Title

The dorsal hippocampus is required for the formation of long-term duration memories in rats

Running head

Dorsal hippocampus and duration memory

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ABSTRACT

Interval timing—the perception of durations mainly in seconds or minutes—is a ubiquitous behavior in organisms. Animal studies have suggested that the hippocampus plays an essential role in duration memory; however, the memory processes involved are unclear. To clarify the role of the dorsal hippocampus in the acquisition of long-term duration memories, we adapted the "time-shift paradigm" to a peak-interval procedure. After a sufficient number of training sessions with an initial target duration (20 s), the rats underwent "shift sessions" with a new target duration (40 s) under a muscimol (0.5 μg per side) infusion into the bilateral dorsal hippocampus. The memory of the new target duration was then tested in drug-free "probe sessions," including the empty trials in which any lever presses were not reinforced. In the probe sessions, the mean peak function of the muscimol group was located leftward to the control group, but these two functions were superimposed on the standardized time axis, suggesting a scalar property. In the session-by-session analysis, the mean peak time (an index of timing accuracy) of the muscimol group was lower than that of the control group in the probe sessions, but not in the shift sessions. In the trial-by-trial analysis, the mean start time and stop times (indices of timing accuracy) were lower than in the control group in the probe sessions. These findings suggest that the dorsal hippocampus is required for the formation of long-term duration memories within the range of interval timing.

Keywords: *Interval timing, Time perception, Peak interval, Dorsal hippocampus, Long-term memory, Short-term memory, Time-shift paradigm*

1. Introduction

Interval timing—the perception, estimation, and discrimination of durations in seconds, minutes, or hours—is a fundamental ability in many species to perform functions such as foraging and decision making (Buhusi & Meck, 2005). For instance, the nectar-feeding birds Amakihi (*Loxops virens*) periodically revisit the flowers from which they had previously collected nectar for efficient foraging (Kamil, 1978). Pigeons and rats underestimate the value of delayed reinforcement as a function of the duration of the delay (Mazur, 1987; Richards, Mitchell, & de Wit, 1997; Green, Myerson, Holt, Slevin, & Estle, 2004). For humans, instant noodles would be soggy if we could not estimate the interval it takes for them to be ready. Thus, interval timing is a ubiquitous behavior for organisms.

Animal studies have shown that hippocampal dysfunction alters interval timing behavior (for review, Lee, Thavabalasingam, Alushaj, Cavdaroglu, & Ito, 2019). The "classic effects" (Yin & Troger, 2011) of hippocampal or hippocampus-related regions dysfunctions on interval timing are to alter the accuracy of the estimated durations—a leftward shift of the response rate function during the peak-interval (PI) procedure (Hata & Okaichi, 1998; Meck, 1988; Meck, Church, & Olton, 1984; Olton Meck, & Church, 1987; Tam, Jennings, & Bonardi, 2015; Yin & Meck, 2014) and an interresponse time (IRT) distribution in the differential reinforcement of low rate (DRL) schedules (Jaldow & Oakley, 1990). These findings suggest that hippocampal lesions do not impair the interval timing itself (i.e., the regulation of behavior depending on the passage of time), while lesions alter the length of the estimated durations.

Other lines of evidence from trace conditioning, electrophysiology, and computational models suggest that the hippocampus and hippocampal neurons are involved in the

memory or coding of durations (see Table 1 in Lee et al., 2019). In trace conditioning, an unconditioned stimulus (US) is delivered after the termination of a conditioned stimulus (CS). The CS offset – US onset interval (CS–US interval) is usually set in the millisecond-to-second range. Many studies using a second CS–US interval range (i.e., range of the interval timing) have reported that hippocampal lesions, especially on the dorsal parts, impair the acquisition and retention of trace fear conditioning (McEchron, Bouwmeester, Tseng, Weiss, & Disterhoft, 1998; McEchron, Tseng, & Disterhoft, 2000; Quinn, Oommen, Morrison, & Fanselow, 2002; Quinn, Loya, Ma, & Fanselow, 2005; Lin & Honey, 2011; Raybuck & Lattal, 2011; Sellami, Abed, Brayda-Bruno, Etchamendy, Valério, Oulé, … Marighetto, 2017). Lee et al. (2019) stated that trace conditioning studies largely substantiate the hippocampus in representations of temporal duration memory. The hippocampal "time cell," which fires at a specific moment in a temporally structured experience, has received much attention in the last decade (Pastalkova, Itskov, Amarasingham, & Buzsaki, 2008; MacDonald, Carrow, Place, & Eichenbaum, 2013; Saltz, Tiganj, Khasnabish, Kohley, Sheehan, Howard, & Eichenbaum, 2016; for review, Eichenbaum, 2014). Moreover, recent computational studies suggest that the temporal distance between the to-be-learned time criterion and the time of the peak activity of each time cell provides an error signal to correct the synaptic weight (Oprisan, Aft, Buhusi, & Buhusi, 2018), and the memory of the duration is stored in the hippocampus (Oprisan, Buhusi, & Buhusi, 2018). Taken together, these recent findings suggest that the hippocampus plays an important role in duration memory.

However, these studies are insufficient to determine whether the hippocampus plays a vital role in the acquisition of duration memories. First, studies on trace conditioning did not selectively focus on duration memory. Thus, the impairment in the acquisition of trace

93 conditioning following hippocampal dysfunction can be explained not only by the
94 inability to acquire the memory of the duration but also by other impairments, such as the
95 disappearance of the memory trace of the CS presentation, or impairment of the
96 continuation of the timing proposed in the ICAT model of temporal processing (Petter,
97 Lusk, Hesslow, & Meck, 2016). Second, due to the nature of electrophysiological
98 correlational studies, it cannot be concluded in principle that the activity of the time cell
99 is the source of the duration memory. Lesion or inactivation studies should be performed
100 to answer this question. Third, models, including computational models, must be
101 supported by experimental observations. Taken together, an experimental design that
102 improves these deficiencies should be designed to determine whether the hippocampus
103 plays an important role in the acquisition of duration memory.

104 To meet these requirements, we applied the "time-shift paradigm" (Hata, 2011) to the
105 Peak-interval (PI) procedure. In the time-shift paradigm, after sufficient training with the
106 initial target duration (e.g., 20 s), the target duration was changed to 40 s in a "shift
107 session." In previous studies, a drug was injected into the striatum (MacDonald, Cheng,
108 & Meck, 2012) or plasticity-related gene expression levels in the striatum were evaluated
109 (Dallérac, Graupner, Knippenberg, Martinez, Tavares, Tallot, … Doyère, 2017) in the
110 shift session to clarify the relationship between the acquisition of duration memory and
111 neuronal processes. We believe that this paradigm can dissociate the effects of a drug
112 with regard to the acquisition of the duration memory from its impact on other processes
113 (e.g., task rules, which may be included in the early stage of learning during the first target
114 duration).

115 An important modification of the time-shift paradigm in our current experiment is to
116 adopt the probe sessions provided from the day after the shift sessions to evaluate the

117 effects of the drug infused during the shift sessions. As mentioned above, some indirect
118 evidence suggests the involvement of the dorsal hippocampus in duration memory.
119 However, the processes or stages of temporal memory in which the hippocampus is
120 involved remains unclear. An early study that applied the time-shift paradigm to the PI
121 procedure did not report impairments in the acquisition of the second duration memory
122 in hippocampal lesioned rats (Meck, 1988). However, this finding does not necessarily
123 mean that the hippocampus does not play a crucial role in acquiring duration memory.
124 This result may be explained by the notion that the hippocampus is important for the
125 acquisition of long-term, but not short-term, duration memories. In general, the PI
126 procedure is comprised of two types of trials— the "food" and "empty" trials. The food
127 trials have a discrete fixed interval (FI) schedule to allow learning of the target durations.
128 Empty trials are used to test the extent of duration memory. These trials are presented in
129 random order. Only data from the empty trials are usually analyzed. In empty trials, the
130 subjects can access not only the long-term duration memories that are acquired through
131 the previous sessions, but also the short-term duration memories that are retrieved from
132 the recent food trials within the session. In such situations, even if the hippocampal lesion
133 impairs the formation of long-term duration memory, the animals can behave adaptively
134 in the shift sessions, depending on the intact short-term duration memory. In fact, in a
135 previous study, an intra-dorsal hippocampal infusion of muscimol, an agonist of GABA
136 A receptors, impaired the intermediate-term (5 min) memory, but not the short-term (10
137 s) memory in a delayed non-matching to sample (DNMTS) task in the eight-arm radial
138 maze (Lee & Kesner, 2003). If probe sessions that are comprised of only empty trials are
139 prepared after the shift sessions, then the within-session short-term memory is no longer
140 unavailable in the sessions. All the available memories are limited to long-term memories

141 acquired through the previous sessions. In such situations, the impairment of the
142 acquisition of the long-term duration memory assumed by the hippocampal dysfunction
143 in the shift sessions should come to the surface in the probe sessions. To detect this
144 possibility, probe sessions provided from the day following the shift sessions were used
145 to evaluate the effect of the drug.

146 Therefore, the purpose of the present study was to clarify the role of the dorsal
147 hippocampus in the acquisition of long-term duration memory by applying the time-shift
148 paradigm to the PI procedure with probe sessions. After learning the first target duration
149 (20 s), the rats underwent PI training for a new target duration (40 s) during shift sessions
150 under the pharmacological inhibition of the bilateral dorsal hippocampus. The memory
151 of the new target duration was then tested using drug-free probe sessions, including empty
152 trials only. We hypothesized that an intra-hippocampal infusion of muscimol during the
153 shift sessions would impair the acquisition of long-memory of the second target duration.
154 We expected that the effects of the drug would be detected in the probe sessions, but not
155 in the shift sessions.

156

157 **2. Methods**

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159 **2.1 Subjects**

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161 Sixteen experimentally naïve, 10-week-old male Wistar albino rats (Shimizu
162 Laboratory Supplies, Kyoto, Japan) were used, but one animal died during surgery. Each
163 rat was individually housed in a stainless steel cage. The rats were depriving food for the
164 first day of shaping; hence, their body weight decreased to 85% of their free-feeding

weight at the start of the behavioral procedure. To compensate for the natural growth, the body weight of the rats was increased at a rate of 5 g per week. Water was available *ad libitum*. The temperature of the breeding room was maintained at 22 °C ± 2 °C. The light-dark cycle was 12:12 h, and the light phase started at 8:00 A.M. All experimental sessions were executed during the light phase. All experimental procedures were approved by the Doshisha Committee of Animal Experiments (A17075).

2.2 Apparatus

Eight identical operant chambers were individually installed in a sound-attenuating box with a 25 W LED light on the ceiling. Each chamber (220 × 165 × 200 mm in DWH) was equipped with a non-retractable lever on the left side (40 mm from the floor) and a food cup (15 mm from the floor) in the center of the front wall. The floor was made with stainless steel bars with a 3 mm diameter, each bar 10 mm apart. A self-made application developed with XOJO® (XOJO Inc. Austin, TX, USA) was run on two Macintosh PowerBook Air computers (Apple, Cupertino, CA, USA) to control the experiment and collect data. This was interfaced with two programmable controllers (SYSMAC CPM1A-40CDR-A-V1, OMRON, Kyoto, Japan) and two USB I/O controllers to control the eight chambers (RBIO-2U, Kyoritsu Electronic Industry, Osaka, Japan).

2.3 Procedure

Habituation. After handling for 5 min per day for 5 days, each animal was trained on a 20 s fixed time (FT) schedule for five sessions, one session per day. In each session, 30

189 food pellets (F0021-J, Bio-serv, Flemington, NJ, USA) were delivered separately every
190 20 s, irrespective of animal behavior. The house light was continuously turned on during
191 the session.

192 ***Shaping***. Rats were trained to press the lever under a continuous reinforcement
193 schedule until they earned 60 pellets from their lever presses or after 60 min had passed
194 in each session, whichever came first. The house light was turned on during the session.
195 The acquisition criteria were that they earned 60 pellets within 60 min in three successive
196 days.

197 ***PI 20 s training (sessions 1-30)***. The rats were then trained on the PI procedure with
198 one session per day. A session consisted of two types of trials—food and empty trials—
199 in a random order separated by intertrial intervals (ITIs) of 40 ± 10 s. The ratio of
200 food:empty trials was 42:18 in sessions 1-21 and 15:15 in sessions 22-30. Both types of
201 trials started with the onset of the house light and tone (signal stimuli) and finished with
202 their offsets. In a food trial, the first lever press after 20 s from the start of the trial was
203 reinforced with a food pellet, and then the trial finished (discrete trial FI 20 s). An empty
204 trial lasted for 60 s without any reinforcements for any lever presses. If a lever press was
205 emitted during the last five seconds of an ITI, the ITI was extended for 10 s from the
206 press.

207 ***Surgery***. Rats were anesthetized with 2-3% isoflurane (2-3 L/min flow rate, MK-A110,
208 Muromachi, Tokyo, Japan) and fixed on a stereotaxic frame (Kopf Instruments, Tujunga,
209 CA, USA). A guide cannula (C232G-3.8/SPC, Plastics One, Roanoke, VA, USA)
210 attached to a dummy cannula (C232DC-3.8/SPC, Plastics One) was lowered into each
211 hemisphere of the dorsal hippocampus. The target coordinates of the tip of the guide
212 cannula based on a brain map (Paxinos & Watson, 1998) were as follows: -3.6 mm

213 anteroposterior to the bregma, ± 1.9 mm mediolateral, and -2.2 mm dorsoventral from the
214 skull surface. The cannula assembly was fixed to the skull surface using dental cement
215 and two small screws. The dummy cannula was inserted into the guide cannula and fixed
216 using a dust cap (303DC, Plastics One). Rats were allowed to recover for ≥5 d after the
217 surgery. For a few days during the early recovery period, an antibiotic agent (Mycillinsol,
218 Meiji, Tokyo, Japan) was applied to the wound once per day to avoid infections.

219 ***PI 20 s retraining (sessions 31-37)***. After the recovery period, rats were retrained using
220 the PI procedure (seven sessions) similar to the training phase. The ratio of the food:empty
221 trials was 15:15. The last two retraining sessions were defined as baseline sessions (Figure
222 2A).

223 ***Shift session (sessions 38-39)***. Rats were assigned to one of two groups—the artificial
224 cerebrospinal fluid (aCSF) or muscimol (Mus) group—to ensure that the performances
225 during the last three sessions of the retraining phase were as even as possible. Immediately
226 before starting each session, the dummy cannula was replaced with an internal cannula
227 (C232I-3.8/SPC, Plastics One), extending 1 mm from the tip of the guide cannula for
228 infusion. The muscimol solution for Mus rats (0.5 μg/μL, 1 μL per side) or aCSF for
229 aCSF rats (1 μL) was infused into the bilateral dorsal hippocampus at a flow rate of 0.5
230 μL/min for 2 min using a gastight syringe (100 μL, Hamilton, Reno, NV, USA) and
231 microsyringe pump (ESP-32, Eicom, Kyoto, Japan). The cannula was left in place for 1
232 min to allow dispersion of the solution, which was then replaced with a dummy cannula.
233 The dummy cannula was again inserted into the guide cannula and fixed with a dust cap.
234 The significant changes in the procedure from the previous sessions were that lever press
235 behavior was reinforced with a 40 s FI during food trials, and that the duration of the

236 empty trials was extended to 120 s. The other parameters were the same as those in the
237 retraining sessions.
238 ***Probe session (sessions 40-41)***. Rats were tested in two sessions of 15 empty trials
239 without infusions. The other parameters were the same as those in the shift sessions.
240
241 **2.4 Dependent variables**
242
243 *Session-by-session analysis* For each rat's session, the total number of lever-press
244 responses in each 3-s bin (such as 0-3 s and 1-4 s) was counted over all empty trials and
245 converted to the percentage of the maximum number of responses among the bins
246 (response rate). The response rates were plotted as a function of elapsed time (peak
247 function). The peak function was fitted with a Gaussian curve using KaleidaGraph® (ver.
248 4.5.3, Synergy Software, Reading, PA, USA): $R(t) = a + b \times \exp\{-.5 \times [(t - c)/d]^2\}$. Here,
249 t is the time from the start of the empty trial, and $R(t)$ is the response rate in bin t.
250 Parameter c is the time at which the response rate was at its maximum. Thus, c was
251 adopted as the peak time (an index of the subjective length of the to-be-timed duration).
252 The initial values of a, b, and d for the iterative estimation were 1, 100, and 10,
253 respectively. To determine the initial c, the first and last bins, which had response rates
254 of over 80%, were detected. The mean of the two class values of these bins was set at the
255 initial c. The data used for fitting ranged from 1.5 to 38.5 s for the baseline sessions, and
256 1.5 to 78.5 s for the shift and probe sessions, which was performed to cut off the noise
257 observed in the last part of the empty trials. The allowable error of the interactive
258 estimation was set to 0.1%. Curve fitting was also performed for the mean peak functions.
259 For the mean functions, the initial a, b, and d values were the same as for the individual

260 functions, whereas *c* was set at 20 and 40 for the baseline and the shift/probe sessions,
261 respectively. The data used to fit the mean functions ranged from 1.5 to 58.5 s for the
262 baseline sessions and 1.5 to 118.5 s for the shift and probe sessions. For the standardized
263 mean functions of the probe sessions (see Figure 2D), the values of the x (time) axis were
264 converted to the ratio of the estimated peak time in each group. The original time values
265 corresponding to the standardized x of 0.1 steps were substituted into the regression curve
266 formula. The Y values were then converted to the maximum Y value.

267 The discrimination index (*DI*) was also calculated for the individual peak function
268 using *DI* = 100/mean response rate of all bins in the peak function (Meck & Church,
269 1987) as an index of precision. In addition, for the DI calculation, the range of averaging
270 in the denominator was from 1.5 s to 38.5 s for the baseline sessions and from 1.5 s. to
271 78.5 s for the shift and probe sessions, respectively.

272 *Trial-by-trial analysis* In an individual empty trial, rats showed a "low-high-low"
273 response pattern. That is, they started pressing the lever before the required time, kept
274 responding, and then stopped responding after the required time. According to an
275 algorithm proposed by a previous study (Church, Meck, & Gibbon, 1994), the timepoints
276 of the transition from low to high (start time) and high to low (stop time) can be detected.
277 The midpoint is the mean of the start and stop times. The start time and stop times are the
278 indices of timing accuracy, in parallel with the peak time of the session-by-session
279 analysis. The spread was calculated by stop time minus start time, which was the index
280 for the timing precision.

281

282 **2.5 Histology**

283

284 After completing all behavioral sessions, the rats were deeply anesthetized with sodium
285 pentobarbital (130 mg/kg, i.p.) and infused with dye (2% pontamine sky blue) using a
286 procedure similar to that of drug infusion to locate the tips of the internal canulae. They
287 were then perfused intracardially with saline, followed by ALTFiX® (FALMA, Tokyo,
288 Japan). Their brains were removed and soaked in 10% and then 20% sucrose phosphate
289 buffer (0.1 M) until they sunk in each solution. The brains were sectioned in the coronal
290 plane (40-μm thickness) using a cryostat (CM1850, Leica, Wetzlar, Germany). Sections
291 were stained with cresyl violet to assess the location of the cannula tip.
292
293 **2.6 Statistical analysis**
294
295 Two-way mixed ANOVAs followed by the test of simple main effects and Holm's
296 multiple comparisons were performed using "anovakun" (ver. 4.8.5.,
297 http://riseki.php.xdomain.jp/index.php) in R software (ver. 4.0.0, R Core Team, Vienna,
298 Austria).
299
300 **3. Results**
301
302 **3.1 Histological results**
303
304 A photomicrograph of a representative section is presented in Figure 1A. The tips of
305 the guide cannulae (arrows) were located above the dorsal hippocampus, while those of
306 the internal cannulae were extended 1 mm from the guide cannulae. Hence, all tips of the

307 internal canulae were presumed to be located within the dorsal hippocampus (Figure 1B).
308 Apparent injuries beyond the canula tracks could not be observed.
309
310 **3.2 Session-by-session analysis**
311
312 The data from three rats were excluded from the session-by-session analysis because
313 the peak time or R^2 of the fitting curve of their probe or baseline sessions were over ±2
314 SD from the mean of each group. As a result, the number of subjects used in the session-
315 by-session analysis was six for each group.
316 ***Response rate function***. The mean peak function for the two sessions of the last
317 retraining sessions (baseline), shift sessions, and probe sessions were calculated for each
318 rat. The group mean is presented in Figure 2. Qualitative observation showed that the
319 functions in the baseline (Figure 2B) could be seen to overlap with each other. The peaks
320 of both groups were located at approximately 19 s. In the shift sessions (Figure 2C),
321 although the Mus group had a wider distribution than the aCSF group, the peaks of both
322 groups were located around 33 s. Interestingly, the functions from the probe sessions
323 (Figure 2D) showed a dramatic difference between the groups. The function from Mus
324 appeared to the left of the aCSF. The peak of the Mus group was around 26 s, whereas
325 that of the aCSF group was around 35 s. These functions, however, were superimposed
326 as a whole by standardizing the horizontal axis (Figure 2E).
327 ***Peak time***. Mean peak times for the baseline, shift sessions, and probe sessions were
328 calculated for each rat. The group mean (± *SEM*) is presented in Figure 3A. There was no
329 difference in the mean peak time of the baseline and shift sessions, whereas it was lower
330 in the Mus group than in the aCSF group for the probe sessions. Mixed two-way ANOVA

revealed that the interaction of the group × block (baseline, shift, and probe) ($F(1.68, 16.82) = 4.96$, *df*s were adjusted by Huynh-Feldt-Lecoutre's epsilon, $p = .025$, $\eta_G^2 = .267$). The simple main effect of the group type was significant for the probe sessions ($F(1, 10) = 6.85$, $p = .026$, $\eta_G^2 = .407$), but not for the baseline and shift sessions ($Fs(1, 10) = .05$ and .69, $ps = .834$ and .426, $\eta_G^2s = .005$ and .065, respectively). The simple main effects of the blocks were significant in both groups (aCSF: $F(1.03, 5.16) = 15.55$, $p = .010$, $\eta_G^2 = .703$. Mus: $F(1.88, 9.41) = 24.06$, $p < .001$, $\eta_G^2 = .767$). The post-hoc Holm's multiple comparisons test showed that the peak times of the shift and probe sessions were significantly higher than the baseline in each group. Moreover, in the Mus group, the mean peak time of the probe sessions was significantly lower than that of the shift sessions.

DI. The mean DI for the baseline, shift sessions, and probe sessions were calculated for each rat; the mean *DI* values (± *SEM*) are presented in Figure 3B. There was no difference in *DI* between the baseline and probe sessions, whereas it was lower in the Mus group than in the aCSF group in the shift sessions. A mixed two-way ANOVA revealed a significant interaction ($F(2, 20) = 8.02$, $p = .003$, $\eta_G^2 = .27$). The simple main effect of the group type was significant in the shift sessions, but not in the probe sessions ($Fs(1, 10) = 7.48$ and 4.19, $ps = .021$ and .068, $\eta_G^2s = .428$ and .295, respectively).

R^2. The mean R^2 (± *SEM*) of the fitting curves is shown in Table 1. All values were over .820. Mixed two-way ANOVA did not detect any significant effects (interaction and main effect blocks: $Fs(1.05, 10.46) = 2.00, 4.42$, $ps = .186$ and .059, $\eta_G^2s = .095$ and .187, main effect of group type: $F(1, 10) = .819$, $p = .389$, $\eta_G^2 = .038$).

3.3 Trial-by-trial analysis

Data from two rats were excluded from the trial-by-trial analysis because the values of one of three indices (start time, midpoint, stop time, or spread) in the baseline or probe sessions were over ±2 SD from the mean of each group. The midpoint was used only for the excluding the outlier. As a result, the numbers of subjects in the trial-by-trial analysis were 7 and 6 for aCSF and Mus, respectively.

Start time and stop time The medians of the start time and the stop time were calculated for all trials (15 trials × 2 sessions, 30 trials) in the baseline, shift, and probe sessions for each rat. The group mean of the medians (± *SEM*) is shown in Figure 4. For the start time (Figure 4A), the means were different between the groups and the phases. However, a two-way mixed ANOVA (group × phase) revealed that the interactions ($F(1.60, 17.58) = 3.215$, $p = .074$, $\eta_G^2 = .137$) and the main effect of the group ($F(1, 11) = .692$, $p = .423$, $\eta_G^2 = .028$) were not significant. The main effect of the phase was significant ($F(1.60, 17.58) = 3.215$, $p = .074$, $\eta_G^2 = .433$). For the stop time (Figure 4B), the means of the Mus group was higher than that of the aCSF group in the shift sessions, whereas the relationship was reversed in the probe sessions. A two-way ANOVA revealed that the interaction was significant ($F(1.45, 15.94) = 9.067$, $p = .004$, $\eta_G^2 = .355$). The simple main effect of the group was significant in the shift session ($F(1, 11) = 12.292$, $p = .005$, $\eta_G^2 = .528$), but not in the probe session ($F(1, 11) = 4.501$, $p = .057$, $\eta_G^2 = .290$). The simple main effect of the phase was significant in both groups (aCSF: $F(1.19, 7.15) = 27.811$, $p < .001$, $\eta_G^2 = .769$, Mus: $F(1.29, 6.44) = 125.949$, $p < .001$, $\eta_G^2 = .929$). The post-hoc Holm's multiple comparisons test showed that the stop time of the probe sessions was significantly lower than the shift sessions in the Mus group but not in the aCSF group.

Spread. The group means of the medians (± *SEM*) of the spread are shown in Figure 4C. It is noticeable that the spread of the Mus group was shorter than that of the aCSF group in the probe sessions. The interaction and the following test of the simple main effect of the group in the probe sessions were significant ($F(1.65, 18.20) = 6.65$, $p = .009$, $\eta_G^2 = .295$, and $F(1, 11) = 5.71$, $p = .036$, $\eta_G^2 = .342$, respectively). The simple main effect of the phase was significant in both groups (aCSF: $F(1.61, 9.64) = 22.448$, $p < .001$, $\eta_G^2 = .719$, Mus: $F(1.30, 6.48) = 50.694$, $p < .001$, $\eta_G^2 = .880$). The post-hoc Holm's multiple comparisons test showed that the spread of the probe sessions was significantly lower than the shift sessions in the Mus group but not in the aCSF group.

4. Discussion

Two fundamental prerequisites; the homogeneity of the two groups before the important experimental operations and the validity of the task, were suggested to be guaranteed by the data. First, the peak functions of both groups almost entirely overlapped with each other in the baseline sessions (Figure 2B). Moreover, the mean peak time, *DI* (Figure 3), and R^2 (Table 1) in the session-by-session analysis, and the mean start time, stop time, and spread in the trial-by-trial analysis (Figure 4) in the baseline sessions were all similar between the groups. These findings suggest that the differences between groups in the following analysis do not stem from the bias of the assignment of the subjects to the groups. Second, the task's validity was confirmed by the transition of the mean peak time (Figure 3A). In the aCSF group, the peak time of the shift session was significantly higher than that of the baseline, suggesting that the memory of the new target time was acquired by the animals. In addition, the extended peak time was maintained in

402 the probe sessions, as evidenced by the non-significant difference between these two
403 phases. These findings suggest that the memory of the new target duration was maintained
404 for at least 24 h.

405 In the shift session, dysfunction of the dorsal hippocampus was suggested to impair the
406 precision, but not the accuracy of the interval timing. The significant extension of the stop
407 time (Figure 4B) inevitably induced elevated response rates after the peak time in the
408 peak function (Figure 2C). This elevation could account for the significantly lowered *DI*
409 value seen in the Mus group (Figure 3B), suggesting impaired precision (discussed further
410 below). On the other hand, the dysfunction of the hippocampus does not seem to impair
411 the accuracy of the interval timing at the shift sessions, as evidenced by the lack of a
412 difference in peak time in the shift sessions (Figure 3A). This finding, however, does not
413 contradict the "classic effect" (Yin & Troger, 2011, see the Introduction section) of the
414 hippocampal lesion on the interval timing. Many studies have repeatedly reported that a
415 dysfunction of the hippocampus results in shortening of the peak time (Hata & Okaichi,
416 1998; Meck, 1988; Meck et al., 1984; Olton et al., 1987; Tam et al., 2015; Yin & Meck,
417 2014). In these studies, the effect has been reported in sessions in which the required time
418 of the PI procedure was constant and appeared after the chronic dysfunction of the
419 hippocampus. However, in our study, the required time was changed, and the
420 hippocampal function was reversibly inhibited only in two sessions. In this way, the
421 situation in our study was different from those suggestive of "classic" hippocampal
422 dysfunction effects. Therefore, our findings do not contradict those of previous studies.

423 The noticeable findings observed in the probe sessions can be explained by the notion
424 that the dorsal hippocampus is involved in the formation of long-term, but not short-term,
425 duration memories. The most important asset of the probe sessions was that the sessions

426 included only empty trials. Thus, the within-session short-term memory was not available
427 for feedback control of the behavior in empty trials. All of the memories that were
428 available to guide their behavior were limited to those acquired during the
429 training/retraining sessions and/or the shift sessions. It is known that when the required
430 time is increased during peak interval procedures, the peak times gradually increase in
431 normal rats (Lejeune, Ferrarra, Simons, & Wearden, 1997; Meck, Komeily-Zadeh, &
432 Church, 1984). These findings suggest that the shorter the peak time, the weaker the
433 memory of the new required time. Here, our data were as follows: the mean peak function
434 of the Mus group was located to the left of the aCSF group's function (Figure 2D). The
435 mean peak time of the Mus group was lower than that of the aCSF group in the probe
436 sessions (Figure 3A). The significantly extended peak time from the baseline sessions to
437 the shift sessions was maintained in the probe sessions in the aCSF group but not in the
438 Mus group (Figure 3A) . In the trial-by-trial analysis, the mean stop time of the Mus group
439 in the probe sessions was sufficiently (p=.057) lower than the aCSF group (Figure 4B).
440 Moreover, the significantly extended stop time from the baseline sessions to the shift
441 sessions was maintained in the probe sessions in the aCSF group but not in the Mus group
442 (Figure 4B). These results consistently suggest that the animals in the Mus group had a
443 poorer memory of the new required time (40 s) than the aCSF animals in the probe
444 sessions. Other important results were that the mean peak times were similar between
445 groups in the shift sessions (Figure 3A). This can be interpreted to show that the within-
446 session short-term memory from the food trials was available even though the muscimol
447 was infused, which guided the animals' adaptive behavior. Some previous studies support
448 this "short-term/long-term dissociation hypothesis" (Haettig, Stefanko, Multani,
449 Figueroa, McQuown, Wood, 2011; Lee & Kesner, 2003; Nagahara & McGaugh, 1992).

450 In a study on the DNMTS task in the eight-arm radial maze, an intra-dorsal hippocampal
451 infusion of muscimol impaired their intermediate-term (5 min) memory, but not their
452 short-term (10 s) memory (Lee & Kesner, 2003). Intra-dorsal hippocampal muscimol
453 infusion immediately after memory acquisition resulted in impairments in the
454 performance of an object memory test conducted in mice 24 h after the acquisition
455 (Haettig et al., 2011). Moreover, a muscimol infusion before the acquisition of inhibitory
456 avoidance into the septum, which has strong reciprocal connections with the
457 hippocampus, disrupted retrieval after 48 h, but not 15 s. After 15 min of acquisition of
458 the memory, the performance was halfway between 48 h and 15 s conditions (Nagahara
459 & McGaugh, 1992). Although the task used and/or the target region in our study were
460 different from those of the reference studies, it is not surprising that the within-session
461 short-term memory was available in our experiment. Taken together, our evidence
462 strongly suggests that dysfunction of the dorsal hippocampus impairs the formation of
463 long-term memory of a new duration.

464 The short-term/long-term dissociation hypothesis can also explain the discrepancy
465 between our findings, the findings of a previous study, and the lower *DI* of the Mus group
466 in the shift sessions. First, as mentioned in the Introduction, Meck (1988) reported that
467 the hippocampectomized rats did not show a delay in the acquisition of memories of the
468 new target duration. In this experiment, however, the effect of the hippocampal lesion
469 was examined in the shift sessions, each of which comprised both food and empty trials.
470 If this hypothesis is correct, it is quite reasonable that the previous study did not report
471 impairments in the acquisition of the new target memory. Therefore, this "discrepancy"
472 may be ostensible. Second, the lower *DI* of the Mus group in the shift sessions might be
473 explained by the incomplete short-term memory and the resulting dysfunction in feedback

474 control. A previous study suggested that normal animals modulate when to start
475 responding and when to stop in PI procedures, which were regulated according to the
476 information from their own performances in recent food trials (Meck, 1988). The study
477 also reported that this feedback control was impaired by the hippocampal lesion. Even if
478 the within-session short-term memory seemed to be available in the Mus group, the
479 availability might be lower than that of the aCSF group because, according to previous
480 studies (Lee & Kesner, 2003; Nagahara & McGaugh, 1992), the inferred time window in
481 which short-term memory was maintained may vary from several dozen seconds to
482 several minutes. However, in our PI procedure, the animals must maintain a memory for
483 at a least 160 s (40 s for the mean duration of ITI + 120 s for duration of an empty trial)
484 for feedback control. Indeed, the contents of the memories are different from each other
485 (durations, spatial information, and fear emotions), so the estimation may be too
486 simplified. However, it seems reasonable to infer that a weakness of the feedback control
487 resulting from the available but weak short-term memory produced a fluctuation of the
488 "low-high-low" pattern and then lowered the *DI* in the shift sessions. This speculation
489 should be confirmed by further studies.

490 The shortening of the spread and the superimposition of the peak functions can be
491 compatible without contradiction. In the literature on interval timing, it has been
492 repeatedly confirmed that two peak functions produced under different required times are
493 superimposed on the standardized time axis relative to the peak time, that is, the scalar
494 property (Church et al., 1994; Gibbon, 1977; Gibbon & Church, 1990, 1992; Malapani &
495 Fairhurst, 2002). This means that the ratio of the width of the function to the peak time is
496 a constant (i.e., Weber's law). In our data, the two peak functions were superimposed on
497 each other in the probe session (Figure 2E), suggesting that the Mus rats had a normal

interval timing based on their own target times, which were different from those of the aCSF rats. Therefore, the narrower mean spread (Figure 4C) and peak function (Figure 2D) in the Mus group, which had a smaller peak time, were inevitable.

The most noticeable progress of our study was its extension of previous knowledge from trace conditioning, electrophysiology, and computational models. First, data from trace conditioning were believed to be evidence of the role of the hippocampus in temporal duration memory (Lee et al., 2019). However, other explanations, such as the disappearance of the memory trace of the CS presentation or an impairment of the "continuation" proposed by the ICAT model of timing (Petter et al., 2016) can also explain the data. Using a timing-specific task (peak interval task) and a filled duration (i.e., the duration in which the to-be-timed stimulus is continuously presented) and confirming the normal interval timing in the probe sessions in the Mus group, we could exclude alternative interpretations of the data from the trace conditioning. Second, inactivation by the muscimol demonstrated the causal, but not correlational, relationship between the hippocampus and the acquisition of long-term duration memory. Third, our data provided experimental support to the computational models arguing the memory role of the hippocampus in interval timing (Oprisan, Aft, Buhusi, & Buhusi, 2018; Oprisan, Buhusi, & Buhusi, 2018).

In summary, the present findings strongly suggest that the dorsal hippocampus plays an important role in the formation of long-term, but not short-term, duration memory in the supra-second range.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

T.Y. conceptualized the study. T.H. and T.K. supervised the study. T.Y., T.K., and T.H. performed the experiments. T.H. and T.K. reanalyzed the data. T.H. and T.K. wrote the manuscript.

DATA AVAILABILITY STATEMENT

The datasets are available from the open science framework (OSF). https://osf.io/fqngb/?view_only=6d21bea2cec74fa784a9cd79f3fe3774

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Table 1. Mean (± *SEM)* R^2 of the fitting curves

	Baseline	Shift	Probe
aCSF	0.959 (.009)	0.924 (.027)	0.939 (.014)
Mus	0.961 (.011)	0.820 (.079)	0.947 (.013)

663

664 **Figure captions**

665 **Figure 1**. **(A)** A representative photomicrograph showing the tracks of the canulae. The
666 tips of the guide canulae (arrows) were located above the dorsal hippocampus. The tips
667 of the internal canulae were extended 1 mm ventrally from these. Scale bar = 1 mm. **(B)**
668 Placements of the internal cannula tips for the aCSF (open circles) and Mus rats (closed
669 circles) on the brain. Reprinted from Paxinos, G. & Watson, C. *The Rat Brain in*
670 *Stereotaxic Coordinates. 4th ed. [CD-ROM]*, Copyright (1998).

671

672 **Figure 2**. **(A)** Overview of the main parts of the experiment. Each types of trials were (+)
673 or were not (-) included in the sessions. Note that the required time was changed from 20
674 s to 40 s for the shift sessions and the drugs were infused before starting these sessions.
675 In addition, only empty trials were included in the probe sessions. Mean peak function in
676 the baseline **(B)**, shift **(C)**, and probe **(D)** sessions. The vertical dotted lines show the
677 original (20 s) and new (40 s) target times. The arrows show the time at which the
678 response rate was at the maximum for each group. **(E)** Standardized mean peak functions.
679 The horizontal axis was converted to the ratio to the peak time of each peak function. The
680 open and closed circles are the estimated standardized response rates for the aCSF and
681 Mus group respectively. The two curves were overlapping as a whole.

682

683 **Figure 3**. Mean (± *SEM*) peak time **(A)**, and DI **(B)**. * $p < .05$, ** $p < .01$ between the
684 groups. # $p <.05$, ## $p < .01$, ### $p < .001$ *vs.* the baseline of the group.

685

686 **Figure 4**. Mean median (± *SEM*) of the start time **(A)**, the stop time **(B)**, and the

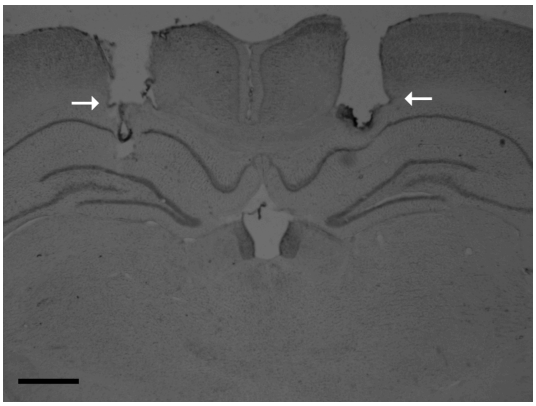
687 spread **(C)**. $p < .05$, ** $p < .01$ between the groups. # $p <.05$, ## $p < .01$, ### $p < .001$

688 *vs.* the baseline of the group.

689

Fig.1

A



B

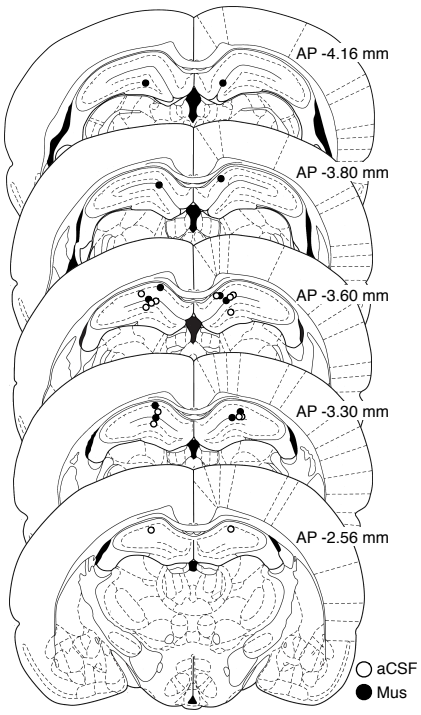
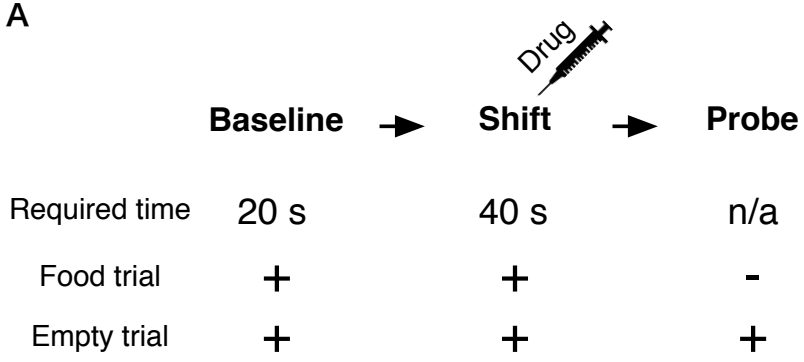


Fig.2

A


Drug
Baseline
Shift
Probe
Required time
20 s
40 s
n/a
Food trial
+
+
-
Empty trial
+
+
+

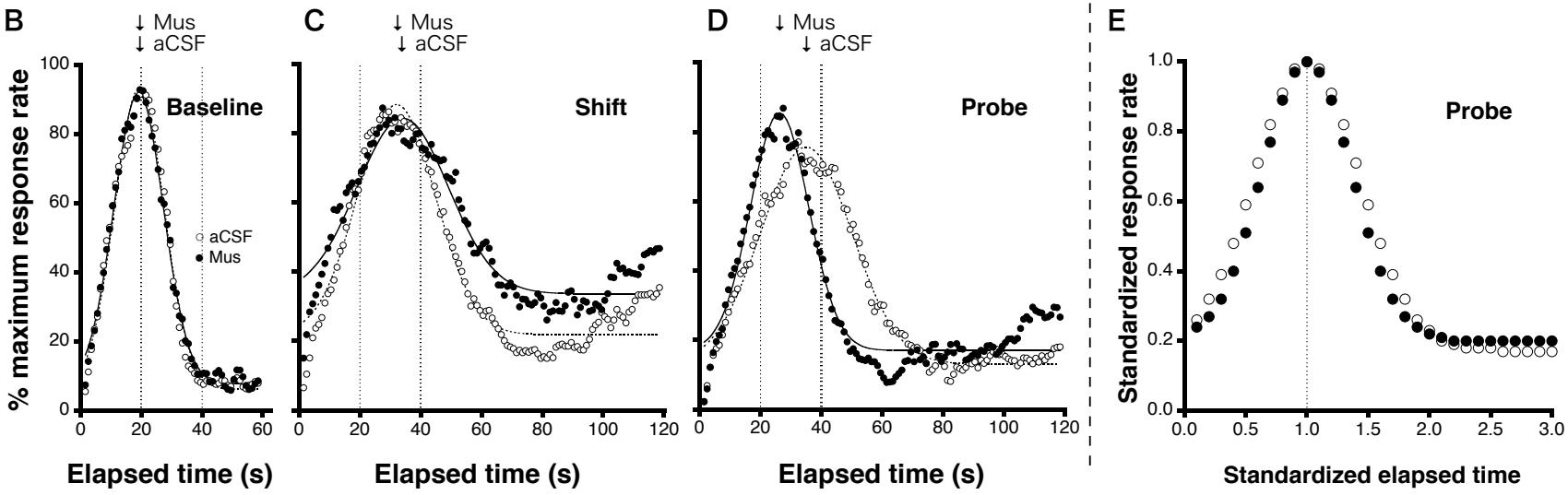

B
↓ Mus
↓ aCSF
Baseline
aCSF
Mus
% maximum response rate
Elapsed time (s)
C
↓ Mus
↓ aCSF
Shift
Elapsed time (s)
D
↓ Mus
↓ aCSF
Probe
Elapsed time (s)
E
Probe
Standardized response rate
Standardized elapsed time

Fig.3

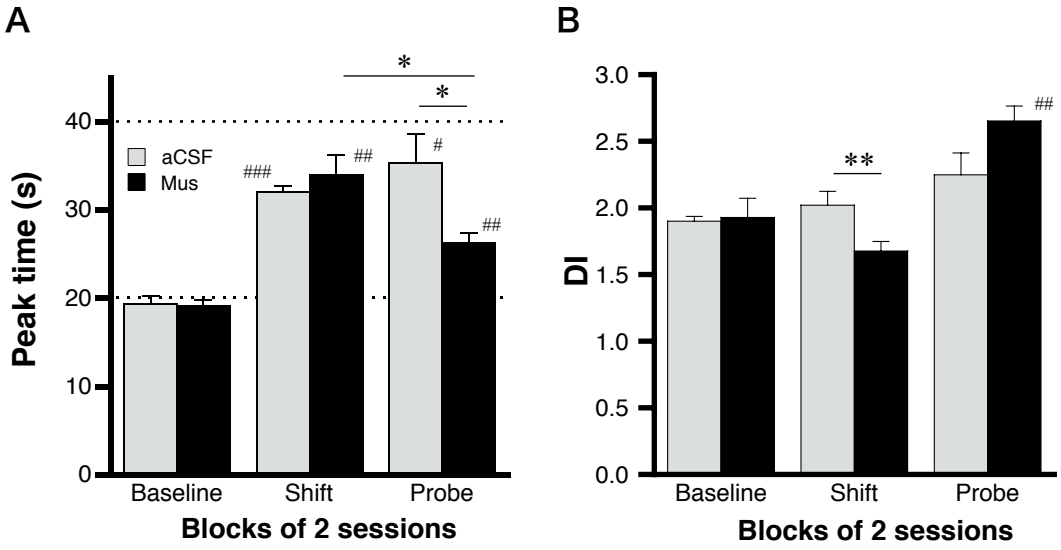
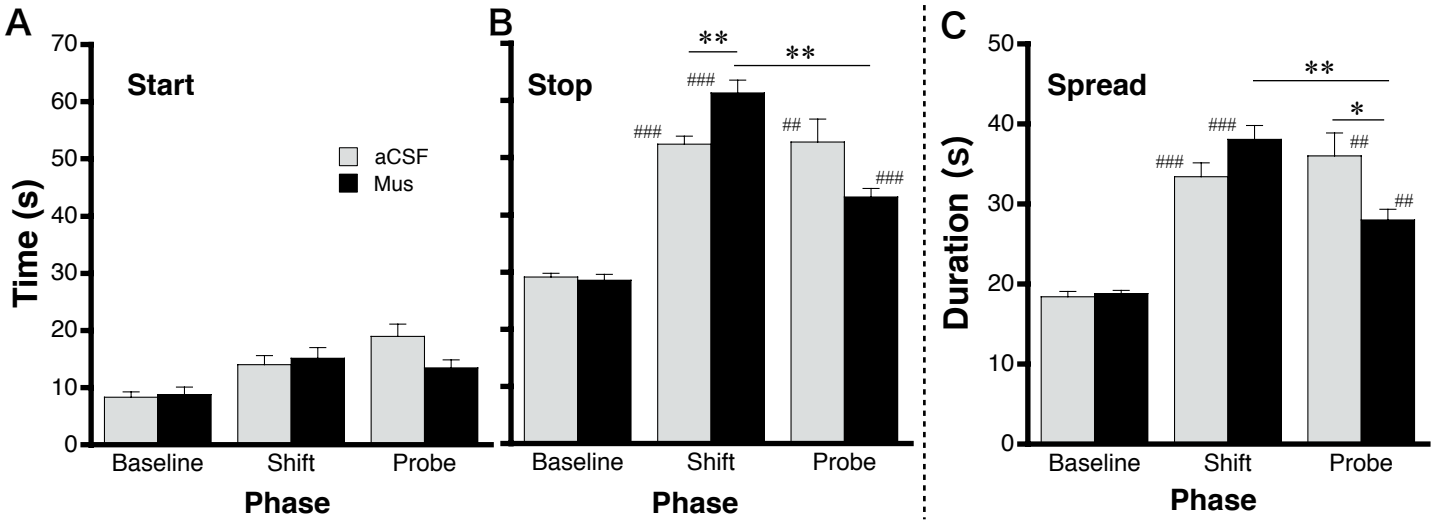

A
B
Peak time (s)
aCSF
Mus
Baseline
Shift
Probe
Blocks of 2 sessions
DI
Blocks of 2 sessions

Fig.4


A
Start
Time (s)
70
60
50
40
30
20
10
0
aCSF
Mus
Baseline
Shift
Probe
Phase
B
Stop
**
**
###
###
##
###
Baseline
Shift
Probe
Phase
C
Spread
Duration (s)
50
40
30
20
10
0
**
*
###
###
##
##
Baseline
Shift
Probe
Phase