Effects of Dorsal Hippocampal Damage on Conditioning and Conditioned-Response Timing: A Pooled Analysis

Shu K.E. Tam,1* Dómhnall J. Jennings,2 and Charlotte Bonardi3

ABSTRACT: Behavioral findings suggest that the dorsal hippocampus (DHPC) plays a role in timing of appetitive conditioned responding. The present article explored the relationship between the extent of DHPC damage and timing ability, in a pooled analysis of three published studies from our laboratory. Initial analyses of variance confirmed our previous reports that DHPC damage reduced peak time (a measure of timing accuracy). However, the spread (a measure of timing precision) was unchanged, such that the coefficient of variation (spread/peak time) was significantly larger in DHPC-lesioned animals. This implies that, in addition to the well-established effect of DHPC lesions on timing accuracy, DHPC damage produced a deficit in precision of timing. To complement this analysis, different generalized linear mixed-effects models (GLMMs) were performed on the combined dataset, to examine which combinations of the different behavioral measures of timing were the best predictors of the degree of hippocampal damage. The results from the GLMM analysis suggested that the greater the DHPC damage, the greater the absolute difference between the observed peak time and reinforced duration. Nevertheless, this systematic relationship between damage and performance was not specific to the temporal domain: paradoxically the greater the damage the greater the magnitude of peak responding. We discuss these lesion effects in terms of scalar timing theory.

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KEY WORDS: pavlovian conditioning; interval timing; peak procedure

INTRODUCTION

There is now compelling behavioral and electrophysiological evidence that the hippocampus is involved in spatial cognition, and moreover, it is the dorsal hippocampus (DHPC) that is crucial. For example, Moser et al. (1995) systematically manipulated the volume of hippocampal damage, starting from either the dorsal or ventral pole of the structure. They confirmed that the DHPC, but not the ventral hippocampus (VHPC), was critical for navigation to a hidden goal using distal visual cues, and that there was a positive relationship between the extent of DHPC damage and spatial navigation deficit (see also Moser et al., 1993). Further evidence from single-unit recording studies suggests that, although pyramidal neurons of both the DHPC and VHPC have location-specific receptive fields (place cells), the DHPC place cells are more stable, coherent, and location-specific than those in the VHPC (Jung et al., 1994; Kjelstrup et al., 2008; Royer et al., 2010).

Nevertheless, the role of the DHPC in cognition is not confined to the spatial domain, and it may also be involved in temporal aspects of learning and performance (Olton, 1986; Kesner, 1998; Sakata, 2006; Howard and Eichenbaum, 2013). For example, McEchron et al. (2003) trained rabbits that a fixed-duration, 3-s conditioned stimulus (CS) was paired with a paraorbital shock after an empty trace interval of either 10 or 20 s. On non-reinforced test trials when the empty trace period was extended, some pyramidal neurons of the DHPC fired maximally at the time point at which the shock had been applied during conditioning. Electrophysiological findings of this type complement an earlier report by Meck et al. (1984), who examined the effect of lesions of the fimbria-fornix (fibers connecting the hippocampus with various subcortical structures) in a similar appetitive instrumental task. A fixed-duration, 20-s stimulus was paired with food at its termination, and subsequently non-reinforced test trials with an extended stimulus period were presented. On the test trials, the time point at which maximal appetitive responding (lever pressing) occurred was estimated. In control animals, maximal responding occurred at the time at which food was delivered on the training trials, but subjects with fimbria-fornix lesions exhibited maximal responding at earlier time points (see also Meck, 1988).

These findings are consistent with the possibility that the hippocampus, or the DHPC, is involved in temporal cognition; but the behavioral evidence, at least, is not conclusive. The subjects in the study of Meck et al. (1984) underwent fimbria-fornix lesions which, by damaging fibers connecting the hippocampus with various subcortical structures, could have had different behavioral effects from those produced by damage to the hippocampus itself (e.g., Sanderson et al., 2006 vs. Aggleton et al., 2009). Until recently...
there was almost no behavioral evidence that could unequivocally establish that damage to the hippocampus, and more specifically the DHPC, is sufficient to produce a temporal deficit (although see Jaldow et al., 1989; Balci et al., 2009; Yin and Meck, 2014). To address this deficiency, we examined the effect of ibotenic-acid lesions of the DHPC in an appetitive conditioning task, in which a fixed-duration, 15-s CS was followed by a food pellet at its termination (Tam and Bonardi, 2012a; Tam et al., 2013). On subsequent non-reinforced test trials we found that, while sham-lesioned subjects showed maximal responding at the time point at which food had been delivered during training, subjects with DHPC damage showed maximal responding at significantly earlier time points (i.e. they underestimated the CS duration). Thus, damage to the DHPC is sufficient to reproduce the temporal deficits observed after fimbria-fornix lesions.

However, other results from our laboratory slightly complicate this picture. In a further study, we examined conditioned responding and timing to a 40-s CS (Tam and Bonardi, 2012b). We reported that while lesioned subjects showed numerically earlier peak times than control animals, this effect was not statistically significant; nevertheless, they did show significantly more absolute error in timing (i.e. |peak time − CS duration|) than control subjects. In other words lesioned subjects overestimated the CS duration, as well as underestimating it. This difference was numerically present, although not statistically significant, in one of the two studies with the shorter CS (Tam et al., 2013).

Taken together these findings suggest that the DHPC plays a role in timing of instrumental and Pavlovian conditioned response. However, the exact relationship between the extent of DHPC neuronal loss and timing performance has never been examined—this was the purpose of the present study. In our earlier reports there was variability in the extent of DHPC damage, and also in both the extent and precise nature of the timing deficits among lesioned subjects (Tam and Bonardi, 2012a, b; Tam et al., 2013). Data of this type thus provide an opportunity to examine the relationship between DHPC damage and response timing in a more quantitative manner, using both actual time of maximal responding (peak time) and absolute error (|peak time − CS duration|) as measures of timing performance.

The present article explored the relationship between DHPC damage and timing performance using data from three earlier reports—Tam et al. (2013); Tam and Bonardi (2012a, b), denoted below as Experiments 1, 2, and 3, respectively. Initially data from the three studies were pooled, and performance was compared in animals with and without DHPC damage. Then a series of generalized linear mixed-effects models (GLMMs) was performed (e.g., Crawley, 2007), in order to examine the degree to which the extent of hippocampal damage could be predicted by different behavioral measures of timing. This approach has two distinct benefits in throwing light on the relationship between DHPC damage and behavioral performance. First, while analysis of variance (ANOVA) can only test the null hypothesis that one specific behavioral measure is unaffected by DHPC damage, GLMM has more in common with multiple regression in that it can simultaneously evaluate the relationships between DHPC damage and several behavioral measures. Second, data were pooled from three different experiments that differed in various respects, such as the duration and modality of the CSs. The relationships between various behavioral measures and amount of hippocampal damage might vary under these different experimental conditions. For example, there is evidence that auditory and visual CSs of the same duration are judged as different, an effect which has been interpreted in terms of different clock speeds for different stimulus modalities (Penney et al., 2000). Critically, GLMM is capable of taking this into account—It permits us to control for the effects of parametric differences among studies, and so can yield meaningful results in spite of the fact that the studies differed in terms of CS duration, modality, degree of hippocampal damage, etc. (Burnham et al., 2002; 2011; Field et al., 2012). Evidence of a significant relationship between damage and timing performance would strengthen the notion that the DHPC is important for temporal processing (Olton, 1986; Kesner, 1998; Sakata, 2006; Howard and Eichenbaum, 2013).

METHODS

Animals

Seventy-two Lister Hooded male rats were used in total (24 animals in each of the three studies). In each experiment 12 animals were assigned to the sham-lesioned group and 12 to the DHPC-lesioned group. Five DHPC-lesioned subjects in Experiment 1 (Tam et al., 2013) were excluded from the pooled analyses because their hippocampal damage was unilateral; one DHPC-lesioned subject in Experiment 2 (Tam and Bonardi, 2012a) was excluded because it did not respond on any of the test trials, resulting in n = 30 in the DHPC-lesioned group in the pooled analyses. One sham-lesioned subject in Experiment 1 was excluded because some of its brain sections were lost during the staining process and hence its hippocampal area could not be determined, resulting in n = 35 in the sham-lesioned group in the pooled analyses. Subjects of the same lesion group were caged in pairs in a colony with a light-dark cycle of 12 h (light phases started at 07:00). The weight of each subject at the start of surgery was ~300 g. Two weeks after surgery animals were deprived to 85% of their ad lib weight, and maintained at this level (with adjustments for natural growth rate) by feeding each subject a restricted daily ration after each experimental session. Subjects were tested 7 days a week during each study. The average weight of subjects at the start of each study was, for Experiments 1–3 respectively, 387 g (range: 350–435 g), 307 g (range: 281–344 g), and 375 g (range: 325–435 g).

Surgery

Subjects were anesthetized with isofluorane, and their scalp was incised along the midline and the facial muscles were

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**Hippocampus**
TABLE 1.

Coordinates of Ibotenic Acid Injection in Tam et al. (2013) and Tam and Bonardi (2012a, b)

| AP  | ML          | DV          |
|-----|-------------|-------------|
| -2.4 | ±1.0 | ±1.0 | -3.0 | ±3.0 |
| -3.0 | ±1.4 | ±1.0 | -2.1 | -2.1 |
| -3.0 | ±1.4 | ±1.4 | -2.9 | ±3.0 |
| -3.0 | ±3.0 | ±3.0 | -2.7 | ±2.7 |
| -4.0 | ±2.6 | ±2.2 | -1.8 | ±1.8 |
| -4.0 | ±2.6 | ±2.2 | -2.8 | ±3.0 |
| -4.0 | ±3.7 | ±3.5 | -2.7 | ±2.7 |

Note. AP = anterior–posterior coordinates relative to bregma in millimetres; ML = medial–lateral coordinates relative to bregma in millimetres; DV = dorsal–ventral coordinates relative to brain surface in millimetres. Coordinates used in Tam et al. (2013) and Tam and Bonardi (2012a, b) are shown on the left in each column, and those used in Tam and Bonardi (2012a, b), the volume of ibotenic acid injected at sites AP = 3.0 mm, ML = 3.0 mm, DV = 2.7 mm and AP = 4.0 mm, ML = 3.7 mm, DV = 2.7 mm was 0.1 µl, and 0.05 µl at all other sites. In Tam and Bonardi (2012a), the volume of ibotenic acid injected at AP = 2.4 mm, ML = 1.0 mm, DV = 3.0 mm was 0.05 µl, and 0.1 µl at all other sites.

retracted. Portions of cranial bone above the DHPC were removed with an electric drill. Dorsal hippocampal lesions were achieved by injecting 0.05 or 0.1 µl of ibotenic acid into 14 sites (Table 1). The concentration of the injected ibotenic acid solution was 63 mM, which was made from dissolving 5 mg of ibotenic acid solids (Sigma-Aldrich, Dorset, UK) into 0.5 ml of 0.1 M phosphate-buffered saline (pH 7.4). Injections were administered by an infusion pump (KD Scientific, Holliston, Massachusetts) at a rate of 0.03 µl min⁻¹ using a 2-µl syringe (Hamilton, Bonaduz, Switzerland) with a 25-gauge, bevel-tip needle. After each injection the needle was left in situ for 1 min before it was withdrawn and moved to the next site. Subjects in the sham-lesioned group received a similar treatment but no injection of ibotenic acid. After all sites were visited, the scalp was sutured. Subjects were injected subcutaneously with 1 ml kg⁻¹ of Rimadyl (Pfizer, Surrey, UK) as analgesic and 0.5 ml of warmed saline to prevent dehydration during surgery, and all of them fully recovered within 2 weeks.

Apparatus and Stimuli

Eight operant chambers (Med Associates, St. Albans, Vermont; length × width × height: 30 cm × 25 cm × 25 cm) were used; each chamber was located inside a sound- and light-attenuating chamber (72 cm × 32 cm × 42 cm) equipped with a ventilation fan. The ambient sound level inside the operant chamber when the ventilation fan was switched on was 65 dB(A). Each operant chamber had two aluminium short walls and two transparent plastic long walls (the front one served as the door). The ceiling was a piece of transparent plastic. The floor consisted of 19 stainless steel bars spaced 1 cm apart; each had a diameter of 0.5 cm and ran parallel to the short walls; located below the floor was a pan containing a layer of sawdust bedding which was changed weekly. A recessed food magazine was located on one of the short walls, equidistant from the long walls and 3 cm above the floor. The magazine was accessible via a rectangular aperture (width × height: 4 cm × 5 cm); an infrared beam was sent from one side of the magazine and received on the other side; each interruption of the beam was recorded as a discrete response. The chambers were not illuminated in Experiments 2 and 3; in these two studies the CS was either a white noise or a 1-kHz click of 75 dB(A), presented via a speaker located at the upper corner of the short wall, opposite to the wall in which the food magazine was located. In Experiment 1 the CS was a 2.8-W houselight, the bottom half of which was shielded, located 11 cm above the magazine; when the CS was not presented, the chambers were not illuminated. In all three studies, the unconditioned stimulus (US) was one 45-mg food pellet (PJAII-0045; Noyes, Lancaster, New Hampshire) delivered into the magazine. Experimental events (delivery of CS and US, and head entry responses) were controlled and recorded by the Med-PC software (version IV) installed on a PC located in another room.

Procedure

Experiment 1: 15-s visual conditioned stimulus (Tam et al., 2013)

In Experiment 1 subjects were first given a 40-min magazine training session in which USs were delivered according to a variable-time, 240-s schedule. There followed six sessions of acquisition, each containing 64 delay conditioning trials on which the CS was a 15-s houselight, and US delivery occurred at CS termination. The intertrial interval (ITI, the interval between the end of one trial and CS onset on the next trial) comprised a random interval (drawn from an exponential distribution) with a mean of 60 s plus a fixed interval of 30 s. Test sessions 7–22 were similar to the acquisition sessions, except that half of the conditioning trials (32 trials) were replaced by the non-reinforced test trials (peak trials), on which the CS lasted for 45 s. These peak trials assessed how well the subjects timed from CS onset. The number of magazine entries was recorded in each 1-s bin of the peak trials. Conditioning and peak trials were presented in a randomized order, with the constraint that each session began with a conditioning trial.

Experiment 2: 15-s auditory conditioned stimulus (Tam & Bonardi, 2012a)

In Experiment 2, after a session of magazine training identical to that in Experiment 1, subjects were given eight sessions of acquisition, each containing 12 delay conditioning trials (they also received eight trace conditioning trials in each session, randomly intermixed with the delay conditioning trials, but the data from these trials were not included in the present analyses). The auditory CS was 15 s, and US delivery occurred at CS termination. Six of the DHPC- and sham-lesioned subjects received the 1-kHz click as the CS and the remainder the white noise. The ITI comprised a random interval with a...
mean of 150 s and a fixed interval of 60 s. After the acquisition phase, subjects were given four sessions of extinction (sessions 9–12), each containing four non-reinforced presentations of the CS (subjects also received an equal number of trace CS extinction trials, randomly intermixed with the delay CS extinction trials in each session). The fixed portion of the ITI was increased to 75 s, but in all other respects these sessions were identical to the acquisition sessions. At this point the animals received some unrelated appetitive training with visual CSs, before retraining sessions 13 and 14 took place, which were identical to sessions 1–8. Test sessions 15–42 were similar to the acquisition sessions, except that each session contained an additional non-reinforced peak trial, on which the delay CS was presented for 45 s (each session also contained one non-reinforced peak trial of the trace CS). Programming issues allow us to present the data from sessions 23–42 only. The ITI in these sessions was a random interval with a mean of 120 s plus a fixed interval of 90 s.

**Experiment 3: 40-s auditory conditioned stimulus (Tam & Bonardi, 2012b)**

In Experiment 3, after a session of magazine training identical to that in Experiment 1, subjects were given 10 sessions of acquisition, each containing 50 delay conditioning trials. The auditory CS was 40 s in duration, and US delivery occurred at CS termination; the identity of the CS (1-kHz click or white noise) was counterbalanced as in Experiment 2. The ITI comprised a random interval with a mean of 40 s and a fixed interval of 40 s. Test sessions 11–40 were similar to the acquisition sessions, except that 15 of the 50 conditioning trials were replaced by the non-reinforced peak trials, on which the CS lasted for 80 s.

**Histology**

Subjects were sacrificed with an overdose of pentobarbitone and perfused intracardially with formal saline. Their brains were stored in formal saline at room temperature for 2 days, subsequently in 20% sucrose solution at a temperature of 4°C for 2 days. Brains were then cut with a cryostat at a temperature of −19°C. Coronal sections were 40 μm in thickness, and every fifth section was collected. The recovered sections were stained with cresyl violet and dried at room temperature. For each subject, the coordinates of the recovered sections were identified using the Paxinos and Watson (2005) atlas. For each identified section, the intact hippocampus in each hemisphere was outlined using ImageJ (version 1.4; National Institutes of Health, Bethesda, MD). The areas of the hippocampus in both hemispheres were quantified (in pixels), and the total hippocampal area was estimated for each subject. Subsequently, the mean total hippocampal area in the sham-lesioned group was calculated (one mean for each experiment), and the extent of hippocampal damage of each subject in the DHPC-lesioned group was estimated relative to the mean hippocampal area of the corresponding sham-lesioned group.

**Data Treatment and Analyses**

**Measures of timing performance**

For each subject, magazine entries in each 1-s bin of the non-reinforced peak trials were pooled across trials and sessions, resulting in a single conditioned-response timing distribution. Each timing distribution was smoothed over four 1-s bins. A Gaussian model with three parameters was then fitted onto each timing distribution,

\[ \hat{R}_j = A e^{-\frac{(x - \mu)^2}{2\sigma^2}}, \]

where \( \hat{R}_j \) indicates the conditioned-response rate in each 1-s bin. Parameter \( A \), the peak conditioned-response rate (peak rate), indicates the strength of appetitive conditioning. \( B \), the spread of the response distribution, reflects timing precision; greater spreads indicate less precise timing. \( C \), the central tendency of the distribution, refers to the time point at which peak conditioned responding occurred (peak time). On the basis of these measures, we computed absolute error and coefficient of variation (CV)—Absolute error was calculated as \(|\text{peak time} - \text{target time}|\), and CV was calculated as spread/peak time; these are summarized in Figure 1. More absolute error indicates less accurate timing or higher timing variability within a group, while CV indicates whether the spread of responding is proportional to the interval timed. If conditioned-response timing is timescale invariant, CV should be approximately constant between groups or across studies. These five measures (peak rate, spread, peak time, absolute error, and CV) were subject to ANOVAs in order to examine effects of DHPC lesion, stimulus duration, and modality.

**Generalized linear mixed-effects models: Data screening**

To examine if there was any relationship between the extent of DHPC damage and different measures, a series of GLMMs was performed in R (R Core Team, 2013) with package lme4 (version 1.0-6; Bates et al., 2014). Prior to the analyses, we screened for multicollinearity by examining partial correlations between different pairs of the variables to be included in the analyses. Bonferroni correction was applied, with \( \alpha = 0.05/n \), where \( n \) is the total number of comparisons to be made. We found that peak time was negatively correlated with CV: \( r = -0.755, P < 0.005 \) (controlling for peak rate, spread, absolute error, and CS duration). In addition, the positive correlation between spread and CV was almost significant after Bonferroni correction was applied: \( r = +0.527, P = 0.006 \) (controlling for peak rate, peak time, absolute error, and CS duration). These correlations are not surprising, given that CV was computed from spread/peak time, and it implies that CV is a redundant variable in the presence of spread and peak time. No other correlation was significant after Bonferroni correction (Fig. 2).

A more formal test for multicollinearity was performed by examining the variance inflation factors (VIFs) of the five
where studies with different stimulus duration and modalities. The mean CV of each group should be approximately constant across peak time. If conditioned-response timing is timescale invariant, the coefficient of variation (CV), which is calculated from spread divided by absolute difference between peak time and target CS duration; 5. Coefficient of variation (CV), which is calculated from spread divided by peak time. If conditioned-response timing is timescale invariant, the mean CV of each group should be approximately constant across studies with different stimulus duration and modalities.

For each variable \( k \), a VIF was computed by \( 1/(1 - R^2) \), where \( R^2 \) represents the coefficient of determination obtained when \( k \) was regressed against all other variables. Only CV had a VIF > 10, which indicates serious multicollinearity. The remaining variables had a mean VIF of 3.74 (range: 1.60–7.36). When CV was removed, VIFs of the remaining variables decreased to a mean of 1.50 (range: 1.20–1.73), confirming that CV is a redundant variable.

**Generalized linear mixed-effects models: Defining models and model selection**

Based on the screening results, CV was removed from further GLMM analyses. We started with a maximal model with peak rate, spread, peak time, and absolute error as fixed-effects predictors, and the extent of DHPC damage as the variable to be predicted. In terms of \texttt{lme4} syntax, the model was defined as:

\[
\text{damage} \sim \text{peak_rate} + \text{spread} + \text{peak_time} + \text{absolute_error} + (1|\text{experiment}) + (0 + \text{CS}|\text{experiment})
\]

This model consisted of fixed- and random-effects components. The first four terms in the model were fixed effects—A slope with a fixed value \( (\beta) \) was estimated for each of the four predictors as in linear regression; \( \beta \) coefficients that are greater than zero indicate positive relationships with the extent of DHPC damage, and values less than zero indicate negative relationships. The last two terms in the model were random effects—\( (1|\text{experiment}) \) represents a random intercept for factor Experiment (1, 2, or 3); and \( (0 + \text{CS}|\text{experiment}) \) represents a random slope for factor CS Duration (15 or 40 s) or Modality (visual or auditory), with factor Experiment nested within it. The random component of the model takes into account, among other things, the different extent of DHPC damage across studies (see Histology results below).

We first assumed that the relationship between DHPC damage and any predictor to be linear, by adopting an identity link function (damage = \( X\beta \)) and a normally distributed error structure in the dataset. We then explored the possibility that there might be nonlinear monotonic relationships, by adopting nonlinear link functions [hyperbolic: \( \text{damage}^{-1} = X\beta \), or logarithmic: \( \log(\text{damage}) = X\beta \)] and a gamma error distribution in the dataset. But as the results (and subsequent discussion of the results) were similar under linear and nonlinear conditions, only the first set of GLMM finding (linearity assumed) was reported in the main article. The GLMM results obtained when nonlinearity was assumed can be found in Supporting Information Table S1.

To assess the importance of each predictor in the maximal model, we adopted the model simplification approach (Crawley, 2007). A series of likelihood ratio tests was conducted, and each test compared a model with a particular predictor \( (k) \) included against a model without that predictor. If the deletion of \( k \) had no significant effect (as indicated by the \( P \) value associated with the \( \chi^2 \) statistic), the model without \( k \) was preferred (i.e., the more parsimonious model was retained). Predictor \( k \) was retained in the model if \( P < 0.05 \).

**FIGURE 2.** Absolute partial correlation \((r^j)\) values between different pairs of the five variables after DHPC damage. The “hotter” the color on the heatmap, the stronger was the relationship between two variables (while “partialling” out all other variables). * indicates that the correlation was almost significant after Bonferroni correction \( (P \) slightly above 0.005); † indicates that the correlation was statistically significant after Bonferroni correction \( (P < 0.005) \). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Hippocampus
Each of the four fixed-effects predictors was tested in turn, progressively reducing the full model to a minimal adequate model (Crawley, 2007). The order of factor deletion across steps was determined by how significant a factor was in predicting damage, as indicated by the t value associated with each β coefficient (i.e. the factor with the lowest absolute t value was deleted first).

We also examined how well the actual values of DHPC damage and the predicted values from the best models were correlated. A strong correlation between the actual and predicted values from the best models were found on CV (F(1, 59) = 10.897, P < 0.005; Fig. 3F), and a significant lesion effect was also found on CV (F(1, 59) = 10.897, P < 0.005; Fig. 3F). There was no Lesion × Experiment interaction for either peak rate or CV (Ps > 0.7), suggesting that the lesion effects were similar across the three studies. No main effect of Lesion or Lesion × Experiment interaction was found for peak rate, spread, or absolute error (Figs. 3B,C,E; Ps > 0.25). These results are summarized in Table 3. In addition, there were main effects of Experiment on peak rate, peak time, and spread (Ps < 0.001), but not on absolute error or CV (Ps > 0.2), effects which are likely to have resulted from the difference in CS duration among the three studies; this was examined further below.

Group mean response distributions during timing of 15-s (Experiments 1 and 2 combined) and 40-s CSs (Experiment 3) on the non-reinforced peak trials are shown in Figures 4A,B. When the target CS was 15 s, the mean response distribution of the DHPC-lesioned group was higher and broader than that of the sham-lesioned group (Fig. 4A); when the target was 40 s, there was no obvious difference between the group mean response distributions (Fig. 4B).

**Effects of Lesion as a Function of CS Duration and Modality: Supplementary Analyses**

(I) CS duration

We also examined the effects of lesion as a function of CS duration across the three experiments. The group mean response distributions during timing of 15-s (Experiments 1 and 2 combined) and 40-s CSs (Experiment 3) are plotted on an absolute timescale in Figures 4C,D; in both groups these functions appeared higher and narrower for the 15-s CS than for the 40-s CS. When plotted on a relative timescale (Figs. 4E,F) the mean response distributions of 15-s and 40-s targets superimposed, as would be predicted by Weber’s law. The degree of overlap of the 15-s and 40-s timing distributions can be quantified in terms of $\eta^2$—a superposition index (range from 0–1) that reflects the proportion of variance accounted for by the mean of the two distributions, with $\eta^2 > 0.8$ indicating relatively good superposition in the literature (e.g., Brown et al., 1992). The values of $\eta^2$ in the sham- and DHPC-lesioned groups were relatively high and almost identical, 0.84 and 0.83 respectively, suggesting that conditioned-response timing was timescale invariant in both groups.

To further examine this issue, group mean values of the different measures were computed as a function of CS duration (Fig. 5), and multiple CS Duration (15 s: Experiments 1 and 2 combined vs. 40 s: Experiment 3) × Lesion ANOVAs were conducted. In accordance with what was observed in Figures 4C,D, longer CSs yielded lower peak rate [F(1, 61) = 10.160, P < 0.005], but greater spread [F(1, 61) = 606.996, P < 0.0005] and peak time [F(1, 61) = 174.492, P < 0.0005]; there was no...
interaction between CS Duration and Lesion ($P > 0.3$), suggesting that the degree of temporal discrimination (15 vs. 40 s) was similar in the sham- and DHPC-lesioned groups (Figs. 5B,C and 5G,H). No main effect of CS Duration or CS Duration × Lesion interaction was found with absolute error or CV (all $P_s > 0.14$; Figs. 5D,E and 5I,J). The absence of an effect of CS Duration on CV (spread/peak time) is particularly important, because it is consistent with the relatively good superposition of timing distributions within both groups, and confirms that DHPC lesion did not affect the timescale-invariance property of conditioned-response timing. (As in the main analyses above, main effects of Lesion were found for peak time and CV, $P_s < 0.02$, but not for other measures, $P_s > 0.16$.)

(II) CS modality

We examined the difference between lesioned and control groups as a function of CS modality by comparing the results from Experiments 1 and 2, which employed 15-s visual and auditory CSs, respectively. Group mean response distributions from

| TABLE 2. |

| Measure   | Exp 1 Sham | Exp 1 DHPC | Exp 2 Sham | Exp 2 DHPC | Exp 3 Sham | Exp 3 DHPC |
|-----------|------------|------------|------------|------------|------------|------------|
| 1. Peak rate* | 21.640 (3.078) | 24.944 (4.998) | 36.125 (6.442) | 42.136 (4.716) | 20.559 (1.828) | 19.334 (2.644) |
| 2. Spread†   | 26.432 (1.441) | 25.821 (1.307) | 28.625 (1.258) | 28.045 (1.932) | 60.833 (1.464) | 63.306 (2.077) |
| 3. Peak time† | 16.172 (0.788) | 12.001 (1.334) | 19.292 (1.900) | 13.386 (1.611) | 39.528 (1.550) | 36.527 (3.288) |
| 4. Absolute error† | 1.808 (0.657) | 3.784 (0.944) | 5.917 (1.448) | 4.750 (0.774) | 4.445 (0.792) | 7.667 (2.562) |
| 5. CV        | 1.645 (0.076) | 2.356 (0.346) | 1.625 (0.148) | 2.439 (0.356) | 1.559 (0.058) | 2.022 (0.329) |

Note. Exp 1 = Tam et al. (2013); Exp 2 = Tam and Bonardi (2012a); Exp 3 = Tam and Bonardi (2012b); * = in responses per minute; † = in seconds.
these studies, plotted on a relative timescale, are shown in Figures 4G,H. The response distributions of the visual and auditory CSs superimposed relatively well during the early and middle portions of the non-reinforced test trials (from relative time 0–2), but not in the later portion of the test trials (shaded regions in Figs. 4G,H). In addition, the distributions of the auditory CS appeared to be broader than those of the visual CS in both groups. The values of $g^2$ in the sham- and DHPC-lesioned groups were almost identical, 0.64 and 0.63 respectively.

Group mean values for the five behavioral measures were computed as a function of CS modality; the resulting data are shown in Figure 6. Multiple CS Modality (visual: Experiment 1 vs. auditory: Experiment 2) × Lesion ANOVAs revealed that auditory CSs yielded higher values of peak rate [Figs. 6A,F; $F(1, 37) = 9.085, P < 0.01$] and absolute error [Figs. 6D,I; $F(1, 37) = 5.459, P < 0.05$]; there was no interaction with Lesion for either measure ($P > 0.15$), suggesting that the effect of CS Modality was similar in the sham- and DHPC-lesioned groups. No main effect of CS Modality or CS Modality × Lesion interaction was found with spread (Figs. 6B,G), peak time (Figs. 6C,H), or CV (Figs. 6E,J), all $P > 0.15$. (As in the main analysis above, main effects of Lesion were found for peak time and CV, $P < 0.003$, but not for other measures, $P > 0.4$.)

Discussion

The analysis of peak time pooled over the three experiments confirmed that DHPC-lesioned subjects underestimated target durations of 15 s and 40 s, consistent with our previous findings (Tam and Bonardi, 2012a; Tam et al.,

Figure 4. Group mean conditioned-response timing distributions. Panels A and B: The mean response distributions of the sham-lesioned group (grey lines) and DHPC-lesioned group (red lines) when the target CS was 15 s (visual and auditory CSs combined) and 40 s (auditory CS); vertical lines indicate target CS duration. The response distribution of the DHPC-lesioned group was higher and broader than that of the sham-lesioned group when the CS was 15 s but not when it was 40 s. Panels C and D: The 15-s and 40-s timing distributions of each group from panels A and B are plotted on the same graph. In both groups, the 15-s distributions (red lines) were higher and narrower than the 40-s response distributions (green lines); vertical lines indicate target CS duration. Panels E and F: The timing distributions from panels C and D superimposed on a relative timescale ($t$/CS duration; 1 indicates target CS duration), suggesting that conditioned-response timing was timescale invariant in both groups. The degree of overlapping of the 15-s and 40-s distributions can be quantified in terms of $\eta^2$, a superposition index (range from 0 to 1) that reflects the proportion of variance accounted for by the mean of the two distributions. $\eta^2 > 0.8$ indicates relatively good superposition in the literature (e.g., Brown et al., 1992). Panels G and H: For both groups, the 15-s timing distributions of the visual CS (red lines) and auditory CS (green lines) did not superimpose well on a relative timescale, especially beyond relative time 2 (indicated by the shaded regions. In general, the visual CS distributions were broader and less smooth than the auditory CS distributions, suggestive of a sensory modality effect on response timing. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
2013; see also Jaldow et al., 1989; Balci et al., 2009; Yin and Meck, 2014), and the size of this lesion effect on timing accuracy did not differ across studies (Tam and Bonardi, 2012a, b; Tam et al., 2013). Within each group, conditioned-response timing was timescale invariant, as indicated by relatively good superposition of 15-s and 40-s timing distributions plotted on a relative timescale, as well as the non-significant effect of CS Duration on CV. However, the DHPC-lesioned subjects had a higher mean CV than the sham-lesioned subjects, implying that DHPC damage...
produced a nonscalar increase in timing variability. But this increase in timing variability in the DHPC-lesioned group was not accompanied by greater absolute error in these animals (cf. Tam and Bonardi, 2012b).

Peak rate and absolute error were higher when timing a 15-s auditory CS than when timing a visual CS of the same duration, although there was no difference in spread, peak time, or CV. The sensory modality effects on peak rate and absolute error were, however, unaffected by DHPC damage. In accordance with these findings, the visual and auditory CS timing distributions did not superimpose well in either group when plotted on a relative timescale, with the auditory CS timing distributions broader and noisier (i.e. less smooth) than the visual CS distributions. However, it should be emphasized that there were other differences between experiments, such as inter-trial interval, total number of conditioning trials, and total number of non-reinforced test trials. Thus it is necessary to be cautious in attributing the differences outlined above to CS modality or duration.

**Relationships Between Extent of Dorsal Hippocampal Damage and Measures of Conditioned-Response Timing**

In all three studies there was variability both in the extent of DHPC damage and in the different measures of timing behavior. Data of this type provide an opportunity to examine the relationship between the extent of DHPC damage and timing performance in a more quantitative manner. Figures 7A–E show the scatter plots of the extent of DHPC damage vs. different aspects of conditioned-response timing.

To take into account the differential amount of DHPC damage and parametric differences across studies, we conducted a series of GLMMs with factor Experiment, CS Duration (15 or 40 s), and Modality (visual or auditory) as random effects. The different measures, peak rate, spread, peak time, and absolute error, were entered as fixed-effects predictors; CV was not included based on the results from multicollinearity screening. The extent of DHPC damage was entered as the variable to be predicted by the above fixed- and random-effects factors.

Among the four fixed-effects predictors in the maximal model, peak time was the weakest predictor of damage ($\beta = 0.195$, standard error $= 0.159$, $t = 1.224$). No significant effect was found when peak time was deleted from the model ($\chi^2 = 1.365$, $P = 0.243$). The removal of peak time resulted in a simpler model in which spread was the weakest predictor of damage ($\beta = -0.244$, standard error $= 0.167$, $t = -1.461$). No significant effect was found when spread was deleted from the model ($\chi^2 = 1.581$, $P = 0.209$).

This resulted in a model with only two fixed-effects predictors, which represents our minimal adequate model, as significant effects were obtained when peak rate was deleted ($\chi^2 = 7.336$, $P < 0.01$) and when absolute error was deleted ($\chi^2 = 5.062$, $P < 0.05$). Furthermore, reference to Table 4 shows that these two models (steps 4a and 4b) were more than 2 AIC (Akaike's information criterion) values greater than the minimal adequate model with peak rate and absolute error included.
(step 3 in Table 4), and hence the model with these two predictors represents the best model in the set (Burnham and Anderson, 2002; Crawley, 2007). Thus, in addition to the contribution of the random component in the model (factor Experiment, CS Duration, and Modality), the extent of DHPC damage was positively predicted by peak rate ($\beta = 0.275$, standard error = 0.089, $t = 3.099$) and absolute error ($\beta = 0.461$, standard error = 0.196, $t = 2.358$). We checked if there was any interactive effect between the two fixed-effects predictors (peak rate $\times$ absolute error) but no significant effect was found when the interaction term was added to the minimal adequate model ($\chi^2 = 0.002, P = 0.967$).

We then examined how well the actual DHPC damage values could be predicted from the minimal adequate model with peak rate and absolute error as fixed-effects predictors. The correlation between the actual and predicted values of damage was strong: $r = +0.813, P < 0.0001$ (Fig. 8A). Importantly, this correlation was of the same magnitude as the correlation between the predicted values from the maximal model (with all four predictors included) and actual DHPC damage: $r = +0.837, P < 0.0001$ (Fig. 8B). Therefore, the severity of DHPC damage was well predicted based on the linear combination of peak rate and absolute error. We also employed a more conservative jackknife approach (Crawley, 2007), to examine how well $\beta$ estimates from a smaller subsample of subjects could be used to predict DHPC damage in subjects not included in the model. More specifically, each animal was left out from the dataset, one at a time, and $\beta$ estimates for peak rate and absolute error were recalculated based on the smaller sample ($n = 30 - 1$). The new set of $\beta$ estimates was then used to predict the extent of DHPC damage of the animal not included in the model; this procedure was repeated for each of the 30 DHPC-lesioned animals. The correlation between the actual and predicted values of damage based on this jackknife approach remained strong, $r = +0.702, P < 0.0001$, demonstrating cross validity of the minimal adequate model. Frequency distributions of the $\beta$ estimates for peak rate and absolute error obtained from the jackknife method are shown in Figures 9A,B respectively.

Finally, we examined if the significant relationships between damage and peak rate and absolute error differed for different stimulus duration or modality, by testing for any significant Damage $\times$ CS Duration or Damage $\times$ Modality interactive effect on the two measures:

\[
\text{absolute_error} \sim \text{damage} + \text{CS} + \text{damage}:\text{CS} \\
\text{peak_rate} \sim \text{damage} + \text{CS} + \text{damage}:\text{CS}
\]

The last terms in the above models represent the interactions of interest. With respect to absolute error, there was no interaction between damage and CS duration ($\beta = 0.309$, standard error = 0.267, $t = 1.161, P = 0.256$), or between damage and modality ($\beta = 0.655$, standard error = 0.801, $t = 0.818, P = 0.421$).

With respect to peak rate, there was no interaction between damage and modality ($\beta = -3.350$, standard error = 2.172, $t = -1.543, P = 0.135$). However, the interaction between damage and CS duration was almost significant ($\beta = -1.078$, standard error = 0.528, $t = -2.040, P = 0.052$), implying that the relationship between damage and peak rate might be different for...
FIGURE 8. Actual vs. predicted hippocampal damage from mixed-effects models. Panel A: Predicted values of damage were obtained from the minimal adequate model with peak rate and absolute error as fixed-effects predictors (step 3 in Table 4); the correlation between actual damage (blue line) and predicted damage (red line) was +0.813. Panel B: Predicted values of damage were obtained from the maximal model with peak rate, spread, peak time, and absolute error as fixed-effects predictors (step 1 in Table 4); the correlation between actual damage (blue line) and predicted damage (red line) was +0.837, which is of the same magnitude as the correlation in panel A. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

15-s and 40-s CSs. Consistent with this idea, when only the 15-s CS data were analyzed, there was a positive relationship between damage and peak rate ($\beta = 1.313$, standard error $= 0.359$, $t = 3.653$, $P < 0.005$), but no significant relationship was evident when the 40-s CS data were analyzed ($\beta = 0.235$, standard error $= 0.337$, $t = 0.696$, $P = 0.502$).

Discussion

The extent of DHPC damage was significantly related to, and could be well predicted by, peak rate and absolute error (Tables 3 and 4), both of which increased with lesion extent. For absolute error, the strength of the positive relationship appeared to be similar for 15-s and 40-s CSs and for stimuli of different modalities, but for peak rate, the positive relationship was more evident when the CS was 15 s than when it was 40 s (although this latter interactive effect only approached significance).

GENERAL DISCUSSION

The DHPC-lesioned subjects displayed maximal responding at significantly earlier time points than the sham-lesioned sub-

jects (Fig. 3D), suggesting that they tended to underestimate the duration of the CS. This is consistent with previous reports on the effect of fimbria-fornix lesions (Meck et al., 1984; Meck, 1988), and confirms that damage to the DHPC is sufficient to produce these effects. Similarly, in a recent study Yin and Meck (2014) reported that DHPC lesions are sufficient to produce earlier peak time. However, in their study the lesioned animals' peak times shifted earlier in time as testing progressed, and the difference between lesioned and control groups was statistically significant only in the last few sessions (their Figs. 1A,B and Table 2). By contrast, in our previous report (Tam et al., 2013) no progressive leftward shift in peak time was observed in our lesioned animals, and the lesion effect was significant in all test sessions (i.e., there was no Lesion × Block of Test interaction; see also Tam and Bonardi, 2012a, b). The reason for this discrepancy is unclear, but it might be related to differences in the time of surgery. In Yin and Meck (2014) surgeries were conducted after animals acquired instrumental responses, whereas in our studies surgeries were conducted before acquisition of conditioned responses. Further studies are needed to examine whether the time of surgery (before vs. after acquisition of conditioned/instrumental responses) is important for the difference in results between studies.

Interestingly, the lower peak time in our lesioned animals was not accompanied by a corresponding decrease in spread that Weber's law would predict (Fig. 3C), with the result that CV was significantly larger in the lesioned than control subjects (Fig. 3F). This indicates that, in addition to an impairment in accuracy, there was a reduction in timing precision after DHPC damage—an effect that has not previously been reported. To our knowledge only two studies have examined this, and both reported that the reduction in peak time produced by lesions was accompanied by a reduction in spread (Meck et al., 1984; Buhusi and Meck, 2002), in complete contrast to the results reported here. However, these studies employed lesions of the fimbria-fornix (Meck et al., 1984) and the entire hippocampus (Buhusi and Meck, 2002). It is possible that these differences could account for the contrast between their findings and the results reported here. Finally, consistent with most previous studies, DHPC lesions had no effect on appetitive conditioning per se (Fig. 3B).

The results from the GLMM analysis complemented these findings, by examining the extent to which different behavioral variables were able to predict the degree of damage that was present. Because all the variables were represented in the same model, it was possible to determine which of them were most influential, while at the same time controlling for parametric variations among the three studies; we are not aware of any other study that has examined the relationship between degree of hippocampal damage and timing performance. Our analysis revealed that damage was not strongly predicted by peak time (Fig. 7C), but that there was a positive relationship between damage and absolute error (Fig. 7D), a measure which is blind to whether animals under- or over-estimated the target duration (cf. Tam and Bonardi, 2012b). A second finding from GLMMs was the positive relationship between the extent of...
DHPC damage and appetitive conditioned responding (Fig. 7A). This result is at face value surprising—Although peak rate was numerically higher in the lesioned than in control subjects in Experiments 1 and 2, this difference was not significant in ANOVA, and there has been almost no previous report of DHPC lesions influencing delay conditioned responding. An exception is Tam and Bonardi (2012b) who found the opposite effect, that DHPC lesions impaired conditioned responding during initial conditioning sessions with the 40-s CS (see also Beylin et al., 2001, who reported a parallel impairment with longer CSs in a delay eyeblink conditioning preparation). The present results confirm and extend existing evidence that the DHPC is important for temporal processing (Olton, 1986; Kesner, 1998; Sakata, 2006; Howard and Eichenbaum, 2013). First, we replicated previous reports that hippocampal damage reduced peak time, and confirmed that damage to the dorsal portion of this structure is sufficient to produce these effects. We also demonstrated a previously unreported effect, that damage to the DHPC also appeared to reduce the precision of timing—CV was greater in the lesioned than in control subjects, which implies the scalar property of timing was violated such that timing precision did not decrease as the observed peak time decreased. Moreover, the GLMM analysis suggested that there was a strong predictive relationship between absolute timing error and DHPC damage. Both findings suggest greater variability in interval timing after DHPC damage. The implication is that DHPC damage might have multiple effects on response timing.

The effect of hippocampal dysfunction on interval timing is usually interpreted in terms of scalar timing theory (Gibbon et al., 1984). According to this model, timing behavior requires a pacemaker which emits pulses that are transferred to an accumulator via a switch when a stimulus is presented. When an US is encountered, the total number of pulses emitted between stimulus onset and US delivery is then transferred into a long-term memory store, multiplied by a constant $K$ that is close, but not equal, to one. When the same stimulus is re-encountered, the number of accumulating pulses in the accumulator is compared with one of the values stored in long-term memory, and conditioned responding begins when these values are sufficiently close. In terms of this model, the behavioral effect of DHPC damage is typically interpreted as a reduction in $K$. This results in the values stored in long-term memory being systematically shorter than those transferred from the accumulator, resulting in animals responding too early (Meck et al., 1984, 2013). However, if a change in $K$ were the only effect of DHPC damage, CV should be unaffected—A reduction in $K$ should produce a reduction in spread as well as peak time, with the result that CV should be constant, but this was not the case. The findings that CV was greater in DHPC-lesioned animals, and that absolute error in timing was systematically related to DHPC damage, suggest that DHPC damage does more than just reduce $K$.

One possibility is that it changes the criterion used to determine whether the current value in the accumulator is sufficiently close to the value taken from long term memory to produce a response. If DHPC lesion changes the threshold for when to start and stop responding, the lesioned subjects would start responding at earlier time points and cease responding later in time, leading to an increase in spread (i.e. less precise timing). The same result would be observed if DHPC lesion affects the time taken to open the switch that controls the

**FIGURE 9.** Histograms showing mean $\beta$ coefficients and variance for the fixed-effects predictors in the minimal adequate model obtained from the jackknife method. Panels A and B: Frequency distributions (grey bars) of $\beta$ coefficients for peak rate and absolute error, respectively; smooth lines (red) represent probability density functions of normal distributions with mean $= 0.277$, standard deviation $= 0.026$ and mean $= 0.465$, standard deviation $= 0.056$, respectively. Mean $\beta$ coefficients were significantly different from the value of zero (null effect) in both panels according to one-sample $t$ tests ($P_s < 0.005$, two-tailed), suggesting that peak rate and absolute error were related to the extent of DHPC damage. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
transfer of pulses from the pacemaker to the accumulator. Either of these effects could produce the pattern of results that we observed.

A second and somewhat surprising finding was the positive relationship between asymptotic rate of conditioned responding and DHPC damage. The first reason this is surprising is because, although an impairment in trace conditioning (CS and US separated by an empty interval) is regarded by some as diagnostic of hippocampal damage, HPC lesions typically have little effect on delay conditioning. However, as mentioned above, there are two reports of conditioned-response disruption in studies in which longer CSs are employed (Beylin et al., 2001; Tam and Bonardi, 2012b). As longer CSs, like trace-conditioned CSs, tend to support relatively low rates of conditioned responding, it may be that the failure of most studies to observe an effect on delay conditioning is simply due to a lack of sensitivity. But the second surprising aspect is the direction of the relationship—the greater the damage the higher the rate of conditioned responding. However, relationships of this type are not without precedent. For example, Baxter and Murray (2001) reported a positive relationship between the magnitude of hippocampal damage and performance in a visual delayed non-matching-to-sample task in macaques (although see Zola and Squire, 2001). They suggested three possible mechanisms that might underlie such an observation. First, partial damage to the hippocampus could alter activity in adjacent regions that are responsible for a particular behavioral output which might enhance performance, while more complete hippocampal damage would minimize such an effect. Second, they suggested that the hippocampus might compete with other regions to achieve a behavioral goal, such that increasing hippocampal damage would increasingly release other regions from this competition, thus enhancing performance. Finally, they proposed that less hippocampal damage might be accompanied by more extra-hippocampal damage caused by misdirected neurotoxin. We think this latter suggestion is unlikely in our studies, in which lesions were small and confined to the DHPC; but the first two are viable possibilities, and could explain why hippocampal lesions have been reported to have a disinhibitory effect on appetitive responding (Cheung and Cardinal, 2005).

In principle the effects of DHPC damage on peak response rate, spread, and absolute timing error could be interrelated. For example, a general disinhibition of conditioned responding that increases with DHPC damage could effectively make the response threshold postulated by scalar timing theory less conservative. Animals would start responding earlier and stop responding later in time on each trial, thereby increasing the spread of the overall response distribution (and probably absolute timing error) when data are combined across multiple trials. Thus, a significant correlation between peak rate and spread, or between peak rate and absolute error in timing, would be expected if these measures are interrelated. There was, however, no statistically significant correlation between pairs of these three variables; correlation coefficients are shown in the heatmap in Figure 2. These negative results do not provide evidence for the idea that the increased timing error was secondary to an increase in peak response rate.

Our behavioral findings complement the increasing body of evidence from single-unit recording studies, that cells in the DHPC show firing patterns that are dependent on the temporal structure of the learning episode—not only anticipating the time of delivery of both signalled (McEchron et al., 2003) and unsignalled USs (Delacour and Houcine, 1987; Young and McNaughton, 2000), but also showing populations of cells that seem to track both absolute and relative time (MacDonald et al., 2011; Naya and Suzuki, 2011). However, the question remains as to whether the role of the DHPC in timing is a primary or secondary one.

A recent extremely influential model of timing is the striatal beat frequency model (Matell and Meck, 2000, 2004; Buhusi and Oprisan, 2013). This model, which is one of the first that attempts to translate theoretical timing principles into neuro-biological substrates, asserts that striatal medium spiny neurons (MSNs) detect the temporal coincidence between reinforcement and activity in a subset of cortical neurons from which they receive inputs. The different intrinsic frequencies of the cortical neurons allow them to oscillate at different periodicities, and by detecting such patterns the MSNs can be trained to respond to different durations. According to this view the role of the hippocampus in timing is a secondary one, perhaps through its tonic inhibitory effect on the striatum (Meck, 1988; Meck et al., 2013). Moreover, Yin and Troger (2011) recently suggested a number of ways in which the hippocampus could interact with this cortico-striatal timing network. They suggested that the hippocampus might modulate the memory stage of the timing process, which could be responsible for the typically observed leftward shift in peak time. But interestingly they also proposed two further mechanisms that could underlie our demonstration that DHPC lesions increased timing variability. First, they suggested that the hippocampus might regulate the dynamic firing thresholds of the striatal MSNs, which they argued could produce more variation in timing performance across trials. Second they suggested that hippocampal dysfunction might affect the decision process that controls responding, arguably equivalent to the response threshold in scalar timing theory. Variation in this threshold could also underlie the greater error in timing that we found here.

A contrasting approach to this issue has recently been suggested by MacDonald (2014). His argument is that there is an inconsistency between the idea that the hippocampus is secondary to the cortico-striatal network in mediating response timing, and its well-established role in encoding the temporal organization of events in tasks that assess episodic memory-like processes. He proposed that the effective relegation of the hippocampus to a supporting role in timing may stem from the fact that most neural and behavioral investigations of hippocampal involvement in timing have relied on prospective memory paradigms, in which memory for a specific duration is required by the structure of the task. The peak procedure is an example of this, as animals are motivated to learn the time point at which food is delivered. By contrast, in retrospective paradigms learning about duration is incidental to the task,
and only probed afterwards. He argued that retrospective procedures are more likely to tap the type of temporal encoding that episodic-type tasks require, and thus to be those in which the hippocampus is critical. These suggestions are intriguing; nonetheless, further work is needed to clarify the role of the DHPC in timing processes (Bonardi et al., in press).

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