwent
558 surgery to transect the hippocampal commissure and corpus callosum. 60 micron-thick
559 coronal sections 6 months post-surgery. Tissue is stained with luxol blue and cresyl
560 violet. cc, corpus callosum; cp, choroid plexus; df, dorsal fornix; fi, fimbria; vhc, ventral
561 hippocampal commissure; VL, lateral ventricle. Red arrows indicate cortical scarring
562 and transection; black arrows indicate transection of the corpus callosum; white arrows
563 indicate transection of the VHC. Scale bar = 1mm.
564
565

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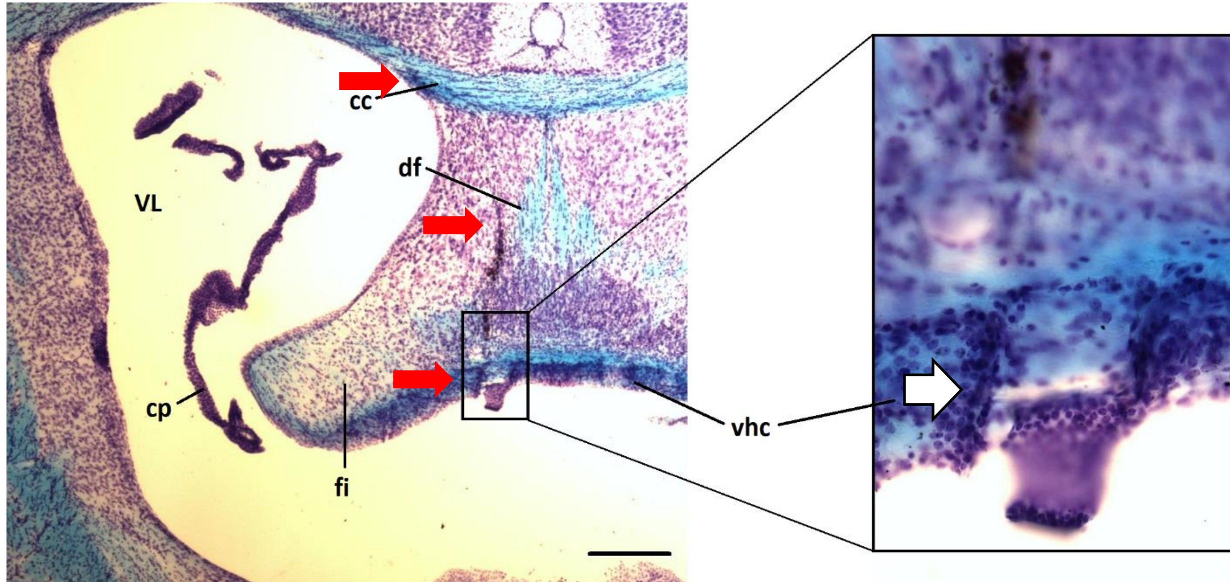

567

568 **Figure 3.** Glial scarring in the VHC and septum with damage to myelin in the VHC of
569 a HC+CC mouse brain. cc, corpus callosum; cp, choroid plexus; df, dorsal fornix; fi,
570 fimbria; vhc, ventral hippocampal commissure; VL, lateral ventricle. Red arrows show
571 glial scarring (in black) following transection; white arrow indicates transection of the
572 VHC. Image on left taken at 4x magnification; image on right taken at 20x magnification;
573 scale bar = 1mm.

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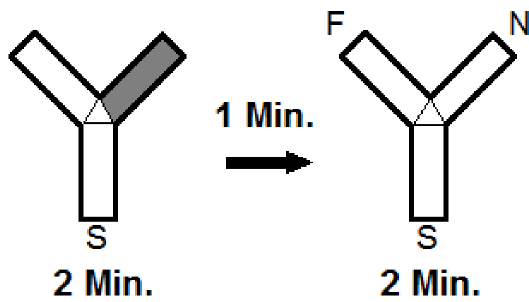
574 **A.**



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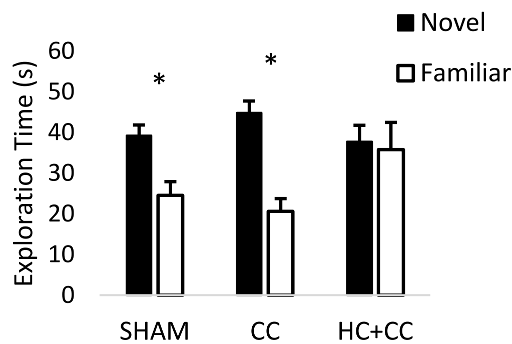
576 **B.**



577

578 **Figure 4.** Y-Maze short-term spatial memory task. ***A***, Experimental design.
579 Following a 2-minute encoding trial with one arm closed off (grey), mice are removed
580 from the maze for a 1-minute intertrial interval and are then re-exposed to the apparatus
581 with all arms open for a 2-minute retrieval trial. ***B***, Time spent in novel and familiar arms
582 during the retrieval trial for each treatment group. S = start arm, F = familiar arm, N =
583 novel arm. * indicates $p<0.05$, novel arm compared to familiar arm.

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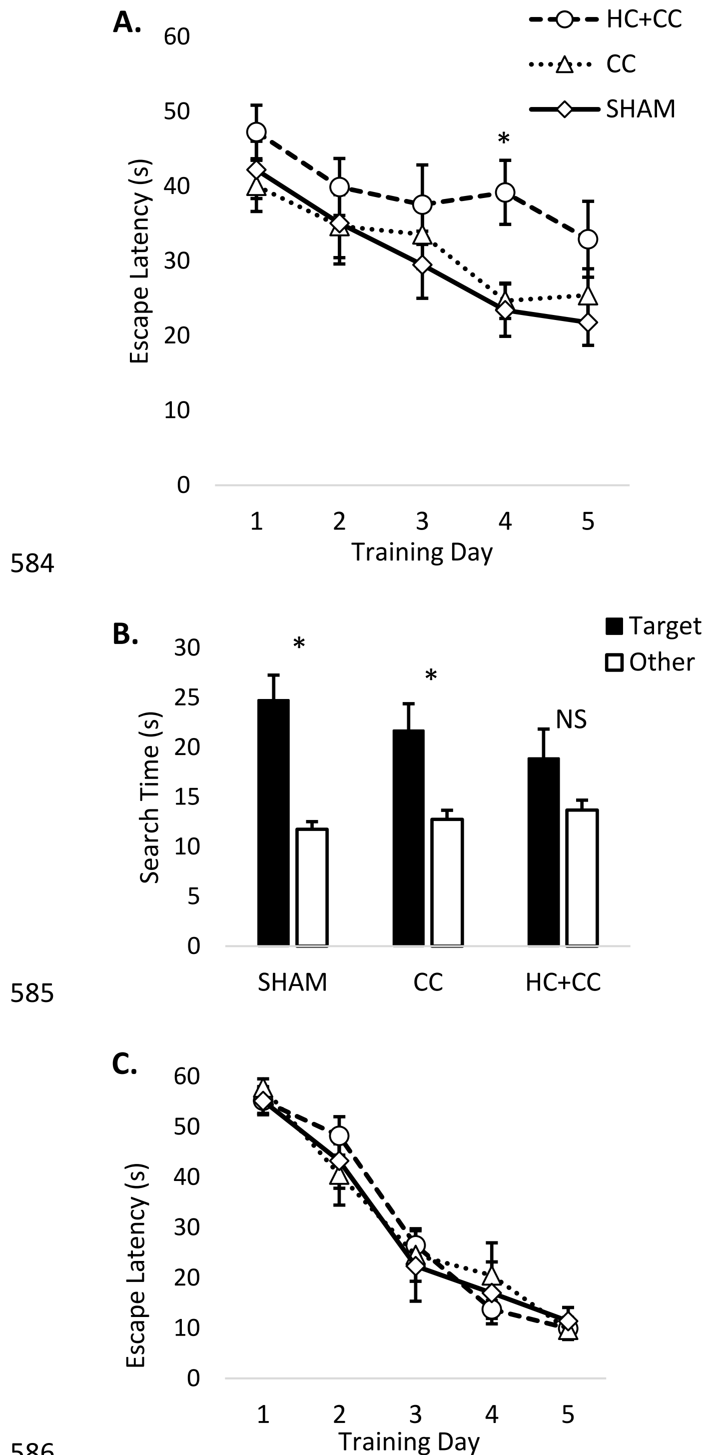

584

585

586

587 **Figure 5.** ***A***, Latency to reach the submerged platform over spatial water maze

588 training. * indicated $p < 0.05$, HC+CC compared to other treatments. ***B***, Time spent

589 searching in each quadrant of the pool during the spatial memory probe trial. * indicates

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590 $p < 0.05$, target quadrant compared to others within a treatment group; NS indicates no
591 significance between target quadrant and others within a treatment group. ***C***, Latency to
592 reach the visible platform over non-spatial water maze training.

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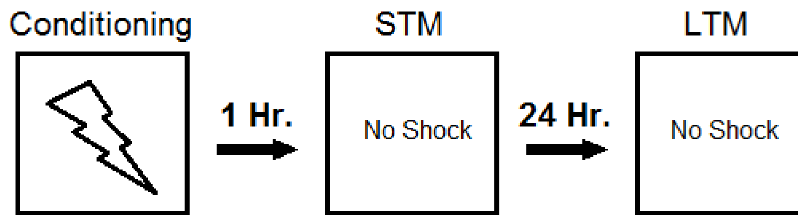
593 **A.**



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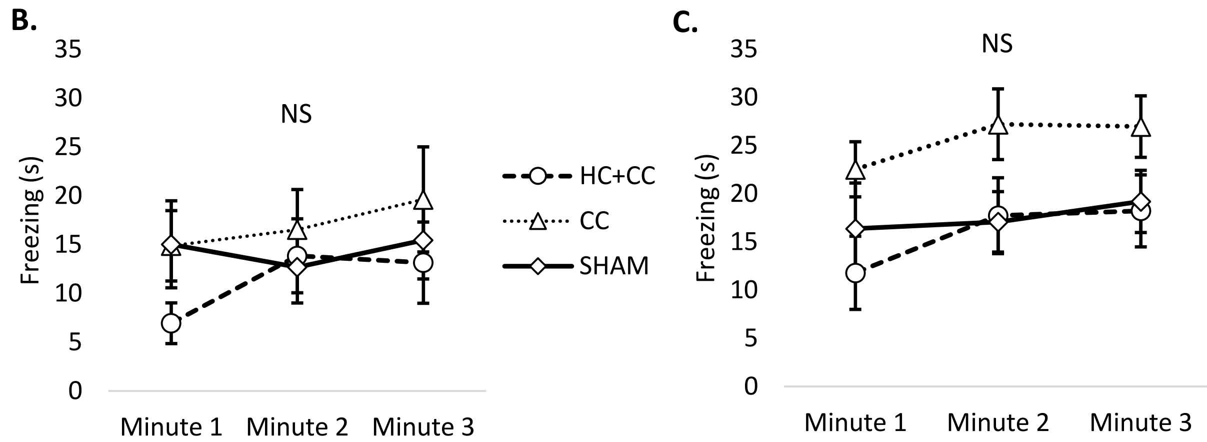
B. **C.**



595

596 **Figure 6.** ***A***, Short- and long-term contextual fear memory paradigm. ***B***, Freezing by
597 minute during context re-exposure following a 1-hour delay. ***C***, Freezing by minute
598 during context re-exposure following a 24-hour delay. NS indicates no significance
599 across treatment groups over the entire course of the retrieval session.

600

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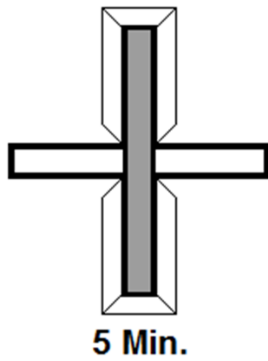
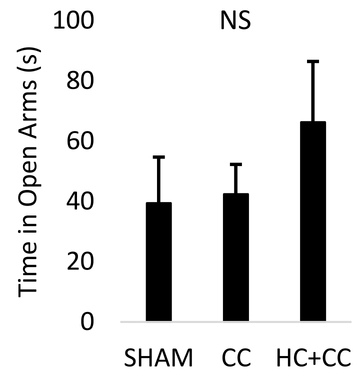
602 **A.** **B.**





603

604 **Figure 7.** ***A***, Elevated plus paradigm to assess anxiety. ***B***, Time spent in the open
605 arms of the apparatus during the 5-minute session. NS indicates no significance across
606 treatment groups.

607