Hippocampal Contributions to the Temporal Dynamics of Behavior

by

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A Dissertation Presented in Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

Approved April 2022 by the
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ARIZONA STATE UNIVERSITY

May 2022

ABSTRACT

The capacity to track time in the seconds-to-minutes range, or interval timing, appears to be at least partially dependent on intact hippocampal (HPC) function. The current dissertation sought to dissociate timed responses, non-timed responses, and motivational aspects of behavior in order to propose a role of the HPC in specific timing sub-processes. In Chapter 2, effects of dorsal HPC (dHPC) lesions on temporal responding in a switch-timing task revealed a critical role of dHPC in the acquisition of interval timing criteria. Following dHPC lesions, the start time of responding was systemically shortened, in a manner that was enhanced and sustained when encoding a novel long interval, consistent with a memory-based account of dHPC function in timed responding. Chapter 3 investigated effects of chronic stress, which has been shown to reliably induce HPC dendritic retraction, on interval timing, utilizing response-initiated schedules of reinforcement, which facilitate deconvolution of timing and motivation. This revealed task-dependent effects on interval timing and motivation, where stress induced transient effects on motivation in a prospective timing task, but transient effects on the variability of timed responding in a retrospective timing task, consistent with an effect on memory function in interval timing. Chapter 4 sought to bring timed responding, motivation, and non-timed behaviors under stronger procedural control, through the implementation of a response-initiated timing-with-opportunity-cost task, in which a cost is imposed on temporal food-seeking by the presence of a concurrent source of probabilistic reinforcement. This arrangement garnered strong schedule control of behavior, and revealed individual-subject differences in the effects of reward devaluation, such that it affected motivation in some rats, but temporal responding in others. Using this

methodology, Chapter 5 investigated initial temporal entrainment of behavior under pharmacological deactivation of dHPC and revealed its critical involvement in updating memory to new temporal contingencies. Together, data from this dissertation contrast with prior conclusions that the HPC is not involved in learning temporal criteria, and instead suggest that its function is indeed critical to encoding temporal intervals in memory.

DEDICATION

For Anupama Gupta and Ajay Gupta, who taught me, by rigorous example, that hard work never goes unnoticed. *लक्ष्य तो हर हाल में पाना है।*

ACKNOWLEDGMENTS

The work completed in this dissertation would not be possible without the support of numerous others. Foremost, my mentor, Dr. Federico Sanabria, for entrusting me with his lab and its research for the better part of five years. I have learned more standing in front of a whiteboard with Fed than I have anywhere else. His kindness, genuine enthusiasm towards data, and dedication to theoretically-driven research are things I hope to emulate for the rest of my career. I am privileged to have experienced his mentorship. My perpetual labmates, Dr. Cristina Dos Santos and Dr. Carter Daniels, who I consider not only wonderful lifelong friends of mine, but mentors in their own right. They modeled for me the attention and rigor that our work deserves, while insisting that I enjoy life along the way. I would not be the same scientist (or person) without their example and care. My sister, Natasha Gupta, my academic role model, whose messages have kept me company at my desk from a distance for the past five years, and who has always delighted in my successes. Dr. Foster Olive, for being exceedingly generous with his resources, knowledge, and time for my whole graduate career. Without his support, this dissertation would not be what it is. Erin Nagy and Paula Overby, who are family to me, and are two of the best scientists I know. Their selflessness and dedication to problem-solving is truly exemplary. Dr. Cheryl Conrad, for her critical support in conducting my dissertation research and effusive enthusiasm for my work. Dr. Sam McClure, for serving on my committee since my qualifying exams and getting me interested in reinforcement learning. Drs. Jason Newbern and Brian Smith, for broadening my research experiences through collaboration. The *dozens* of undergraduates who have served as research assistants in our lab, who are truly the driving force behind all of the research I have

conducted at ASU. My friends and colleagues in the Behavioral Neuroscience and Comparative Psychology program, who have made this truly the best part of my life so far. ASU DACT, particularly Susan McNeil-Eastwood, who makes research on the third floor of Psych run like a well-oiled machine. Finally, perhaps most importantly, the subjects of my research, for the privilege of beautiful data.

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CHAPTER 1

INTRODUCTION

Interval Timing and its Component Processes

Distinct from both circadian timing, which governs wakefulness and hunger, and millisecond timing, which underlies speech and motor control, *interval timing* is defined as the tracking of the passage of time in the seconds-to-minutes range (Buhusi & Meck, 2005; Gibbon, 1977; Killeen & Fetterman, 1988; Roberts, 1981). Such intervals underlie the structure of motivated behavior and subserve capabilities necessary for survival, such as foraging (Bateson, 2003; Fox & Kyonka, 2014; Kacelnik & Brunner, 2002), decision-making (Ariely & Zakay, 2001; Bermudez & Schultz, 2014; Tosun et al., 2016), and reward-seeking (Delamater & Oakeshott, 2007; Galtress et al., 2012; Minamimoto et al., 2009; Petter et al., 2018). Through this fundamental capacity, behavior comes to be entrained to the temporal periodicity of the environment in a biologically-relevant manner (Buhusi & Meck, 2005; Ward et al., 2013).

Even prior to coming under control of a conditioned stimulus (CS), early conditioned behavior in basic Pavlovian and operant conditioning contexts becomes entrained to the temporal properties of the CS (Balsam et al., 2002; Balsam et al., 2010; Delamater & Oakeshott, 2007; Savastano & Miller, 1998). Beyond merely facilitating the eventual CS-US association, evidence from cue competition paradigms suggests that the intervals between CS and US are explicitly learned, as a part of the content a stimulus association (Savastano & Miller, 1998). Consistent with the *temporal coding hypothesis* (Matzel et al., 1988; Savastano & Miller, 1998), the rate at which this temporal learning arises is proportional to the amount of information the CS conveys about the rate of the

US (Balsam et al., 2010), and thus, temporal information appears to be a component of *what is learned.* These temporal regularities in the behavioral expression of associative learning are also reflected in its neural substrates (Bermudez & Schultz, 2014; Daw et al., 2003; Fiorillo et al., 2008), where mesencephalic dopamine neurons display monotonically increasing ramping activity from onset of the reward-predictive CS until the expected time of reward (Fiorillo et al., 2008, 2003; but see Niv et al., 2005).

Though the temporal dynamics of behavior largely emerge and proceed implicitly (Dragoi et al., 2003), interval timing becomes more conspicuous when it is dysfunctional. Aberrant interval timing capacity may manifest as persistent confabulation of memory recall in Alzheimer's disease (El Haj & Kapogiannis, 2016; El Haj & Larøi, 2020), maladaptive perseveration in autism (Allman et al., 2011; Falter et al., 2012), and disorganization and confusion in schizophrenia (Elvevåg et al., 2000). Thus, interval timing is not only an organizing force in behavior, but its study provides insight into the qualia of cognition and perception in psychopathology.

Though theories describing the mechanism underlying temporal entrainment of behavior vary in how the timekeeping signal is defined, the necessary computational processes for interval timing performance are predominantly instantiated within the *pacemaker accumulator* (PA; general structure depicted in Figure 1.1) family of theoretical models (Church et al., 1994; Gibbon, 1977; Killeen & Fetterman, 1988; Sanabria, 2020; Simen et al., 2013; Treisman, 1963; but see Balci & Simen, 2016; Dragoi et al., 2003; Machado, 1997; Simen et al., 2011; Staddon, 2005). PA models assume that interval timing consists of three primary components: an internal clock, a memory storage, and a decision unit (Buhusi & Meck, 2005; Gibbon, 1977). According to this

model, the onset of some stimulus that marks time allows pulses to be emitted by the *pacemaker* to an *accumulator* (*A*) in working memory, which has been first reset by the time-marking stimulus. The rate of pulse emission is the *clock speed* (1/*c*). Pulses in *A* are continually compared to a sample of pulses from long-term memory (*m*). If *A* and *m* are sufficiently similar according to some response threshold (θ), a response is made. If at the time of response, the to-be-timed interval (*t*) has elapsed and reinforcement occurs, *A* is stored in memory. Memory, therefore, consists of an increasingly large number of samples of previously reinforced times (Gibbon, 1977; Gibbon et al., 1984). Thus, through repeated trials and feedback, this mechanism accounts for covariation between the behavior of an organism and the temporal features of its environment. Importantly, this system is adaptive, in that the memory-updating mechanism can compensate for transient disruptions in clock, memory, or decision processes (Meck, 1983).

Simulations of PA models can account for key features of interval timing data generated from commonly utilized interval timing procedures in animal models (Gibbon, 1977; Gibbon et al., 1984). One such common procedure is the *peak procedure* (Barrón et al., 2020; Church et al., 1994; MacDonald et al., 2012; Roberts, 1981). In this procedure, subjects are trained to seek food on a fixed-interval (FI) schedule of reinforcement, wherein the first response after a given interval *t* has elapsed is reinforced. In non-reinforced probe trials, which are at least twice as the length of *t*, responding shows temporal entrainment to *t*, where response rate increases at *t* approaches, and is maximal at times flanking *t*, forming a *peak function* (Balci et al., 2009; Church et al., 1994; Plowright et al., 2000; Roberts, 1981; but see Daniels & Sanabria, 2017; Sanabria et al., 2009). The transitions into and out of this high-response state in each probe trial are

respectively designated as the *start* and *stop* times of peak responding (Church et al., 1994).

Importantly, if the peak procedure is repeated with different absolute values of *t*, the coefficient of variation (CV) of peak functions increases in proportion with the length of *t*, a manifestation of Weber's law of perceptual discrimination (Gibbon, 1977) referred to as the *scalar property* of temporal estimates (Church et al., 1994; Oprisan & Buhusi, 2014; Simen et al., 2013; but see Bizo et al., 2006). In a specific instantiation of the PA model, scalar expectancy theory (SET; Gibbon, 1977), scalar invariance does not derive from any property of the pacemaker itself, but rather from variation in a memory constant during transfer of *A* into reference memory. This memory error presumably increases at larger intervals, yielding increasingly noisy temporal estimates and outputs at the level of the comparator, such that the variance in estimates increases with the square of the mean (Gibbon, 1977). Contrastingly, Behavioral Theory of Timing (BeT; Killeen & Fetterman, 1988) argues that scalar invariance may emerge from a property of the pacemaker, where pulses from a Poisson clock are emitted at a rate proportional to the rate of reinforcement (Killeen & Fetterman, 1988; but see Balci & Simen, 2016; Machado, 1997; Rakitin et al., 1998; Treisman, 1963).

Changes in measures of temporal responding, such as those obtained from the peak procedure, can be mapped back to specific sub-components of the PA framework. For example, correlation between the start and stop time of temporal responding in probe trials increases with increasing variability in memory for the correct time. Contrastingly, changes in the total duration of the response window can be attributed to lax response thresholds (θ; Balci et al., 2009; Church et al., 1994). Variability in θ would be reflected

in a negative correlation between the start and stop of the peak function, where the start time of responding increases as the stop time decreases (Matell et al., 2006). In this manner, specific dimensions of behavior entrained to temporal intervals can be attributed to effects on specific computational processes nested within timing, which then facilitates the identification of neural structures capable of those computations.

Neurobiological Correlates of PA Model Sub-Processes

Although PA models structurally resemble a neural circuit, there exists no definitive mapping between specific brain functions and PA models of interval timing. There exist, however, multiple candidate neural regions whose activity covaries with each PA component. Notably, basal ganglia dopaminergic activity is associated with clock speed modulation (De Corte et al., 2019; Drew et al., 2003; but see Balci et al., 2010). This is demonstrated by effects of systemic administration of dopamine antagonists, which yield an overestimation of temporal estimates, consistent with a decrease in clock speed (Drew et al., 2003). In rats trained on a peak-timing procedure, D2 receptor blockade in the dorsomedial striatum delays both the start- and stop-times of responding, suggesting that dopaminergic afference in the dorsomedial striatum may modulate the speed with which the pacemaker emits pulses (De Corte et al., 2019). Further, ramping neurons with negative acceleration in the dorsomedial striatum display maximal activity at times proportional to the to-be-timed interval, and these neurons encode time more accurately and at higher proportions compared to ramping neurons in the medial prefrontal cortex (mPFC; Emmons et al., 2017). Insofar as these populations of neurons change their activity patterns to encode information about elapsed time and scale this activity across multiple intervals, such findings suggest that clock and

accumulator timing sub-processes are, at least in part, supported by dopaminergic activity in the basal ganglia network (Fontes et al., 2016; Matell & Meck, 2000).

Decision processes within the PA model have two main neurobiological correlates. The first of these is the dorsomedial striatum, in which D1 receptor blockade (in contrast to D2 receptor blockade, discussed above), selectively delays stop times in the peak interval procedure (De Corte et al., 2019). This selectivity to stop-times suggests that D1 (direct pathway) activity in the dorsomedial striatum tunes the decision threshold for ratio-based judgments. The decision mechanism is also correlated with activity in the mPFC, which shows similar neuronal ramping activity to the dorsomedial striatum, in conjunction with a to-be-timed interval (Bakhurin et al., 2017). Although the code for time in mPFC is relatively imprecise compared to that to that in the dorsomedial striatum, mPFC deactivation attenuates both interval timing accuracy *and* dorsomedial striatum neuron ramping, suggesting that mPFC serves as executive control for timing-related processes and decisions (Bakhurin et al., 2017; De Corte et al., 2019).

Hippocampal Function in Interval Timing

A foundational inference that the hippocampus (HPC) is involved in temporal cognition derives from trace conditioning, for which HPC only appears to be critical when there is a long delay between CS and US onset (Moustafa et al., 2013; Tam & Bonardi, 2012a), and when the trace interval is at least 20 s long (Chowdhury et al., 2005). This delay-dependent recruitment of HPC in learning is also expressed in Pavlovian conditioning to a visual cue, where HPC lesions disrupt precision of conditioned approach only when the initial position of the CS is interrupted by a long gap duration (Tam et al., 2013). Such temporally-graded involvement of HPC in trace

conditioning is generally attributed to the critical function of the HPC in encoding and retrieval long-term memory (Eichenbaum et al., 1992), and this attribution carries over to studies of explicit interval timing.

The classical behavioral expression of disrupted HPC function in the peak procedure is a leftward shift in the peak function, indicating an underestimation of previously entrained intervals (MacDonald et al., 2014; Meck et al., 2013; Olton et al., 1987; Palombo & Verfaellie, 2017; Yin & Meck, 2014; Yin & Troger, 2011; but see Dietrich & Allen, 1998). At face value, aberration in several candidate components of the PA model could account for this consistent effect. Critically, Meck (1988) observed that, following peak procedure training on a 20 s FI, HPC lesions yielded stable, but early peak responding. In a subsequent condition, the FI requirement was shifted down to 10 s, and HPC lesion rats still underestimated the interval, and peak responding again stabilized at some constant proportion of the to-be-timed interval (e.g. 8 s). Meck and colleagues (1988, 2013) concluded that this constant underestimation of intervals following HPC lesions reflects its role as a feedback mediator between clock and memory, tuning the temporal expectancy of events associated with reinforcement across the interval, but that the proportionality of the early peak to the time of reinforcement indicated that HPC is *not* involved in learning new temporal criteria (Lustig & Meck, 2005; MacDonald & Meck, 2004). The plausibility of this account is tempered by the persistence of early responding following HPC lesion in assessments of *acquisition*, where novel temporal intervals are underestimated for an extended period of training (Gupta et al., 2019; Yin & Meck, 2014), suggesting a failure of memory to update to temporal contingencies over time.

Alternatively, it has been suggested that HPC may function in the decision-making component of interval timing, where persistent underestimation of intervals reflects a sustained decreased in the threshold for timed responding (MacDonald et al., 2012; Meck, 2005; Meck & Church, 2003; Yin & Meck, 2014; Yin & Troger, 2011). Importantly, the discordance of these accounts suggests that interpretation of HPC effects on temporal responding is contingent on 1) the length of testing and 2) whether one is assessing acquisition or maintenance of the to-be-timed interval.

In addition to compelling evidence from behavioral data, there is strong neurobiological support for a representation of time in HPC. Time cells in HPC appear to track intervals between discontinuous events and fire at relevant intervals in temporally-structured event sequences across multiple timescales, on the order of seconds and even several hours (Eichenbaum, 2013, 2014; MacDonald et al., 2014; Mau, Sullivan, Kinsky, Hasselmo, Howard, Mau, et al., 2018). In a differential reinforcement of low rates (DRL) task, where reinforcement is delivered for responses separated by a criterial interval, HPC CA1 and CA3 neurons show an initial ramp up in activity, with a ramp down as the interval progresses, leading up to the reinforced response (Young & Mcnaughton, 2000). Importantly, these time-signaled responses appear to be independent of non-temporal (spatial and sequential) task dimensions (Gill et al., 2011).

Modulatory Role of Interval Length and the Structure of Temporally-Entrained Behavior on HPC Function in Interval Timing

Though its provenance is unclear, both empirical data from timing studies investigating HPC function and models formed to explain these data implicate a role for interval length in modulating the HPC's role in interval timing. One such computational

model developed by Oprisan et al. (2018) structures the storage of temporal memories as a topological map in the HPC, with memory cells for longer durations stored towards the dorsal hippocampus (dHPC) and those for shorter durations stored towards the ventral HPC (vHPC). Removal of memory cells in this model, as would be experimentally-induced by an HPC lesion, shifts the peak interval, such that the relative shift is determined both by the lesion size and its location, and peak offset increases as a function of lesion ventrality (Oprisan, Aft, et al., 2018). A more flexible version of this model (Oprisan, Buhusi, et al., 2018) modifies the map such that the HPC stores weights which reflect the contribution of each time cell to the average temporal field that determines a response.

The theoretical ascending structure proposed by Oprisan and colleagues is demonstrated in a range of experimental data. Sabariego et al. (2020) trained rats in a spatial temporal discrimination task, in which a 10-s interval was reinforced by a left turn within a maze, and a 20-s interval was reinforced by a right turn. dHPC lesions dropped performance to chance level. Although lesioned rats gradually improved their performance on 10s trials, performance on 20-s trials did not rise above chance. Jacobs et al. (2013) demonstrated this interval-length dependence of dHPC function at a longer timescale, where following pharmacological deactivation of dHPC, rats were unable to discriminate 8-12 min intervals, whereas discrimination of 60-90 s intervals remained accurate. When a similar methodology was implemented in patients with medial temporal lobe damage, they showed impairment in temporal estimation for long (> 90 s), but not short intervals (Palombo et al., 2016). Importantly, this duration-dependent effect was

found primarily in patients with lesions localized to HPC, suggesting that it is not related to extra-HPC or HPC-adjacent structures.

Evidence from Palombo et al. (2016) and Jacobs et al. (2013) — that shorter duration judgements are intact following HPC lesion—is somewhat at odds with evidence of the time cell activity in HPC (Eichenbaum, 2014; MacDonald et al., 2014; Mau, Sullivan, Kinsky, Hasselmo, Howard, Mau, et al., 2018; Young & Mcnaughton, 2000), which shows that time cell activity is present at all tested durations. Other procedural differences deepen this incongruity: rats with fornix and HPC lesions show no deficit in a Pavlovian temporal task, where the CS+ was a 12 s interval and the CS – was a 3 s interval with a 12 s (Kyd et al., 2008), suggesting that highly discriminable intervals may be less HPC-dependent than less disparate intervals, or alternatively, that Pavlovian and operant preparations garner differential HPC involvement. Allen & Bunnel (1996) showed that HPC lesions did not affect 20 s peak times, suggesting that in the absence of a concurrent schedule to discriminate or a novel schedule to learn, HPC function may not be important for accurate temporal responding. Collectively, these findings demonstrate apparent heterogeneity in the conditions under which HPC function supports the entrainment of behavior to timed intervals, in a manner that appears dependent on task contingencies (R. A. Block & Zakay, 1997; MacDonald et al., 2014; Tam et al., 2013; Zakay & Block, 2004), specific lengths of the to-be-timed intervals (Jacobs et al., 2013; Oprisan, Buhusi, et al., 2018; Palombo et al., 2016; Sabariego et al., 2020), and non-timing processes (Meck et al., 2013; Tam et al., 2015).

Characterizing hippocampal function within the PA model framework is further complicated by the structure of behavior entrained to long intervals, which is subject to

interference from motivational control of behavior, as well as the invariable presence of non-timed responses in otherwise temporally-entrained behavior (Daniels & Sanabria, 2017; Galtress et al., 2012; Sanabria & Killeen, 2008). Motivation can be procedurally dissociated from timing-related behaviors through the implementation of *response-initiated trials*, in which the initiating response is distinct from the timed responses (Daniels, 2018; Daniels & Sanabria, 2019; Fox & Kyonka, 2015). Because timing tasks inherently impose a delay-to-reward, longer intervals, which seem to implicate increased HPC-dependence, can induce schedule strain and reduce motivation to engage in the task, indexed by decreased frequency of trial initiation and increased latency-to-initiate (LTI) trials (Daniels & Sanabria, 2017; Fox & Kyonka, 2015). In response-initiated schedules of reinforcement, LTIs scale with the interval requirement, suggesting reduced motivation to engage in tasks with long interval requirements (Holter et al., 2019; Watterson et al., 2015). This suggests that prior demonstrations of HPC lesion and inactivation effects in timing may have been misinterpreted: they may reflect the involvement of HPC function not in tracking long intervals, but in engaging a timing task with a relatively low rate of reinforcement. As HPC has been shown to act in inhibiting non-task related motivations and enhancing motor readiness (Jarrad, 1973; Tracy et al., 2001), lesions may interfere with motivation to engage in a reinforced behavior, which may then manifest as aberrant interval timing. Further, the totality of behavior in timing tasks is not attributable to timing processes alone (Daniels & Sanabria, 2017; Sanabria et al., 2009), but rather to fluctuations between a timing state and a non-timing state. This generative mechanism yields a mixture of timed and non-timed responses, which are gamma- and exponentially-distributed, respectively (Daniels, Fox, et al., 2015; Daniels et

al., 2018; Mazur et al., 2014; Sanabria & Killeen, 2008; Watterson et al., 2015). The proportion of non-timed responses increases as a function of interval requirement (Daniels, Fox, et al., 2015; Watterson et al., 2015), indicating that an increase in the to-be-timed interval brings with it a decrease in schedule control.

Therefore, although evidence points at HPC involvement in interval timing, the function of other processes in mediating this involvement has not been ruled out. The heterogeneity of conditions under which HPC function is associated with temporal entrainment of behavior is incongruous with the consistency of biological evidence for temporal coding in HPC. It is thus possible that this heterogeneity derives not from some property of the specific function of HPC in interval timing, but rather from the conflation of temporal behavior with extra-timing processes. Accordingly, the present dissertation hypothesizes that the engagement of the HPC in entrainment of behavior to long temporal intervals is subject to modulation from non-timing components of operant responding, including changes in motivation, the background richness of reinforcement, and whether assessment is of acquisition or maintenance of temporal entrainment. Experiments in this dissertation will procedurally and analytically deconvolve timed responses, non-timed responses, and motivation, thereby increasing the temporal signal of interval timing behavior under hippocampal manipulations. Data from these experiments will then elucidate a specific function of the HPC within theoretical models of interval timing.

Summary of Experiments and Findings

Chapter 2 investigates the role of the dorsal hippocampus (dHPC) in the temporal entrainment of behavior, while addressing limitations of previous evidence from procedure experiments. Rats were first trained on a switch-timing task in which food was obtained from one of two concurrently available levers; one lever was effective after 8 s and the other after 16 s. After performance stabilized, rats underwent either bilateral NMDA lesions of the dHPC or sham lesions. After recovery, switch-timing training resumed. In a subsequent condition, the switch-timing task was modified such that food was available after either 8 or 32 s. Although dHPC lesions had little effect on when rats stopped seeking for food at the 8-s lever (departures), it reduced the time when rats started seeking for food at the 16-s and 32-s lever (switches). No systematic effect of dHPC lesions were observed on the coefficient of quartile variance (normalized dispersion) of latencies to departures and switches. Within the context of the PA framework of interval timing, these findings suggest that partially or wholly independent response thresholds control the initiation and termination of timed responses, and dHPC is primarily involved in the initiation of timed responses.

Chapter 3 examines the sensitivity of various components of the PA framework of interval timing to chronic variable stress (CVS), which has consistent effects on the HPC, and preliminarily identify candidate neural correlates underlying this sensitivity. Following stable training on a response-initiated prospective (switch-timing) or retrospective (temporal bisection) timing task, rats underwent either a 21-day CVS regimen or normal handling, with behavioral testing continuing daily during stress. Chronic stress selectively reduced motivation to engage in timing in a prospective timing

task (response-initiated switch-timing), but selectively increased the variability of timing judgments in a retrospective timing task (response-initiated temporal bisection). Findings support a memory-based interpretation of the effect of chronic stress on timing sub-processes.

Chapter 4 aims to validate a response-initiated *timing-with-opportunity-task* (Sanabria et al. 2009) in which rats are trained to seek food at one location based on time (fixed-interval) and at another location based on probability (random ratio). This design facilitates response-initiated timing behavior, even at long delays (>48-s). Importantly, the response-initiated design of this task allows for procedural dissociation of motivation and timing, such that effects of interval length and pre-feeding manipulations on trial initiation frequency can be measured. Consistent with prior findings, performance on the FI component tracked schedule requirement and displayed scalar invariance; the removal of the RR component yielded more premature FI responses. For some rats, pre-feeding reduced the number of trials initiated without affecting timing performance; for other rats, pre-feeding delayed responding on the FI component but had a weaker effect on trial initiation.

Chapter 5 examines the role of the dHPC in the entrainment of behavior to long intervals in a response-initiated timing-with-opportunity-cost task. Rats were tested in the response-initiated *timing-with-opportunity-cost* task, where the terminal condition was either a 48-s interval (long-interval condition) or a 12s interval (short-interval condition), during targeted administration of a GABA agonist or vehicle in the dHPC. -

CHAPTER 2

THE DIFFERENTIAL ROLE OF THE DORSAL HIPPOCAMPUS IN INITIATING AND TERMINATING TIMED RESPONSES

***Published in Behavioural Brain Research, Volume 376 ,* 2019**

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Introduction

Interval timing is the entrainment of behavior to stimulus periodicity in the minutes-to-seconds range, which entails a representation of time in the central nervous system (Meck et al., 2008; Wittmann, 2013). Recent computational models have suggested various roles of the hippocampus and its subregions in encoding, maintaining, and retrieving those representations, e.g.,(Buzsáki & Tingley, 2018; Mau, Sullivan, Kinsky, Hasselmo, Howard, & Eichenbaum, 2018; Oprisan, Aft, et al., 2018; Oprisan, Buhusi, et al., 2018). Much of the behavioral data supporting these models is obtained from laboratory animals trained in the peak-interval procedure (Buhusi et al., 2009; Roberts, 1981). In this procedure, subjects are trained in a discrete-trials fixed-interval

(FI) schedule of reinforcement, in which the first response after a fixed interval elapses is reinforced. Interspersed among these FI trials are unsignaled extinction probe trials, three-to-four times longer than FI trials. Subjects well-trained in the peak-interval procedure produce a Gaussian-like distribution of responding across time in probe trials. The properties of this Gaussian-like distribution may be assessed to determine both timing accuracy and precision, where timing accuracy is defined by central tendency measures (*peak times*) and timing precision is defined by dispersion measures (standard deviation and coefficient of variation; CV = standard deviation / mean) of the distribution of responses. Peak times are approximately equal to the length of the trained interval and the CV is approximately invariant over broad ranges of trained intervals (Buhusi et al., 2009; Gibbon, 1977); but see (Bizo et al., 2006).

Early peak-interval studies in rats showed that lesions to the fornix-fimbria, which connects the hippocampus to various subcortical structures, permanently shorten peak times without systematically affecting the CV of response distributions (Meck, 1988; Meck et al., 1984; Olton et al., 1987). More recent studies (Tam & Bonardi, 2012b, 2012a; Tam et al., 2013;see pooled analysis in Tam et al., 2015) found similar sustained reductions in peak times in lesions to the dorsal hippocampus (dHPC), but also found systematic lesion-induced increases in the CV of response distributions. Although these results suggest that dHPC lesions yield sustained underestimations of the length of time intervals, Dietrich and Allen (1998) showed that dHPC lesions did not disrupt peak times and that the hippocampus was not necessary for the acquisition of peak-interval performance.

These inconsistent effects of dHPC lesions on timing precision (Tam et al., 2015), and on timing in general (Dietrich & Allen, 1998), may reflect procedural differences, including the size of lesions, task parameters, and the timing of the lesions relative to training (Tam et al., 2015). However, these inconsistencies may also reflect limitations in the standard interpretation of peak-interval performance. It is now well established that the Gaussian-like shape of the distribution of responding emerges from averaging step-like functions obtained in individual trials (Church et al., 1994). These step-like functions involve variable start-times (abrupt transitions from a low to a high response rate) and stop-times (abrupt transitions from a high to a low response rate), which typically envelop the trained interval. Typical start- and stop-times are positively correlated, and the interval between them is negatively correlated with start-times, suggesting a complex relation among components of the mechanism governing peak-interval performance (Church et al., 1994; Rakitin et al., 1998; Sanabria et al., 2009; but see Matell et al., 2006). Peak times and CVs do not account for these important aspects of the structure of peak-interval performance, which are differentially sensitive to manipulations of various forms of motivation, e.g., (Balci, 2014; MacDonald et al., 2012).

Yin and Meck (2014) assessed the effect of dHPC lesions on the start- and stop-times of mice in a *bi-peak* procedure. This procedure is similar to the peak-interval procedure, except that training trials consist of two randomly alternating FI schedules with different interval requirements, programmed on separate but concurrently available levers. Averaged bi-peak extinction-probe performance typically shows Gaussian-like distributions of responding on each of the levers around the FI requirements of their assigned schedules. Similar to standard peak-interval procedures, the average distribution

of responses on each lever in the bi-peak procedure appears to emerge from step-like functions obtained in individual trials (Buhusi & Meck, 2009). Yin and Meck's analysis indicates that underestimations of time intervals induced by dHPC lesions are circumscribed to central tendency measures of start-times. These results suggest that the dHPC is more involved in the initiation than in the maintenance and termination of timed activities.

However, Yin and Meck's (2014) analysis of bi-peak performance does not address a second limitation in the standard interpretation of peak-interval performance: measures of interval timing are highly sensitive to changes in motivation (Galtress et al., 2012; Plowright et al., 2000). For instance, peak-interval start-times are delayed and stop-times are expedited when a concurrent random-ratio schedule, in which each response has a constant probability of reinforcement, is effective (Sanabria et al., 2009). This shows that start- and stop-times are sensitive not only to the passage of time, but also to proportion of reinforcers obtained from the peak-interval procedure. Also, based on FI-performance data, Daniels and Sanabria (2017) suggest that start-times are sampled from a mixture of time-sensitive and time-insensitive distributions, and that the latter become longer and more prevalent with prefeeding; see also (Daniels et al., 2018). It is thus possible that the effect of dHPC lesions on peak-interval and bi-peak performance reflects a sustained increased motivation for the reinforcer, and not the underestimation of the timed interval.

To address some of these limitations, the present study implemented a *switch-timing* procedure in rats that had undergone bilateral NMDA-induced lesions of the dHPC. This procedure is similar to the bi-peak procedure, but without extinction probe

trials (Balci et al., 2008). The analytical approach, however, is substantially different. Instead of assuming two independent timing processes in each lever, the switch timing procedure assumes that well-trained subjects transition from the short-FI to the long-FI when the subjective probability of reinforcement in the former is lower than in the latter. Therefore, on each switch timing trial, inferences about interval timing are drawn from the time to the last response on the short-FI lever, or *latency-to-depart* (LTDs; Daniels, Fox, et al., 2015), and from the first response on the long FI lever, or *latencies-to-switch* (LTSs; Balci et al., 2008). Finally, potential lesion effects on schedule control were evaluated on the proportion of trials initiated in the short-FI lever (discrimination ratio, or DR) and the proportion of trials in which the LTS was longer than the LTD (i.e., in which the rat did not switch back to the short-FI lever; persistence ratio, or PR).

Methods

Subjects

Twenty male Wistar rats (Charles River Laboratories, Hollister, CA) served as subjects. Rats arrived on post-natal day (PND) 60 and were pair-housed immediately upon arrival. Rats were housed on a 12:12 h light cycle, with dawn at 1900 h; all behavioral training was conducted during the dark phase of the light cycle. Behavioral training and food restriction protocols were implemented shortly after arrival. Access to food was reduced daily from 24, to 18, 12, and finally 1 h/day. Chow was placed on the home cages of rats during the dark phase of the light cycle. During behavioral training, food was provided 30 min after the end of each training session, such that at the beginning of the next session weights were, on average, 75% of mean ad libitum weights estimated from growth charts provided by the breeder. Water was always available in-

home cages. All animal handling procedures in this study followed National Institutes for Health guidelines and were approved by the Arizona State University Institutional Animal Care and Use Committee.

Apparatus

Experiments were conducted in 10 MED Associates (ST. Albans, VT, USA) modular test chambers (10 chambers measured 305 mm long, 241 mm wide, and 210 mm high; 6 chambers measured 305 mm long, 241 mm wide, and 292 mm high), each enclosed in a sound- and light-attenuating box equipped with a ventilation fan that provided masking noise of approximately 60 dB. The front and back walls and the ceiling of test chambers were made of Plexiglas; the front wall was hinged and served as a door to the chamber. One of the two aluminum side panels served as a test panel. The floor consisted of thin metal bars positioned above a catch pan. The reinforcer receptacle was a square opening (51-mm sides) located 15 mm above the floor and centered on the test panel. The receptacle provided access to a dipper (MED Associates, ENV-202M-S) fitted with a cup (MED Associates, ENV-202C) that could hold 0.01 cc of a liquid reinforcer (33% sweetened condensed milk diluted in tap water; Great Value brand, Walmart, Bentonville, AK).

The receptacle was furnished with a head entry detector (MED Associates, ENV-254-CB). A multiple tone generator (MED Associates, ENV-223) was used to produce a 15-kHz tone at approximately 75 dB through a speaker (MED Associates, ENV-224 AM) centered on the top of the wall opposite the test panel and 240 mm above the floor of the chamber. Two retractable levers (MED Associates, ENV-112CM) flanked the reinforcer receptacle. Lever presses were recorded when a force of approximately 0.2 N was applied

to the end of the lever. Three-color light stimuli (ENV-222 M) were mounted above each lever; they could be illuminated yellow, green, and red (only yellow was used in the present experiment). A house light located behind the wall opposite to the test panel could dimly illuminate the test chambers. Experimental events were arranged via a MED-PC® interface connected to a PC controlled by MED-PC IV® software.

Procedure

Sessions were conducted daily and were 75-min long, unless noted otherwise. All sessions began with a 3-min warmup period during which the house-light was illuminated. The house-light was then turned off, signaling the beginning of experimental events.

Behavioral Training

Box Acclimation and Lever-Shaping. On PND 65, all rats were acclimated to the chambers for two days. During this procedure, the reinforcer (2.5 s of access to sweetened condensed milk solution) was delivered on a fixed-time 60-s schedule. After two days, this procedure was amended such that both levers were inserted 10 s prior to the delivery of reinforcement. This phase continued for seven sessions, at which point all animals were reliably lever-pressing.

Pretraining. Trials began by turning off the house-light, inserting either the left or right lever into the chamber, and illuminating the yellow LED above the active lever (pseudo randomly selected from a 6-item list with $p = .5$). A single lever press on the active lever resulted in termination of the yellow LED above the lever, retraction of the lever, reinforcement, and onset of a 15-kHz tone signaling and lasting throughout reinforcement. Reinforcement was followed by a 5-s inter-trial interval (ITI), during

which the tone was turned off and the house-light was illuminated. Pretraining continued until all rats had been weaned onto the food restriction protocol and had successfully earned more than 50 reinforcers in each of two consecutive sessions. This phase was completed in eight sessions.

Switch-Timing Baseline (FI 8-s FI 16-s). All rats were trained on a dependent concurrent fixed-interval (FI) 8-s FI 16-s schedule of reinforcement. Sessions were arranged in blocks of 16 trials, half of which were FI 8-s trials, and half of which were FI 16-s trials; trial types were pseudo-randomly selected. In each trial the first press on the active lever after its corresponding FI elapsed turned on the houselight and delivered a single reinforcer. That is, the first press at the FI 8-s lever after 8 s had elapsed was reinforced on FI 8-s trials, and the first press at the FI 16-s lever after 16 s had elapsed was reinforced on FI 16-s trials (Figure 2.1a). A head-entry into the reinforcer receptacle broke an infrared beam, upon which the reinforcer was delivered. Baseline training on the FI 8-s FI 16-s schedule was completed in 20 sessions (Figure 2.1b).

Dorsal Hippocampus Surgery. After 20 days of baseline training, rats underwent bilateral cytotoxic lesions (NMDA; N = 10), or sham surgery (SHAM; N = 9), targeting the dorsal hippocampus. Animals were assigned to conditions based on baseline training performance, such that mean median LTSs and LTDs were approximately equal between NMDA and SHAM groups. Rats were administered 1.0 mg/kg of 5 mg/mL of meloxicam (i.p.) dissolved in sterile saline for pain management 30 minutes prior to surgery. The rats were then anesthetized with a cocktail of 45mg/kg of sodium pentobarbital and 0.4 mg/kg of atropine (i.p.). and given up to 0.1 mL boosters of the cocktail, as needed. The head was shaved and disinfected with chlorhexidine surgical scrub (Fort Dodge Animal

Health, Fort Dodge, IA, USA) and the head was secured into the stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). 0.4 mL of bupivacaine, a local anesthetic, was administered underneath the scalp prior to scalpel incision. The skull was then exposed, holding the skin with tissue forceps. Lambda and bregma were located and the head was leveled. Glass Hamilton Syringes (5 μL; National Scientific Company, Rockwood, TN, USA) were used to infuse N-Methyl D-aspartic acid (NMDA; Sigma-Aldrich, St. Louis, MO, USA) dissolved in sterile saline (20 μg/μl), or sterile saline. In order to lesion the majority of the dorsal hippocampus, 4 infusions were made (2/hemisphere) at the following coordinates (in mm from bregma): anterior/posterior (A/P), -2.8; medial/lateral (M/L), ±1.6; dorsal/ventral (D/V), -3.6; A/P, -4.2; M/L, ±2.6, D/V, -3.6. Small burr holes were drilled with a Dremel drill at each location. Hamilton syringe was carefully lowered, and approximately 0.5 μL of NMDA or sterile saline was infused over the course of 5 min at each infusion site. The syringe was left in place for another 4 minutes to allow for solution diffusion. After the last infusions, the incision was sutured using sterile coated vicryl sutures (Ethicon, Inc., Somerville, NJ, USA) and rats were placed in a cage on a heating pad until they awoke. Figure 2.2 depicts the range of dHPC lesion sizes as well as representative images of SHAM and lesion surgery results.

Rats were administered buprenorphine (0.05 mg/kg of 0.3 mg/mL of drug dissolved in saline) after awaking from surgery and received another dose the following day for pain management. Rats were returned to their home cages and placed back in the colony room. For post-operative care, the scalpel site was inspected daily and treated with triple antibiotic immediately after surgery and for the following 7 days. Additionally, rats were given post-operative doses of meloxicam (1.0 mg/kg of 5 mg/mL

dissolved in saline) daily for 3 days following surgery. Surgery and recovery took a total of 7 days.

Switch-Timing Extinction Testing. Following surgical recovery, animals were re-tested in extinction. This was similar to baseline training except that trials were not reinforced, and each trial was followed by a 7.5-s ITI. The lack of reinforcement in this phase induced schedule strain and noticeably reduced the number of trials the animals completed. This phase was therefore abandoned after three sessions and data were not included in analysis.

Switch-Timing Reacquisition. NMDA and Sham lesion animals were re-tested on the concurrent FI 8-s FI 16-s schedule just as in baseline training. Reacquisition was completed in 12 sessions.

Novel Long-FI Training (FI 8-s FI 32-s). NMDA and Sham lesion animals were trained on a concurrent FI 8-s FI 32-s switch-timing schedule. This was similar to baseline training, except in that the FI 16-s component was substituted with a FI 32-s schedule. The purpose of this condition was to detect potential lesion effects on the acquisition of a novel timing task, which would suggest a role of the dorsal hippocampus on interval encoding (Jacobs et al., 2013).

Results

Data Analysis. Latencies to depart (LTDs; last short-FI lever press) and to switch (LTSs first long-FI lever press) were tracked on individual trials in each of the first 5 (initial) and last 5 (asymptotic) sessions from Reacquisition, and Novel Long-FI training, and only from trials in which the long-FI schedule (16-s or 32-s) was active. Asymptotic baseline data were analyzed separately in order to verify that performance between the

two groups was approximately equal prior to dHPC surgery. Figure 2.3 displays a sample of individual-trial data from a representative animal.

Analyses were based on median and coefficient of quartile variation (CQV) of LTDs and LTSs obtained from individual initial and asymptotic sessions. Medians served as measures of central tendency and CQVs as measures of normalized dispersion. Coefficients of quartile variation were calculated as (Q3–Q1)/(Q3 + Q1), where Q1 and Q3 represent the quartiles 1 and 3 of the dependent measure (Q2 is the median). Discrimination ratios (DRs; proportion of trials initiated in the short-FI lever) and persistence ratios (PRs; proportion of trials in which LTS was longer than LTD) were also obtained from individual sessions. All dependent measures were log-transformed (median LTSs and LTDs) or log-odds-transformed (CQVs, PRs, DRs) before undergoing statistical analysis. Measures of timing performance are typically analyzed in logarithmic scale, because Weber's law suggests that intervals are encoded in logarithmic scale (Kim et al., 2013).

One SHAM animal was removed from analyses, as histological analysis indicated severe damage to the hippocampus, as well as more ventral structures, such as the thalamus. Consequently, the final number in each group was SHAM, n= 8, NMDA, n= 10.

The similarity of baseline performance prior to treatment was first verified using *t*-tests that compared asymptotic measures between treatments (NMDA vs. SHAM). Treatment effects on switch-timing performance were then evaluated using 2 (Treatment) × 2 (Condition: Reacquisition, Novel Long-FI) ANOVA on initial and asymptotic measures, with Treatment as a between-subject variable and Condition as a within-

subject variable. Follow-up *t*-tests were used to probe significant Treatment × Condition interaction effects.

Verification of Similarity of Baseline Performance. No significant differences in asymptotic dependent measures between treatments were observed during baseline ($0.165 < p < 0.876$), indicating that NMDA and SHAM animals performed similarly in switch-timing prior to dHPC surgery.

Analysis of Median LTDs. Rats departed from the short-FI lever sooner in the Reacquisition condition than in the Novel Long-FI condition (Figure 4 top left panel); initial LTDs: F (1, 16) = 415.88, $p < 0.01$, $\eta^2 = .96$; asymptotic LTDs: F (1, 16) = 274.24, $p < 0.01$, $\eta^2 = .92$. A significant Treatment × Condition interaction effect on asymptotic LTDs, F (1, 16) = 5.07, $p < 0.05$, indicates that, even though this measure increased between conditions regardless of treatment, it did so more for NMDA rats than for SHAM rats.

Analysis of Median LTSs. Rats switched to the long-FI lever sooner in the Reacquisition condition than in the Novel Long-FI condition (Figure 2.4, top right panel); initial LTSs: F (1, 16) = 60.16, $p < 0.01$, $\eta^2 = .78$; asymptotic LTSs: F (1, 16) = 112.28, $p < 0.01$, $\eta^2 = .88$. NMDA rats appeared to switch to the long-FI lever sooner than SHAM rats: the effect of treatment on initial LTSs was significant, F (1, 16) = 4.9, $p < 0.05$, $\eta^2 = 0.24$, but despite a large effect size, the effect of treatment on asymptotic LTSs did not reach significance, F (1, 16) = 4.12, $p = 0.06$, $\eta^2 = .21$. No significant Treatment × Condition interaction effects were detected on median LTSs ($0.35 < p < 0.65$).

Individual-Subject Performance. Figure 2.5 displays P-P plots of initial and asymptotic median LTSs from individual rats. In baseline performance, data points fall

along the identity line, indicating a similar baseline performance in NMDA and SHAM rats. Two aspects of Reacquisition and Novel Long-FI data are noteworthy. First, whereas initial and asymptotic data substantially overlap in Reacquisition, they do not in Novel Long-FI data. This difference reflects the relatively steady performance over the course of Reacquisition testing, compared to Novel Long-FI performance, where LTSs became longer over sessions (cf. Figure 2.5, top-right panel). Second, and more importantly, in both Reacquisition and Novel Long-FI testing, data points consistently fall above the identity line—only 2 of 16 points fall below the identity line. This indicates that NMDA lesions to the dHPC consistently reduced LTSs. Interestingly, the divergence from identity appears to be less even in Reacquisition than in Novel Long-FI. On a previously-trained switch-timing schedule, NMDA lesions appear to induce very short LTSs in some rats, but less so in others. This variability in lesion effects may explain why they did not reach significance on asymptotic LTSs.

Analysis of LTD CQVs. Initial CQVs of LTDs were larger in the Reacquisition condition compared to the Novel Long-FI condition (Figure 2.4, bottom left panel), F (1, 16) = 20.21, $p < 0.01$, $\eta^2 = .54$. No significant Treatment or Condition effects were observed on asymptotic CQVs of LTDs ($.15 < p < .38$). Because visual examination of Figure 4 suggests a potential effect of Treatment on that asymptotic CQVs of LTDs in Novel Long-FI testing, a post hoc t-test was conducted, and revealed a significant effect of Treatment, t (16) = 2.7, $p < 0.05$, Cohen's d = 1.00, where NMDA animals had lower LTD CQVs in this condition compared to SHAM animals.

Analysis of LTS CQVs. Initial CQVs of LTSs were larger in the Reacquisition condition than in the Novel Long-FI condition (Figure 2.4, bottom right panel), F (1, 16)

= 6.75, $p < 0.05$, $\eta^2 = .3$. No other significant Condition or Treatment effects were observed on asymptotic LTS CQVs ($0.67 < p < 0.96$).

DR Analysis. Discrimination ratios were lower in the Reacquisition condition than in the Novel Long-FI condition; initial DRs: F (1, 16) = 12.92, $p < 0.05$, $\eta^2 = .44$; asymptotic DRs, F (1, 16) = 14.7, $p < .05$, $\eta^2 = .46$ (Figure 2.6). No significant Treatment effects were detected on DRs, indicating that dHPC deactivation did not substantially compromise the rats' capacity to initiate the switch-timing sequence correctly.

PR Analysis. Persistence ratios were higher in the Reacquisition (M = 0.52 ± 0.03) condition than in the Novel Long-FI condition (M = 0.26 ± 0.09); initial PRs: F (1, 16) = 32.07, $p < 0.01$, $\eta^2 = .67$; asymptotic PRs: F (1, 16) = 18.27, $p < 0.01$, $\eta^2 = .51$ (Figure 6). Initial and asymptotic PRs were higher for SHAM rats (M = 0.45 ± 0.04) than for NMDA rats; initial PRs: F (1, 16) = 16.46, $p < 0.01$, $\eta^2 = .51$; asymptotic PRs: F (1, 16) = 23.42, $p < 0.01$, $\eta^2 = .59$. These results suggest a general tendency of NMDA rats to persist less in the long-FI lever once they initiated seeking food in that lever. No significant Treatment × Condition interaction effects were detected on PRs ($0.23 < p < .79$).

Latencies to First Response. Latencies to the first response were defined as the time between trial onset and first lever press, when the first lever press was on the short-FI lever; trials in which the first lever press was on the long-FI lever were not considered when computing median latencies to first response. These latencies were log-transformed and analyzed as a potential measure of motivational effects. At baseline, asymptotic median latencies to first response were substantially shorter for NMDA rats (M = 2.56 ±

0.22 s) than for SHAM rats (M = 3.56 ± 0.32 s). Therefore, log-transformed median latencies to first response were analyzed in the Reacquisition and Novel Long-FI conditions as their difference relative to asymptotic baseline. A significant Treatment × Condition interaction effect of initial latencies to first response, F (1, 16) = 6.99, p < 0.05, η^2 = .304, indicates that these latencies to first response increased more between initial Reacquisition and initial Novel Long-FI conditions in SHAM rats (by 1.08 ± 0.11 s; t = 3.44, p < 0.01) than in NMDA rats (by 0.38 ± 0.20 s, t = 1.35, p = .10).

Discussion

The effects of dHPC NMDA lesions were examined on the reacquisition and maintenance of a previously trained switch-timing task, as well as on the acquisition and maintenance of a novel schedule. Although these effects involved a range of performance measures, their discussion focuses first on latencies-to-switch (LTSs), because these are easier to interpret and are consistent with prior findings. Other effects are then discussed primarily to qualify the interpretation of NMDA lesion effects on LTSs.

NMDA lesions on the dHPC had little impact on performance in a familiar switch-timing schedule (FI 8-s vs. FI 16-s), except for reducing the time to seek food in the long FI schedule (LTS) in most rats (Figure 2.4, central panel). Moreover, this effect appears to be somewhat transient, confined primarily to the early sessions after reinstating the previously trained schedule. Interestingly, this effect is replicated in a novel schedule (FI 8-s vs. FI 32-s), where it appears to be more permanent (Figure 3, top-right panel; Figure 2.4, right panel). The relative dispersion of LTSs (i.e., their CQV) was more resilient to dHPC NMDA lesions. These results are consistent with early peak-interval studies showing that fimbria-fornix lesions shortened peak times while leaving

the relative dispersion of responding intact (Meck, 1988; Meck et al., 1984; Olton et al., 1987). It is also consistent with Yin and Meck's (2014) finding that dHPC lesions selectively shortened start-times, without affecting its relative dispersion, in the bi-peak procedure.

The effects of dHPC NMDA lesions on LTSs may have important implications for unveiling the role of dHPC in interval timing. To interpret them, these implications must be formulated in terms of a theoretical framework of interval timing; the pacemaker-accumulator model provides a fairly parsimonious framework in algorithmic form (Simen et al., 2013; Treisman, 1963). This model assumes that (a) trial onset resets a counter and initiates the emission of pulses from a pacemaker to an accumulator; (b) when the pulse count in the accumulator crosses a similarity threshold relative to a criterion sampled from memory, a response (switching to long-FI lever) is emitted, and (c) upon reinforcement, pulses in the accumulator update the memory criterion. According to this model, the shortening of LTSs reported here may result from (a) a faster emission or accumulation of pulses, (b) a lower similarity threshold, or (c) a biased memory of past pulse counts.

A pulse-speed-based interpretation of the reported results is improbable, because a faster emission or accumulation of pulses entails two effects on performance that were not reliably observed: LTSs would be only transiently shortened (faster accumulation entails larger pulse counts updating memory, yielding longer intervals) and, after the length of LTSs rebound, their relative dispersion would be reduced (law of large numbers: a larger count of pulses with variable inter-pulse intervals yields more

consistent counts). The first effect was observed in the Reacquisition LTSs but not in the Novel Long-FI LTSs; the second effect was not observed in either condition.

A threshold-based interpretation of the reported results is also somewhat improbable, because a permanent reduction in the similarity threshold cannot account for the seemingly transient effect of dHPC NMDA lesion on Reacquisition LTSs. A memory-based explanation, on the other hand, may account for these effects. Because the Reacquisition schedule was trained before the lesion, the seemingly transient shortening of LTSs in this condition may reflect a transient lesion-induced failure to retrieve temporal information from memory. In contrast, the Novel Long-FI schedule was trained after the lesion, so the more permanent shortening of LTSs in this condition may reflect a more permanent lesion-induced failure to encode new (or longer) intervals in memory. The expression of memory failures as shorter LTSs may reflect proactive interference from preceding training conditions, when food in the Long-FI lever was available after a shorter interval, or simply a reduced capacity to learn new intervals.

There are important caveats to the proposed interpretation of LTS data. First, although there is strong statistical support for lesion effects on median LTSs early in each condition, evidence for its transiency in the Reacquisition condition and for its permanence in the Novel Long-FI condition are less compelling. The lack of stronger statistical support for the encoding-specific role of the dHPC in interval timing makes this hypothesis somewhat provisional; in particular, the threshold-shifting hypotheses cannot be entirely ruled out.

Another caveat involves the multiple differences between experimental conditions. The Novel Long-FI condition included a FI 32-s component that, relative to

the preceding Reacquisition condition, was new and longer than the FI 16-s component it replaced, both in absolute terms (16-s longer) and relative to the alternative FI 8-s component (four times its length, instead of twice its length). Therefore, differences in performance between conditions, such as those on which the encoding-specific hypothesis is based on, may be attributed to the novelty of the FI 32-s or to its absolute length, or to its enhanced discriminability from the alternative FI component. Further research may clarify the relative contribution of each of these factors to the difference in performance between conditions.

Finally, a complex pattern of dHPC NMDA lesion effects on non-LTS measures of performance paints a potentially more complex picture. First, dHPC NMDA lesions reduced the probability of staying in the long-FI lever after switching. This effect, however, may result from the increased opportunity to return to the short-FI lever afforded by lesion-associated shorter LTSs. Also, the new, longer FI component was expected to either have no effect on the latency to first response on the short-FI lever (because the short-FI schedule was not changed) or to lengthen it (because the rate of reinforcement was reduced). Whereas the performance of lesioned rats was more consistent with the former effect; the performance of control rats was more consistent with the latter effect, perhaps reflecting a reduced sensitivity to the novel long-FI schedule in lesioned rats. The effect of introducing the longer FI component on the variability (CQV) of the time to stop seeking food in the short FI lever (latency-to-depart, or LTD) was also more muted in dHPC NMDA lesioned rats than in SHAM rats (Figure 3, bottom-left panel). These effects are unlikely to reflect a dHPC NMDA lesion-induced reduction in sensitivity to the change of schedule, because the change in schedule

supported a larger increase in median LTDs from dHPC NMDA lesioned rats compared to SHAM rats (Figure 2.3, top-left panel).

Although the pattern of treatment effects on LTDs and latencies to first response are difficult to interpret, it is clear that the mechanisms that govern these aspects of performance operate somewhat independently from those that govern LTSs. Whereas the dHPC appears to play a complex and unclear role in the decision to discontinue a timed activity, it appears to play a more prominent and distinct role in encoding the time to initiate an activity.

CHAPTER 3

PROSPECTIVE AND RETROSPECTIVE TIMING TASKS DISSOCIATE THE EFFECTS OF CHRONIC STRESS ON MOTIVATION AND INTERVAL TIMING

Introduction

Sustained disruption of homeostasis, such as that induced by chronic stress, can compromise a wide range of cognitive functions, including spatial memory, decision-making, attentional allocation, and fear learning (Blanchard et al., 2001; Bondi et al., 2008; Conrad, 2010; Hoffman et al., 2014; Maroun & Richter-Levin, 2003; Peay et al., 2020; Simpkiss & Devine, 2003). Such disruption also dysregulates the stress response system, resulting in excess of stress-induced hormone-release, the primary mechanism in stress-induced dendritic remodeling in several neural structures, including the prefrontal cortex (PFC), striatum, and hippocampus (HPC; Conrad et al., 2017). Chronic stress provides a useful model in to assess the role of these brain regions in various cognitive functions.

These brain regions, which show reliable sensitivity to stress, are also consistent neural correlates of the capacity to track time in the seconds-to-minutes range, or *interval timing* (Buhusi & Meck, 2005; Kleen et al., 2006a; Matell & Meck, 2000). Interval timing underlies the structure of motivated behavior and subserves basic cognitive capacities such as foraging, decision-making, and conscious time estimation (Buhusi & Meck, 2005), and requires synergistic interaction of attention, working memory, and long-term memory (Buhusi & Meck, 2005; Sévigny et al., 2003). Aberrant time perception, theoretically driven by dysregulation in one of more of these sub-processes, is a proposed underlying mechanism of impulsive decision-making, and is associated with a

wide array of psychopathologies (Carroll et al., 2009; Sévigny et al., 2003; Wittmann & Paulus, 2008). Taken together, this framework suggests that impaired time perception is a potential mediator of the effect of stress on behavior and cognition.

Components of interval timing and their underlying neural substrates appear to be sensitive to acute stress, where acute stress immediately prior to and during the target interval induce overestimation of the interval length (Bar-Haim et al., 2010; Droit-Volet & Meck, 2007; Meck, 1983; Meck & MacDonald, 2007; Watts & Sharrock, 1984). Reports of timing disturbances related to chronic stress and stress-associated disorders, unlike those related to acute stress, are inconsistent. Whereas some human studies indicate that chronic stress is associated with greater variability in timed responses (Mezey, 1961; Zhang, 2015), other reports indicate that chronic stress is associated with temporal underestimation but intact variability (Droit-Volet & Meck, 2007).

Additional accounts indicate that symptoms of depression, which is linked to chronic stress (Hammen et al., 2009; Hammen, 2015; Robles et al., 2005), is also associated with a slower experience of time (i.e. time *feels* slower), while temporal estimation is retained (Bschor et al., 2004; Mezey, 1961). This subjective slowing of time was also widely reported as a result of the collective stress endured at the onset of the COVID-19 pandemic (Holman & Grisham, 2020). Data from some studies using animal models is somewhat at odds with these findings; as sustained stress and social defeat in rats seems to induce longer temporal estimates (Kant et al., 1997; Yin et al., 2016). However, these studies lacked the longitudinal design required to derive more reliable inferences regarding shifts in timing sub-processes across the stress response.

One possible explanation for these observed inconsistencies is the adaptive nature of timing performance (Meck, 1983). Notably, increased length of exposure to the aversive stimulus eliminates interval under-estimation (Bar-Haim et al., 2010), suggesting that timing processes may continually adapt as stress progresses. This suggests a nonlinear relationship between timing performance and the parameters of the mechanism that underlies it, where performance is mediated by experience and feedback (Block & Zakay, 1996). The absence of experimental control of stress-associated variables in human studies is expected to yield divergent performance, even when underlying mechanisms are systematically responsive to stress.

Another potential explanation for the varied effects of chronic stress on timing is the consistent link between timing processes and motivation (Daniels & Sanabria, 2017), where target responses in timing tasks are similarly sensitive to changes in incentive value (Plowright et al., 2000; Sanabria & Killeen, 2008; Ward & Odum, 2006; Ward et al., 2016). Thus, shifts in the central tendency or variability of ostensibly timed responses may reflect shifts in motivation to engage in a timing task. This consideration is especially critical when assessing chronic stress effects, as chronic stress devalues reinforcement (Kleen et al., 2006b). Procedural dissociation of changes in motivation from changes in timing allows for clearer assessment of effects.

In order to reveal the systematic effects of chronic stress on the mechanisms underlying interval timing and its potentially associated neural structures, the current study assessed temporal cognition prior to and a during chronic variable stress (CVS) procedure, implementing two complementary response-initiated timing assessments. In both tasks, trials were initiated using a nose-poke response to a device mounted in the

back wall of the operant chamber. Allowing rats to initiate their own trials effectively separates changes in motivation to engage in the task from changes in interval timing (Daniels & Sanabria, 2017; Fox & Kyonka, 2013). One task, response-initiated switch-timing, involves prospective timing: interval timing is inferred from the entrainment of the target response to the periodicity of reinforcement (R. K. Cheng et al., 2016; Fanselow, 1994). In contrast, a response-initiated temporal bisection task involves retrospective timing: interval timing is inferred from the control that an elapsed interval exerts over a "short" vs. "long" choice response (Allan & Gibbon, 1991; Church & Deluty, 1977).

Utilizing both prospective and retrospective timing tasks is especially critical as they are differentially sensitive to attentional and memory processes (Block & Zakay, 1997; Marshall & Kirkpatrick, 2015; Zakay & Block, 2004). This is particularly important for this study as chronic stress may interfere with attentional and memory systems (Bangasser et al., 2019, 2018; Bondi et al., 2008). Thus, implementing these two complimentary tasks allows for a clearer assessment of the impact of chronic stress on these processes, and testing during the onset and continuation of stress facilitates the evaluation of changes in temporal responding across the stress response

Experiment 3.1 Methods

Subjects

Forty male and 40 female Sprague-Dawley rats (Charles River Laboratories, Hollister, CA) served as subjects. Rats arrived on postnatal day 60 and were pair-housed immediately upon arrival. Rats were housed on a 12:12 light cycle, with dawn at 1900 h. All behavioral training was conducted during the dark phase of the light cycle starting at

approximately 1200 h and ending at approximately 1400 h. Following three days of acclimation to the colony room, food access was reduced daily in 6 h increments from 24 to 1 hour per day. One hour of food access was given in home-cages starting 30 min after the end of each experimental session. This feeding schedule ensured that, at the beginning of each session, weights were, on average, 85% of *ad libitum* weights, as estimated from growth charts provided by the breeder. Water was always available in home cages. After applying inclusion criteria following baseline testing (details explained in Data Analysis below), 51 rats were excluded from the experiment, and results are based on the performance of 29 rats. All animals handling procedures used during this study followed National Institutes for Health Guidelines and were approved by the Arizona State University Institutional Animal Care and Use Committee.

Apparatus

Experiments were conducted in 16 MED associated (St. Albans, VT, USA) modular operant chambers (305-mm long, 241-mm wide, and 210-mm high), each enclosed in a sound and light-attenuating box equipped with a ventilation fan that provided approximately 60 dB of masking noise. The front and back walls and the ceiling of the test chambers were made of Plexiglas. The front wall was hinged and served as a door to the chamber. One of the two aluminum side panels served as the test panel. The floor consisted of thin metal bars positioned above a catch pan. The reinforcement port was a square opening (51-mm sides) located 15 mm above the floor and centered on the test panel. The receptable provided access to a dipper (MED Associates, ENV-202M-S) fitted with a cup (MED Associates, ENV-202C) that could hold 0.01 cc of a liquid reinforcer (~33% sweetened condensed milk diluted in tap water; Kroger, Cincinnati,

OH). The receptacle was furnished with a head-entry detector (ENV-254-CB). A multiple-tone generator (MED associates, ENV-223) was used to produce a 15-kHz tone at approximately 75 dB through a speaker (MED Associates, ENV-224 AM) centered on the top of the wall opposite the test panel and 240 mm above the floor of the chamber. Two retractable levers (ENV-112CM) flanked the reinforcement port. Lever presses were recorded when a force of approximately 0.2 N was applied to the end of the lever was applied at the end of the lever. Three-color light stimuli were mounted above each lever, which could be illuminated yellow, green, and red. The opposite wall was furnished an illuminated nose-poke device (MED Associates, ENV-114BM) at the bottom of the panel. A house light located behind the wall opposite to the test panel could dimly illuminate the test chambers. Experimental events were arranged via a MED PC ® interface connected a PC controlled by MED-PC IV ® software.

Procedure

Training sessions were conducted 7 days/week. Each session began with a 3-min acclimation period during which the house light was illuminated, but no other manipulanda or stimuli were activated.

Reinforcer Consumption Training

All rats were first trained to consume the reinforcer (sweetened condensed milk) from the liquid dipper in the reinforcement port. Following the 3-min acclimation period, a reinforcer was made available at the liquid dipper. All subsequent reinforcers were made available at variable intervals, with a mean inter-trial interval (ITI) of 45 s. During the ITI, no stimuli or manipulanda were activated. When a reinforcer was delivered, the house light turned on and the liquid dipper was activated. Head entries into the

reinforcement port activated a 15-kHz tone. The dipper and tone were deactivated 2.5 s after the head entry. Reinforcer consumption training continued for 7 days, when rats received 100 reinforcers per session and the median time to retrieve a reinforcer was shorter than 4 s. Two sessions of reinforcer consumption training were conducted.

Manipulandum Shaping

Following two days of reinforcer consumption training, lever-pressing and nose-poking were shaped, using a Pavlovian conditioned approach (auto-shaping) procedure. After the 3-min acclimation period, a single reinforcer was delivered, as described in reinforcer consumption training. At the end of each ITI thereafter, a single manipulandum (left lever extended, right lever extended, nose-poke device illuminated) was pseudo-randomly selected from a list, such that no manipulandum could be selected consecutively in more than six trials. The selected manipulandum was then activated. If 8 s elapsed after manipulandum activation, it was deactivated (lever retract or nose-poke light turned off) and reinforcement was delivered as described in reinforcer consumption training. If, alternatively, a response was made on the manipulandum before 8 s had elapsed, the manipulandum was deactivated immediately following the response and reinforcement was delivered as described. This phase continued until all rats responded in at least 100 trials per session Three sessions of manipulandum shaping were conducted.

Lever and Nose Poke Training

The lever and nose poke shaping procedure was modified such that reinforcement was only delivered following a single response on the active manipulandum, as described. This continued until all rats were reliably lever pressing and responding on the

nose-poke device in at least 100 trials per session. Two sessions of lever and nose poke training were conducted.

Response-Initiated Switch-Timing

Following lever and nose poke training, rats were trained to initiate switch-timing trials. After the 3-minute acclimation period, the nose-poke device was activated. Following trial initiation by nose poke, the nose-poke device was deactivated, the house lights turned on, and both levers were extended. Either a fixed-interval (FI) 1-s (Short FI) or FI 3-s (Long FI) schedule was pseudo-randomly sampled from a list such that neither schedule occurred consecutively in more than four. For some rats, the left lever delivered reinforcement according to the Short FI and the right lever delivered reinforcement according to the Long FI, and vice versa for the remaining rats. The first press on the active FI lever after the active FI elapsed turned off the house light and delivered a single reinforcer. Reinforcement was then delivered as described.

Because the optimal strategy in the switch-timing task is to respond first on the Short FI lever and then switch over to the Long FI lever, certain measures were taken during switch-timing training to encourage this sequence. Thus, during training, rats were punished, and trials were terminated without reinforcement if (a) the first response made after trial initiation was on the Long FI lever, (b) if a response was made on the Long FI lever during a time when the Short FI was active, and (c) if a response was made on the Short FI lever after a response on the Long FI lever.

Timing performance was inferred by the first response on the Long FI lever, or latency-to-switch (LTS). Median LTS was used as a measure central tendency, and coefficient of coefficient of quartile variation (CQV) was used as a measure of

dispersion. CQVs were calculated using the first (Q1) and third (Q3) quartiles of the dependent measure as (Q3-Q1)/(Q3+Q1). Stable performance in FI 1-s FI 3-s, was defined by a non-significant linear regression over the median LTS and CQV over the last five sessions, with <1% of trials being punished. Once rats achieved stable performance, they were then trained up to the terminal timing condition in the following order: FI 2-s FI 4-s, FI 3-s FI 9-s, FI 4-s FI 12-s, and then the terminal condition, FI 8-s FI 16-s. Punishment continued as described until subjects experienced a minimum of 5 sessions of training in the FI 8-s FI 16-s condition and until performance was deemed stable. Training on FI 8-s FI 16-s then continued without punishment until subjects experienced a minimum of 5 sessions in this condition and performance was deemed stable.

Chronic Variable Stress Procedure

Upon achieving stable performance on response-initiated FI 8-s FI 16s, rats were divided into control (CON) and stress (STR) groups. Groups were assigned such that mean median LTS and mean CQV were similar between CON and STR groups. STR rats received a 21-day long variable and non-habituating physical and social stress regimen, designed to be unpredictable (Taylor et al., 2020; Tõnissaar et al., 2008). Each day of stress consisted of two stressors, from a list of nine possible stressors (described below). There was a morning stressor (administered between 800 h and 1100 h, prior to behavioral testing) and an afternoon stressor (administered between 1300 h and 1600 h, after behavioral testing). Morning and afternoon stressor times and stressor types were randomized in order to make them as unpredictable as possible. Only two stressors (Cage Tilting and Dirty Bedding) were assigned exclusively to the afternoon stress period, as

they were conducted overnight. CON rats were handled for a few minutes during stress periods. Rats were tested on response-initiated switch-timing throughout the 21 days of chronic variable stress, between the morning and afternoon stress periods.

Stressors

Wire Mesh Restraint. Rats were enclosed in a wire-mesh restraint within their home cages. After rats entered the restraint, it was closed on both ends using binder clips. Wire-mesh restraint sessions lasted 1 h.

Orbital Shaker with Restraint. Similar to the wire-mesh restraint alone stressor, except that rats were also placed in a plastic tub on top of an orbital lab shaker (Roto Mix, Type 50800) with a 22.23 × 22.23 cm platform set to a speed of 120 rpm and fastened in place using a bungee cord. Orbital shaker with restraint sessions lasted 30 min.

Orbital Shaker with Social Crowding. A maximum of 8 non-cage-mate rats were placed in an empty plastic tub placed on top of an orbital lab shaker (Roto Mix, Type 50800) set to a speed of 120 rpm and fastened in place by a bungee cord. Rats were observed during this stressor for any indications of aggressive behavior. If aggressive behavior were observed, the experimenter intervened and removed the dominant rat. Orbital shaker with social crowding sessions lasted 1 h.

Warm water (30° C) Forced Swim. Rats were stressed by placing them in warm (30° C) water. The apparatus was an opaque cylinder 5-gallon bucket (30.25 cm in diameter, 36.8 cm in height, 30 cm water height). Rats were continually monitored during this stressor. Rats that showed signs of drowning were immediately removed from the

bucket. Afterwards, rats were towel dried and placed on a heating pad to prevent hypothermia. Warm water forced swim sessions lasted 20 mins.

Cold water (18° C) Forced Swim. This stressor was conducted similarly as warm water forced swim, but the water was cold (18° C). Cold water forced swim sessions lasted 10 mins.

Tail Pinch in Wire-Mesh Restraint. Rats were stressed by placing them in wire-mesh restraints and by applying a wooden clothespin 1 cm from the base of their tail. Tail pinch in wire-mesh restraint sessions lasted 10 mins.

Footshock (0.5 mA, 5s on/5s off). Rats were stressed by placing them in a fear conditioning chamber. Current was uniformly distributed through a metal grid floor (1.5 mA, 5s on 5s off). Footshock sessions lasted 15 mins. Foot shock stressors were delivered in a 25 × 29 × 26 cm fear conditioning chamber (Coulborn Instruments, E10-18TC). Shock was equally distributed through a metal grid floor (1.5 mA, 5-s on 5-s off; *Coulborn Animal Shock Generator, H13-H15).*

Cage Tilting. Rats were stressed by tilting their cage 45°, by placing a binder underneath it. This stressor was carried out overnight.

Dirty Bedding. Rats were stressed by placing them in a dirty cage of a conspecific overnight. This is a social stressor, as rats are unable to escape from an environment that has been marked by other rats.

Tissue Collection

Following 21 of CVS and testing, rats were euthanized using isoflurane and rapidly decapitated. Adrenal glands, thymus, and uterus were excised and weighed for a

secondary measure of stressor effectiveness. Data from tissue collection was pooled across both experiments, and their analysis is reported at the end of Experiment 2.

Experiment 3.1 Data Analysis

Timing performance was assessed from the distribution of the first lever press on the Long FI lever, or latency-to-switch (LTS), and from the distribution of the last lever press on the Short FI lever, or latency-to-depart (LTD). Additionally, motivation was indexed by measures the time to initiate each trial, or latency-to-initiate (LTI). Finally, start ratios (SR) were used to evaluate subjects' ability to correctly start each trial on the Short FI lever, and persistence ratios (PR) were used to determine the extent to which subjects remained on the Long FI lever after switching. Table 3.1 provides a precise operationalization of these dependent measures.

For all dependent measures, the median was used as a measure of central tendency and the coefficient of quartile variance (CQV) was used as a measure of dispersion. CQV were calculated as (Q3 – Q1)/(Q3 + Q1), where Q1 and Q3 represent the quartiles 1 and 3 of the dependent measure (Q2 is the median). Because Weber's law suggests that time intervals are encoded on a logarithmic scale, all dependent variables were log-transformed (LTSs, LTIs, and LTDs) and log-odds-transformed (CQVs, PRs and SRs) before statistical (Kim et al., 2013). In order to minimize the effect of Baseline differences in dependent variables prior to CVS, analyses were performed on differences in log-transformed measures relative to Baseline. All analyses were conducted in JASP.

During data analysis, we noticed an abnormal amount of LTSs at exactly 16.01 seconds. Because LTSs were recorded at the end of each lever press (the time when the

lever was released) reward was made available any time that the lever was in the "down" position at 16 seconds or beyond. Some animals learned this and were able to receive reward at exactly 16 seconds by holding down the long-FI lever until then. There were 28 animals that were unaffected by this anomaly, and fewer than 1% of trials were affected for 66 animals. In order to better capture timing performance and account for this error, all LTSs at 16.01 seconds were excluded from analyses.

Baseline Exclusion Criteria

During the last five sessions of training before CVS, all individual dependent measures were tracked to measure Baseline performance. Rats whose SRs and PRs were greater than 0.5 were classified as "compliant", in that their performance was likely reflecting interval timing processes. Data from non-compliant animals was excluded from analysis. There were also three rats who completed fewer than 70 trials and were excluded from data analysis. The final number of (compliant) animals in each group was CON, n=16; STR, n=16. Figure 3.1 displays the SRs and PRs of individuals animals deemed Compliant and Non-Compliant according to these criteria**.**

On sessions where a rat failed to initiate any trials, their LTI was recorded as the length of an entire session, or 3420 seconds. In order to account for these extreme scores, the last LTI for each rat was replaced with the number 3420. However, there were some animals that initiated very few or no trials in an entire week. Median LTI scores from these animals biased the data in a way that did not reasonably reflect group performance. In order obtain measures that most accurately represented group performance, the animals with the highest and lowest median LTI on the third week of treatment (CVS vs. control) were excluded from all stress analyses. After exclusions, the final number in

each group for LTI analyses was CON, n=15 and STR, n=14. A Bayesian repeated-measures ANOVA with between-subject factors of Sex (Male, Female) and Treatment (CON, STR), and a within-subject factor of Stress Week (Week 1, Week 2, Week 3) was used to analyze group differences.

Bayesian analyses were conducted because they reduce the impact of outliers and allow for support for hypotheses to be quantified for both null and alternative hypotheses. This allowed for a model selection approach, wherein each Bayesian analyses were used to calculate Bayes Factors, a metric which characters the strength of evidence for a model relative to the null model. The log of the Bayes Factor ($LogBF_{10}$) was used, where a positive $LogBF_{10}$ value indicates support for the model (alternative hypothesis or H_1) and a negative $LogBF_{10}$ value indicates support for the null hypothesis (H_0). $LogBF_{10} \geq 1.098$ were considered as having strong evidence support for H_1 (Kass & Raftery, 1995). All models with evidence (positive $LogBF_{10}$) were probed using Bayesian One-Way ANOVAs and Bayesian t-tests.

Experiment 3.1 Results

LTS and LTD

There was no evidence for effects of Week, Sex, or Treatment on median LTSs or LTD or LTS or LTD CQVs relative to Baseline. Figure 3.2 shows median LTSs and LTDs and their CQVs at Baseline and across the three weeks of CVS. Because visual examination of Figure 3.2 suggests a potential Week × Treatment interaction on median LTSs, a post-hoc Bayesian ANOVA was conducted, but revealed no evidence for this effect (highest $LogBF_{10} = -0.239$).

LTI

There was evidence for an effect of Treatment on median LTIs ($LogBF_{10}$ = 0.305), and post-hoc ANOVA revealed strong evidence ($LogBF_{10}$ = 1.961) for an effect of Treatment on Week 1 where STR rats took longer to initiate trials relative to Baseline compared to CON rats in first week of stress (Figure 3.3). This indicates a stress-induced reduction in their motivation to engage in timing, but returned to initiating with frequency similar to Baseline in Weeks 2 and 3 (Figure 3.3).

Start and Persistence Ratios

There was no evidence for effects of Week, Sex, or Treatment on start or persistence ratios.

Experiment 3.2 Methods

Subjects

16 male and 16 female Sprague-Dawley rats (Charles River Laboratories, Hollister, CA) served as subjects. Rats arrived on postnatal day 60 and were pair-housed immediately upon arrival. Rats were housed on a 12:12 light cycle, with dawn at 1900 h. All behavioral training was conducted during the dark phase of the light cycle starting at approximately 1200 h and ending at approximately 1400 h. Following three days of acclimation to the colony room, food access was reduced daily in 6 h increments from 24 to 1 hour per day. One hour of food access was given in home-cages starting 30 min after the end of each experimental session. This feeding schedule ensured that, at the beginning of each session, weights were, on average, 85% of *ad libitum* weights, as estimated from growth charts provided by the breeder. Water was always available in home cages. After applying inclusion criteria following baseline testing (details explained in Data Analysis below), 8 rats were excluded from the experiment, and results are based

on the performance of 24 rats. All animals handling procedures used during this study followed National Institutes for Health Guidelines and were approved by the Arizona State University Institutional Animal Care and Use Committee.

Apparatus

The apparatuses in this experiment were identical to those used in Experiment 1.

Procedure

Training sessions were conducted 7 days/week. Each session began with a 3-min acclimation period during which the house light was illuminated, but no other manipulanda or stimuli were activated.

Response-Initiated Temporal Bisection- Anchor Training

Following lever and nose poke training, rats were trained to discriminate between two anchor intervals. After the 3-minute acclimation period, the nose-poke device was activated. Following trial initiation by nose poke, the nose-poke device was deactivated, and the house lights turned on. Either an 8-s (short) or 16-s (long) interval was pseudo-randomly sampled from a list such that neither interval was sampled for more than 6 consecutive trials. After the interval had elapsed, the houselight turned off, and both levers on the test panel were extended. The left lever was associated with the short interval for half of the rats, and with the long interval for the remaining rats. When the rat pressed one of the levers, a correct choice was marked by a 15-kHz tone, which was deactivated by a head entry into the reinforcement port. The dipper was deactivated 2.5 s after the head entry, followed by a 7.5-s ITI. Incorrect choices were followed by a 10-s ITI during which no stimuli or manipulanda were activated. Anchor interval training continued until 80% of choices in each session were correct for five consecutive sessions.

Stability in performance was confirmed by a non-significant linear regression of the proportion of correct choices over those days. Anchor interval training took 14 sessions.

Response-Initiated Temporal Bisection – Probe Testing

After anchor discrimination performance stabilized, rats were tested on five intermediate probe durations (9, 10, 11.3, 12.7, 14.4 s). Probe testing sessions were arranged in blocks of 13 trials. In each block, 6 trials were anchors (4 short, 4 long), and 5 were intermediate probe intervals. Correct responses after anchor intervals were reinforced as before; responses after probe intervals were no reinforced but initiated a 7.5-s ITI. Intervals were selected pseudo-randomly by sampling without replacement from a 13-item list. Probe testing continued for 14 sessions; stable anchor discrimination was assessed as before.

Chronic Variable Stress Procedure

Upon achieving stable performance on response-initiated temporal bisection probe testing, rats were divided into control (CON) and stress (STR) groups. Groups were assigned such that mean proportion of correct choices on anchor intervals was similar between CON and STR groups. Chronic variable stress was then conducted in the same manner as in Experiment 1, where rats were tested on response-initiated temporal bisection throughout the 21 days of CVS, between the morning and afternoon stress periods.

Tissue Collection

Following 21 of CVS and testing, rats were euthanized using isoflurane and rapidly decapitated. Adrenal glands, thymus, and uterus were excised and weighed for a

secondary measure of stressor effectiveness. Data from tissue collection was pooled across both experiments, and their analysis is reported at the end of Experiment 2.

Experiment 3.2 Data Analysis

Baseline Exclusion Criteria

During the last five days of training before CVS, all individual dependent measures were tracked to measure Baseline performance. Rats that completed fewer than 100 trials during Baseline testing and performed below 75% correct on anchor trials were excluded from analysis. The final number of animals in each group was CON, n =12, STR, n = 12.

Point of Subjective Equality (PSE) and Coefficient of Quartile Variation (CQV)

Psychophysical functions for probe testing Baseline, and Weeks 1, 2, and 3 of stress were drawn using the mean proportion of "long" responses across sessions for each interval. Dependent measures were estimated by fitting a generalized 4-parameter logistic function to the empirical psychophysical functions for each animal, where the logistic function was of the form

$$y = b + \frac{a}{1+e^{-k(x-h)}}, \quad (3.1)$$

where *b* is the lower asymptote of the function, *a* is the upper asymptote of the function, *k* is the slope of the curve, and *h* is the x-value at which $y = (a + b)/2$, which constitutes the point of subjective equality (PSE). Functions were fit in Wolfram Systems Mathematica using maximum likelihood estimation. The PSE served as measure of central tendency. The coefficient of quartile variation (CQV), a measure of dispersion, was calculated by solving the fitted function at y = 0.25 and y = 0.75. Additionally, motivation was indexed by measures the time to initiate each trial, or latency-to-initiate (LTI).

Like in Experiment 1, all dependent variables were log-transformed (LTIs and PSEs) and log-odds transformed (CQVs) before statistical analysis. Analyses were performed on differences in log-transformed measures relative to Baseline.

Experiment 3.2 Results

Baseline Analysis

A 2 (Treatment: Stress, Control) × 2 (Sex: Male, Female) Bayesian ANOVA revealed no evidence for Baseline differences in PSE, CQV of "long" choices, or median LTI between groups.

Stress Analysis (Experiment 2)

Treatment data were grouped into three weekly blocks (each week of CVS) where the difference median measure of each variable relative to Baseline was calculated for all animals compared across groups.

A Bayesian repeated-measures ANOVA with between-subject factors of Sex (Male, Female) and Treatment (CON, STR), and a within-subject factor of Stress Week (Week 1, Week 2, Week 3) was used to analyze group differences. Models selected by the Bayesian analysis as having evidence for H_1 were probed using Bayesian One-Way ANOVAs and Bayesian t-tests.

PSE of Psychophysical Functions

Psychophysical functions for Baseline, Week 1, Week 2, and Week 3 are displayed in Figure 3.4. Although visual inspection indicates that these functions are shifted upward for STR animals on weeks 1 and 2, which correspond to lower PSEs, there was no evidence for effects of Week, Sex, or Treatment on PSEs.

CQV of Psychophysical Functions

There was evidence for an effect of Treatment on CQVs ($LogBF_{10} = 0.425$), and post-hoc ANOVA revealed moderate evidence ($LogBF_{10} = 0.762$) for an effect of Treatment on Week 1 (Figure 3.5), where STR rats made more variable timing judgments relative to Baseline compared to CON rats in first week of stress, but returned to CQVs similar to Baseline in Weeks 2 and 3.

LTI

There was no evidence for effects of Week, Sex, or Treatment on Median LTIs.

Physiological Measures

Adrenal weights, thymus weights, and uterus weights from Experiments 1 and 2 were pooled and analyzed using a Bayesian 2 (Treatment: Stress, Control) by 2 (Sex: Male, Female) Bayesian ANOVA as a measure of stressor effectiveness. Analysis of uterus weights did not include Sex as a factor. There was evidence that stress decreased thymus weight ($LogBF_{10} = 0.782$). Although uterine weights were lower in STR rats, they were not statistically different between treatment groups.

Discussion

The effects of chronic variable stress (CVS) on interval timing were examined using two complementary behavioral response-initiated timing assessments, for which training began prior to start of CVS and continued as CVS progressed. In Experiment 1, rats were trained in a switch-timing task, in which they responded as the to-be-timed interval elapsed; in Experiment 3.2, rats were trained in a temporal bisection task, in which the intervals were judged after they had elapsed. In both experiments, rats initiated each trial by making a nose-poke response, distinct from the timed responses, which facilitated analytical dissociation of motivation and timing processes.

In switch-timing performance (Experiment 3.1) CVS had no impact on temporal responding, as the time to start seeking food on the long FI schedule (LTS), time to depart from the short FI schedule (LTD), and the variability of those responses on an FI 8-s FI 16-s switching schedule were not affected by the onset of CVS. Rather, CVS increased the time to initiate trials (LTIs) for STR rats relative to Baseline performance (Figure 3.3), indicating a stress-induced reduction in motivation to engage in timing. Importantly, this effect was somewhat transient, present primarily in Week 1 of CVS, suggesting that rats' stress response to the CVS paradigm may have habituated by Weeks 2 and 3 (Figure 3.3).

Contrastingly, CVS had no impact on LTIs in response-initiated temporal bisection (Experiment 3.2). Additionally, although central tendency of timing judgments was not systematically affected by CVS, the relative dispersion of these judgements (i.e. their CQV) was increased for STR rats relative to Baseline performance (Figure 3.5) in Week 1 of CVS, which declined in Weeks 2 and 3.

Whereas the effect of CVS on LTIs in switch-timing reflect an established relationship between stress and motivation for food reward, the implications of the effects of stress on variability in temporal bisection performance can be formulated in terms of the sub-processes nested within interval timing. These processes are described in the pacemaker-accumulator (PA) model of interval timing (Gibbon et al., 1984) which assumes that timing consists of three primary sub-processes: a clock, memory, and decision process. According to this model, a gate is closed by the stimulus that marks the onset of the timed interval, and pulses are then emitted by the pacemaker to an accumulator in working memory; the rate of pulse emission is referred to as the clock

speed. The number of pulses in the accumulator at that time is stored in reference memory, and then a decision process determines response requirements based on the current sum in the accumulator, the sample from memory, and a response threshold. A ratio rule then determines whether to make a response or which response to make (Gibbon, 1977; Gibbon et al., 1984). Changes in any of these components are reflected in the central tendency and dispersion of temporal judgments (Balci, 2014; Daniels, Watterson, et al., 2015).

The transiently increased CQV induced by CVS in the temporal bisection task does not support a clock-speed-base interpretation of these results, as a change in the speed of pulse emission would predict at least a transient effect on the central tendency (PSE) of timing judgments, which would resolve as memory updated with accurate pulse counts (Meck, 1983). It is also unlikely that this result is driven by a stress-induced increase or decrease in the similarity threshold for response, as this would also affect central tendency, and be more sustained across stress treatment. As such, the most likely PA-model-based account of these results is an effect of stress on reference memory. Rats were trained on response-initiated temporal bisection prior to the start of CVS, and thus, the transient increase in CQVs may reflect temporary noisiness of pulse-counts sampled from memory, or temporary failure to retrieve reinforced samples from memory. This account also aligns with the established memory effects of chronic stress, where stress-induced hippocampal dendritic retraction decrements performance in spatial memory tasks (Conrad, 2006; Isgor et al., 2004; Jarrard, 1993; Peay et al., 2020), as well as previous finding from our lab in which dorsal HPC lesions induced changes in timing performance consistent with changes in memory processes (Gupta et al., 2019a).

The discrepancy between procedures in the effects of CVS on performance highlights the complex nature of timing and timing-adjacent processes (working memory, attention, etc.), as well as their different sensitivities. Importantly, although temporal bisection and switch-timing are complementary tests of interval timing, and their responses presumably share generative mechanisms, the effect of CVS on motivation in switch-timing and on timing variability in temporal bisection suggest that the tasks are differentially sensitive to non-timing influences on performance. Because temporal judgments in temporal bisection are made after the interval has elapsed, stress-induced changes in attention may contribute to spurious judgments, which could be expressed as the observed increase in variability. Further, because LTIs were only affected in switch-timing, we can speculate that the relatively higher response requirement of prospective timing mediated a stronger effect on motivation to engage in timing, and that the task requirements in temporal bisection were not sufficient to devalue reward during stress.

Results of this study also have important implications for chronic stress research, as each interval timing task revealed impacts in both male and female rats. This is notable as, while young adult male rats often exhibit cognitive deficits following chronic stress (Hoffman et al., 2011; Ortiz et al., 2015), young adult female rats often exhibit resilience to stress-induced cognitive impacts (Duarte-Guterman et al., 2015; Jaric et al., 2019; Luine et al., 2017; Ortiz et al., 2015). Those intending to investigate chronic stress-induced cognitive and/or motivational impacts should further probe the neural substrates of timing and motivation, as there are clear vulnerabilities to chronic stress in both sexes.

One potential caveat to our interpretations is that the stress response was not measured throughout the study. With repeated exposure to a stressor, corticosterone

levels are often attenuated (Babb et al., 2014; Grissom et al., 2007). Some argue that this attenuation reflects increased predictability of the challenge and an adaptive process to reduce negative consequences (Grissom & Bhatnagar, 2009; Koolhaas et al., 2011). Stress effects were only observed during Week 1 of CVS, and the lack of stress effects in subsequent weeks may reflect a habituated response to the CVS paradigm.

These results provide preliminary target timing sub-processes, which if studied further, could establish a potential link between chronic stress, interval timing, and stress-related psychopathology. Further establishment of the mechanism by which cognitive functions involved in timing are affected by a dysregulated stress response system will inform neurocomputational hypotheses of stress effects on cognition, and potentially guide pharmacological and behavioral therapies to reduce these impacts.

Conflicts of Interest

The authors declare no conflicts of interest.

Funding

This research was funded by the National Institute of Mental Health (MH115245) and by the College of Liberal Arts and Sciences at Arizona State University.

Acknowledgments

We thank Jessica Judd, Anna Yee, Julia Guido, Jinah Kim, Ramisa Alam, Amara Miller, Amanda Acuña, Cindy Reynolds, Elliot Smith, Megan Donnay, Kiram Tung, and David Renteria for their support in data collection.

CHAPTER 4

MOTIVATED TO TIME: EFFECTS OF REINFORCER DEVALUATION AND OPPORTUNITY COST ON INTERVAL TIMING

Introduction

Interval timing—the capacity to track the passage of time in the seconds-to-minutes range—is often assessed using the peak-interval procedure (Barrón et al., 2020; Buriticá & Alcalá, 2019; Church et al., 1994; Gibbon, 1977; Roberts, 1981; Sanabria & Killeen, 2007). In this procedure, subjects are trained on a fixed-interval (FI) schedule of reinforcement, in which reinforcement is contingent on the first response after an interval elapses. Temporal control is demonstrated in longer unsignaled extinction probe trials, where response rate typically rises abruptly before the criterial interval (at the *start* time) and declines abruptly after the criterial interval (at the *stop* time) (K. Cheng & Westwood, 1993; Church et al., 1994). The *midpoint* time—the average of start and stop times—typically falls near the criterial interval, indicating the *accuracy* of behavior in tracking that interval. The *width* of the period of high response rate—the difference between start and stop times—indicates the *precision* of behavior in tracking the criterial interval. Alternative measures of temporal precision may include the dispersion of start times, stop times, midpoints, and widths (Daniels, Watterson, et al., 2015; Gupta et al., 2019a).

An examination of temporal precision in the peak-interval procedure reveals the loose control that interval schedules of reinforcement exert over responses: it is not so tight that animals only respond at the time of reinforcement (i.e., width > zero), but also not so loose that animals respond at a constant rate through the interval (i.e., width < FI

requirement). Sanabria, Thrailkill and Killeen (2009) suggest that such loose control reflects both a limit in the precision of a timing mechanism (which keeps width > zero) and the competition between scheduled and contextual reinforcement over the control of behavior (which keeps width < FI requirement). Consistent with this hypothesis, they demonstrated in pigeons that all measures of temporal precision improve when a concurrent non-timing schedule of reinforcement is programmed. They called this preparation *timing with opportunity cost* because, with each timed (FI) response, the subject incurs the cost of potentially missing reinforcers from the concurrent schedule.

The present experiment sought two objectives. The first objective was to replicate Sanabria and colleagues' (2009) main findings in another common laboratory species: rats. The second objective was to examine the effect of reinforcer devaluation on motivation and performance in the timing-with-opportunity-cost procedure. Because devaluation affects both the FI schedule and the concurrent schedule, it was not expected to impact the competition between them over control of behavior. Therefore, all devaluation-induced changes in timing indices (start times, stop times, width, midpoint) may be more reliably interpreted as changes in the mechanism governing interval timing.

To further isolate motivational from timing effects, rats in the present experiment initiated each trial with a distinct response (nose poke). Reinforcer devaluation appears to reduce the rate of self-initiated trials in various timing procedures, leaving performance within each trial relatively intact (Daniels & Sanabria, 2017; Fox & Kyonka, 2015). Thus, this arrangement provided a positive control, showing the efficacy of the devaluation manipulation.

Methods

Subjects

Six male Sprague-Dawley rats (Charles River Laboratories, Hollister, CA) served as subjects, and were pair-housed upon arrival on approximately PND 120. Rats were housed in a 12:12 h light cycle, with lights on at 1900 h. Behavioral training and testing was always conducted in the dark phase of the light cycle. Following 1 week of acclimation to their housing, access to food was reduced daily from 24, to 18, 12, and finally 1 h/day. Food was placed in the hopper of rat home cages during the dark phase of the light cycle. During behavioral training, food was provided 30 min after the end of each training session (except during pre-feeding; see *Procedure* section), such that at the beginning of the next session weights were, on average, 75% of the mean ad libitum weighs estimated from growth charts provided by the breeder. Water was always available in home cages. All animal handling procedures in the proposed studies follow National Institutes of Health guidelines and were approved by the Arizona State University Institutional Animal Care and Use Committee.

Apparatus

All testing was conducted in 6 MED Associates (St. Albans, VT, USA) modular test chambers (305 mm long, 241 mm wide, 210 mm high). Each chamber was enclosed in a sound- and light-attenuating cabinet equipped with a ventilation fan that provides approximately 60 dB of masking noise. The front and back walls and the ceiling of the chambers were made of Plexiglas, and the front wall was hinged and served as the door

to the chamber. The two side panels were made of aluminum. The floor consisted of thin metal bars positioned above a catch pan. On the right side panel, the reinforcement port was a square opening (51-mm sides) located 15 mm above the floor and centered on the test panel. The port provided access to a dipper (MED Associates, ENV-202M-S) fitted with a cup (MED Associates, ENV 202-C) that can hold 0.01 cc of a liquid reinforcer (33% sweetened condensed milk diluted in tap water; Kroger, Cincinnati, OH). The port was furnished with a head entry detector (MED Associates, ENV-254-CB). A multiple tone generator (MED Associates, ENV-223) was used to produce 1-20 kHz tones at approximately 75 dB through a speaker (MED Associates, ENV-224 AM) centered on the top of the left side panel and 240 mm above the floor of the chamber. Two retractable levers (MED Associates, ENV-112CM) flanked the reinforcement port. Lever presses were recorded when a force of approximately 0.2 N is applied at the end of the lever. The opposite side panel was furnished with an illuminated nose-poke device (MED Associate, ENV-114BM) at the bottom panel. A house light on this side panel could dimly illuminate test chambers. Experimental events were arranged via a MED PC ® interface connected to a PC controlled by MED-PC IV ® software.

Procedure

Training sessions were conducted 7 days/week in 2-h sessions. Each session began with a 3-min acclimation period during which no manipulanda or stimuli were activated.

Pre-Experimental Training

Reinforcer Consumption Training. Prior to training on the switch-timing task, all rats were trained to consume the reinforcer (sweetened condensed milk) from the

liquid dipper in the reinforcement port. Following the 3-min acclimation period, a reinforcer was made available at the liquid dipper. All subsequent reinforcers were made available at variable intervals, with a mean inter-trial interval (ITI) of 45 s. During the ITI, no stimuli or manipulanda were activated. When a reinforcer was delivered, the house light turned on and the liquid dipper was activated. Head entries into the reinforcement port activated a 15-kHz tone. The dipper and tone were deactivated 2.5 s after the head entry. Reinforcer consumption training continued for 7 days, until rats received 100 reinforcers per session and the median time to retrieve a reinforcer was 4 s of less, which took two sessions.

Manipulandum Shaping. Following two days of reinforcer consumption training, lever-pressing and nose-poking were shaped, using a Pavlovian conditioned approach (auto-shaping) procedure. After the 3-min acclimation period, a single reinforcer was delivered, and reinforcer delivery was followed by a 7.5-s ITI. At the end of each ITI thereafter, a single manipulandum (left lever extended, right lever extended, or nose-poke device illuminated) was pseudo-randomly selected from a list, such that no manipulandum could be selected consecutively in more than six trials. Following the activation of the selected manipulandum, reinforcement was delivered (as described in reinforcer consumption training) and the manipulandum was deactivated either after a response was made on the manipulandum (lever press, nose poke), or after 8 s had elapsed, whichever happened first. This phase continued until all rats completed at least 100 trials per session, which took 5 sessions.

Lever and Nose Poke Training. The shaping procedure was modified such that reinforcement was only delivered following a single response on the active

manipulandum. This continued until all rats were reliably lever pressing and responding on the nose-poke device, completing at least 100 trials per session, which took 3 sessions.

Timing-with-Opportunity-Cost Procedure

Once rats were consistently responding to the active manipulandum, the timing-with-opportunity-cost procedure was implemented. This procedure consists of a dependent concurrent random-ratio (RR) fixed-interval (FI) schedule of reinforcement (Figure 5.2). Each component of the schedule—RR, FI—was assigned to a different lever. Following trial initiation by nose poke, the houselight turned on and both levers were extended. Either an FI-active or RR-active trial was pseudo-randomly sampled from a 16-item list such that neither trial type occurred consecutively in more than 8 times; the active schedule was not signaled.

On RR-active trials, each press on the RR lever was reinforced with probability $1/x$, where x is the RR requirement; pressing the FI lever was extinguished. On FI-active trials, the first press on the FI lever after an interval t elapsed was reinforced; pressing the RR lever was extinguished.

Experimental Training Conditions

Random-ratio (RR) Adjustment. Figure 1 depicts the sequence of training conditions that all rats underwent, starting with pre-experimental training. Across experimental training conditions t was increased from 12 to 24 to 48 s. Across the initial sessions of each condition, x was adjusted individually to each rat, increasing it progressively until it took about $2t$ on average to obtain a reinforcer on RR-active trials. The number of these RR-adjustment sessions is detailed in Table 5.1.

Baseline. Assessment of baseline stability and data analyses were restricted to presses on the FI lever during *long RR-active trials,* in which the RR schedule was active and lasted at least 2*t*, which accounted for approximately 25% of all trials. In each condition, assessment of performance stability started after 15 sessions. Stability was defined as a non-significant linear regression of peak time and IQR of responses across 5 sessions for every rat. Peak times and IQRs were determined using the mean responses on the FI lever in each 1-s bin of long RR trials in each session. Peak times were defined as the bin with maximum average responding; IQRs were calculated as the difference between the time bins in which 75% and 25% of total responses were accumulated.

In the timing-with-opportunity-cost procedure, rate of reinforcement is maximized by continually responding on the RR lever, pressing the FI lever just once at time *t*. Such strategy, however, assumes a very precise tracking of time. To the extent that time tracking is imprecise, start times (when rats switch from RR to FI) indicate the perceived proximity of *t*, whereas stop times (when rats switch from FI back to RR) indicate the perceived likelihood that *t* elapsed (Sanabria et al., 2009).

Pre-Feeding. Upon achieving stable performance with a criterion *t*, post-session feeding was discontinued and substituted with 1 h of *ad libitum* access to food immediately before each of three consecutive sessions.

FI 48-s Only. The timing-with-opportunity cost procedure was modified for 10 consecutive sessions after testing on $t = 48$ s such that all trials were FI-active trials. This constituted a "no opportunity cost" condition, allowing an assessment of the effect of opportunity cost on FI 48-s performance.

Data Analysis

Parameter Estimation

The analysis of performance in the timing-with-opportunity-cost procedure assumed that temporal control is expressed in each trial as a sequence of three response states, in which rats transition from an initial low rate of responding to a high rate of responding at a *start* time, then maintain this higher rate of responding for a period (*width*) that typically encapsulates the criterial interval *t*, and then transitions back to a low rate of responding at a *stop* time (K. Cheng & Westwood, 1993; Church et al., 1994; Sanabria et al., 2009). Start and stop times were estimated from FI responses during *long RR-active* trials—those in which the RR schedule was active and lasted at least 2*t*.

Estimates of start and stop times were obtained using the method described by Church and colleagues (1994). Briefly, in each long RR-active trial, every possible combination of two responses on the FI schedule was tested as potentially occurring at the start time (s_1, the earlier of the two responses) and at the stop time (s_2, the later of the two responses). Times s_1 and s_2 were estimated as those that maximized the expression

$$s_1(r - r_1) + (s_2 - s_1)(r_2 - r) + (v - s_2)(r - r_3), \quad (4.1)$$

where r is the response rate on the FI lever computed over the whole trial, r_1 is the (low) FI response rate computed over the interval between trial onset and s_1, r_2 is the (high) FI response rate computed over the interval between s_1 and s_2, and r_3 is the (low) FI response rate computed over the interval between s_2 and the end of the trial (v). This estimation procedure was implemented in Wolfram Systems Mathematica v12. Estimates of widths and midpoints were derived from the estimates of s_1 and s_2; namely, $width = s_2 - s_1$ and $midpoint = (s_1 + s_2)/2$.

Stable Estimates

Analyses of start, stop, midpoint times and widths were restricted to long RR-active trials from stable trials at the end of each baseline condition. For each parameter, stable trials were identified for each rat in each baseline condition by an iterative process conducted in Wolfram Systems Mathematica v12. This process started by building a dataset containing the estimates from every trial, arranged in chronological order. This dataset was then split into two blocks, each containing an equal number of estimates. A t-test for independent means was then conducted comparing these blocks. If the t-test revealed a significant difference between blocks ($p < 0.05$), about 1% of estimates were removed from the earlier part of the dataset. This split-and-compare process was repeated until the t-test failed to detect a significant difference between blocks. Estimates of central tendency and dispersion of each parameter were obtained from the remaining dataset.

Latency to Initiate Trials (LTIs)

The latency to initiate a trial (LTI) was defined as the intervals between the illumination of the nose-poke device and the subsequent trial-initiating nose poke. When the end of the session truncated this interval, the LTI was coded as greater than the truncated interval and included in the computation of median LTI. LTIs from all trials in stable sessions, including FI-active trials were calculated and included in analyses.

Effects Tested

Effects of FI requirement *(t).* The first analysis verified temporal control of behavior in baseline performance. It tested whether (a) median midpoints tracked *t*; (b) median s_1, s_2, and widths covaried with *t*; (c) CQV of midpoints, s_1, and s_2 covaried with

t. CQVs were calculated as (Q3 – Q1)/(Q3 + Q1), where Q1 and Q3 represent quartiles 1 and 3 of the parameter (Q2 is the median). The effect of *t* was also assessed on the median LTI. Because LTIs were expected to lengthen with lower rates of rate of reinforcement, and longer *t* implied lower rates of reinforcement, a positive correlation between median LTIs and *t* was expected.

Effects of Pre-Feeding on LTIs. Pre-feeding was expected to lengthen median LTIs and drastically reduce the number of trials initiated per session. These effects were assessed using 2 × 3 (pre-fed vs. not pre-fed × *t* = 12 vs. 24 vs. 48 s) Bayesian repeated-measures ANOVAs. Bayesian analyses were conducted because they reduce the impact of outliers and allow for support for hypotheses to be quantified for both null and alternative hypotheses (Dienes & Mclatchie, 2018; Keysers et al., 2020). This allowed for a model selection approach, wherein each Bayesian analyses were used to calculate Bayes Factors, a metric which characters the strength of evidence for a model relative to the null model. The log of the Bayes Factor ($LogBF_{10}$) was used, where a positive $LogBF_{10}$ value indicates support for the model (alternative hypothesis or H_1) and a negative $LogBF_{10}$ value indicates substantial evidence for the null hypothesis (H_0). $LogBF_{10} \geq 1.098$ were considered as having substantial evidence support for H_1 (Kass & Raftery, 1995; Kruschke, 2014). Selected models with evidence for H_1 (most positive $LogBF_{10}$) were probed using Bayesian one-way ANOVAs and Bayesian t-tests.

Effects of Pre-feeding on Temporal Responding. The reduction in number of trials initiated implied a reduction in the size of the sample from which to estimate central tendency and dispersion measures of timing parameters (see Table 1). Because this reduction could inflate dispersion measures (i.e., CQVs), pre-feeding effects on timing

parameters were assessed using a bootstrapping analysis conducted on Wolfram Systems Mathematica v12. For each parameter, *t* requirement, and rat, 10,000 samples were taken from the stable baseline dataset, where the size of each sample was the number of long RR-active trials that rat completed in the following pre-feeding condition. For example, if a rat completed 17 long RR-active trials in the t = 12 s condition under pre-feeding, 10,000 samples of 17 trials were drawn from the stable baseline t = 12 s datasets; parameter estimates were then drawn from those trials (i.e., 17 s_1, 17 s_2, etc., per sample). The median and CQV of each sample was then calculated, creating a bootstrap distribution of medians and CQVs for each parameter estimate. The size of the pre-feeding effect on a median or CQV of a parameter estimate was computed as the difference between the pre-feeding estimate and the corresponding mean of the bootstrap distribution, expressed in standard deviations (σ) of the bootstrap distribution (see Appendix A for a more detailed description of this procedure). Differences greater than 2σ were deemed statistically significant.

Effects of Opportunity-Cost. The removal of an opportunity cost to timing in the FI 48-s Only condition was expected to yield worse measures of temporal precision. Because reinforcement at 48 s truncated trials in this condition, the measures of temporal precision available were only the median and CQV of s_1. Stable estimates of these parameters were obtained using the split-and-compare process (see *Stable estimates* section above) from performance in the FI 48-s Only condition. These parameters were compared with those obtained from the preceding baseline t = 48 s condition using Bayesian t-tests. In the FI 48-s Only condition, median s_1 estimates were expected to be shorter and their CQV larger than in the comparable baseline condition.

Results

Figure 5.3 shows a representative set of raster plots obtained from one subject. It shows that, in the timing-with-opportunity-cost procedure, FI lever presses typically clustered around FI requirement *t*, that the widths of these clusters were roughly proportional to *t*, and that continual streams of RR lever presses flanked these clusters.

Effects of Fixed Interval (FI) Requirement (*t*)

Bayesian repeated-measures ANOVA revealed strong evidence for an effect of FI-requirement ($LogBF_{10} = 27.275$) on median midpoints (Figure 4.4a). Post-hoc testing confirmed that midpoints increased as *t* increased (smallest $LogBF_{10} = 4.414$), indicating that they tracked *t*. Start and stop times (s_1 and s_2) also covaried with *t* (Figure 4.4b,c), for which there was also strong evidence for effects of FI-requirement (smallest $LogBF_{10} = 18.731$), where s_1 and s_2 increased as *t* increased (smallest post-hoc $LogBF_{10} = 2.800$).

The relative dispersion (i.e., CQV) of midpoints (Figure 4.4e) and s_2 (Figure 4.4g) did not covary with *t* (largest $LogBF_{10} = 0.344$), but there was substantial evidence that dispersion of s_1 covaried with *t* ($LogBF_{10} = 1.661$; Figure 4.4f). Post-hoc testing indicated that relative dispersion of s_1 increased when *t* was raised from 12 to 24 s ($LogBF_{10} = 1.153$), but not when *t* was raised from 48 to 24 s ($LogBF_{10} = -0.621$). Similarly, latencies to initiate (LTIs) covaried with *t* ($LogBF_{10} = 8.501$; Figure 5) increasing when *t* was raised from 12 to 24 s ($LogBF_{10} = 4.311$), but less so when *t* was raised from 24 to 48 s ($LogBF_{10} = 0.399$).

Effects of Pre-Feeding on LTIs

Although median LTIs covaried with *t*, there was insufficient evidence for a main effect of deprivation level ($LogBF_{10} = 0.640$; Figure 4.6a). However, there was evidence

for an interaction effect of deprivation level and FI requirement *t* on median LTIs ($LogBF_{10} = 9.899$), where pre-feeding increased LTIs when $t = 12$ ($LogBF_{10} = 1.536$), but not when $t = 24$ s ($LogBF_{10} = 0.124$) and only moderately when $t = 48$-s ($LogBF_{10} = 1.084$). In contrast, there was strong evidence for an effect of pre-feeding on the number of trials initiated per session across FI requirements ($LogBF_{10} = 4.337$; Figure 6b; smallest $LogBF_{10} = 2.030$).

Effects of Pre-feeding on Temporal Responding.

Effects of deprivation level on measures of temporal precision were assessed using a bootstrapping analysis (described in *Data Analysis* and Appendix A). Table 2 lists the mean $d(\theta)$ (the difference between pre-feeding and baseline values expressed in number of standard deviations) for the median and CQV of each timing parameter, weighted by the number of trials completed by each subject in each pre-feeding condition. Figure 7 depicts individual and mean unweighted $d(\theta)$ values.

Although the analysis of weighted $d(\theta)$ suggests that pre-feeding right-shifts the start time and midpoint of the high-response state when $t = 24$ s, individual (unweighted) $d(\theta)$ values suggest a more complex effect (Figure 4.7). Individual unweighted $d(\theta)$ values indicate that although pre-feeding significantly affected central tendency and dispersion of FI lever responding for some subjects, these changes are not systematic across subjects or measures of temporal responding. Further, significant weighted mean $d(\theta)$ values appear to be driven by individual $d(\theta)$ values from subjects who completed a relatively high number of trials under pre-feeding.

In order to further characterize this relationship, a set of post-hoc Bayesian Pearson's correlations were conducted to assess the relationship between the number of

trials completed under pre-feeding and individual $d(\theta)$ values. Indeed, there was evidence for a positive relationship between the number of trials completed during pre-feeding and individual $d(\theta)$ for median start times (Figure 4.8a – c) when t = 12 s (R = .850; LogBF_{10} = 1.153), t = 24 s (R = .996; LogBF_{10} = 4.556), and t = 48 s (R = 0.704; LogBF_{10} = .116), median stop times (Figure 8d – f) when t = 24 s (R = .801; LogBF_{10} = .168), and median midpoints when t = 24 s (R = .984; LogBF_{10} = 1.291) and t = 48 s (R = .817; LogBF_{10} = .553). These positive correlations suggest that the subjects that produced more trials under pre-feeding also displayed a stronger pre-feeding-induced right-shift in the time during which the FI lever was engaged. Analyses also revealed a strong negative correlation between number of pre-feeding trial and $d(\theta)$ of midpoint CQVs when t = 48 s (R = -.854). This negative correlation suggests a pre-feeding-induced enhancement of temporal precision in subjects that completed more trials under pre-feeding.

Effects of Opportunity-Cost

When the RR component was removed following the t = 48 s condition, analysis revealed strong evidence that this loss of opportunity-cost induced earlier median s_1 (LogBF_{10} = 9.14), but did not affect their CQV (LogBF_{10} = -0.660; Figure 4.9).

Discussion

A timing-with-opportunity-cost task (Sanabria et al., 2009) was implemented in rats. In its present implementation, this task involved a response-initiated dependent concurrent schedule of reinforcement with two components, RR and FI. Timing performance was assessed primarily in the FI component across three requirements (t = 12, 24, 48 s) and only when it was inactive (*long RR-active* trials). This task was thus like the peak-interval procedure (Fuat Balci et al., 2009; Matell et al., 2006; Roberts, 1981),

but trials were self-paced and timing involved an opportunity cost—the cost of missing potential RR reinforcers.

On each long RR-active trial, rats generally responded first on the RR component, then abruptly switched over to the FI component before *t* elapsed (start time, or s_1), and finally switched back to the RR component after *t* elapsed (stop time, or s_2, Figure 3). This pattern tracked *t*, with the midpoint of the pattern [(s_1 + s_2)/2] falling close to *t* (Figure 4a), dwelling times in the FI component (*widths*) proportional to *t* (Figure 4d), and the relative dispersion (CQVs) of most performance parameters remaining approximately constant over *t* (Figure 4e-h). These results are consistent with typical findings in the peak-interval procedure, showing the scalar invariance of timing precision (Gibbon, 1977; Oprisan & Buhusi, 2014; Simen et al., 2013).

It is, nonetheless, somewhat surprising that, despite the large changes in RR requirement over *t* (from RR 65 to RR 235; Table 1), the largest deviation from scalar invariance was a slight increase in the CQV of s_1 when *t* was raised from 12 to 24 s (Figure 4f). This robustness of scalar invariance was confirmed in the Only FI 48-s condition. The removal of opportunity cost (i.e., of the RR component) in this condition had no substantial impact on the CQV of s_1 (Figure 9b). This is inconsistent with previous findings in pigeons, in which the removal of opportunity cost yielded less precise timing (Sanabria et al., 2009). The provenance of this discrepancy is unclear; we can only point out three differences between the Sanabria et al. (2009) study and the present study that may account for it: the species (pigeons vs. rats), the pacing of trials (externally- vs. self-paced), and the FI requirement *t* (15 vs. 12-48 s).

Nonetheless, the removal of opportunity cost influenced when rats sought food in the FI component (s_1)—it did not increase its relative variability, but it prompted rats to seek food earlier (Figure 9). This effect is consistent with previous finding in pigeons, and with the notion that schedule performance reflects the competition between scheduled and contextual reinforcement over the control of behavior (Hernnstein, 1970; Sanabria et al., 2009).

Prior research suggests that the efficacy of reinforcement of a pattern of responding is reflected in the rate at which the pattern is initiated (Brackney et al., 2011, 2017; Daniels & Sanabria, 2017, 2019; Mazur et al., 2014; Rojas-Leguizamón et al., 2018). Two findings from the present study support this hypothesis. First, as *t* increased (and rate of reinforcement declined) the latency to initiate trials (LTIs) also increased (Figure 5). Second, pre-feeding reduced the number of trials initiated regardless of *t* (Figure 6b).

Because pre-feeding devalued FI and RR reinforcement equally, it was not expected to affect their competition over control of behavior. Pre-feeding-induced changes in performance, therefore, may be interpreted as reflecting changes in timing mechanisms. Unexpectedly, results suggest individual-subject variability in the sensitivity of timing mechanisms to deprivation state.

For most subjects, pre-feeding significantly reduced the number of trials completed (Figure 6b), but left performance within each trial relatively intact (Figure 7). This effect is consistent with the notion, drawn from behavioral systems theory (Timberlake, 2001) that trial self-pacing protects timing performance from motivational fluctuations (Daniels & Sanabria, 2019). However, some subjects showed the opposite

pattern: pre-feeding delayed and widened the intervals spent on the FI component (see data points above +2 significance threshold in Figures 7 and 8) but only slightly reduced the number of trials completed. This latter effect is inconsistent with the expected protective effects of trial self-pacing, but is consistent with the notion that reduced arousal slows down the internal clock (Galtress et al., 2012; Killeen & Fetterman, 1988; Ludvig et al., 2007).

Given the small number of subjects tested, it would be premature to draw strong conclusions regarding individual differences in the sensitivity of interval timing on motivational variables. However, it is not unreasonable to speculate that, for some subjects, trial initiation may fall under habitual action control and display robustness to changes in motivation, akin to the resistance of habitual sign-tracking to reinforcer devaluation (Keefer et al., 2020; Morrison et al., 2015). In these subjects, reinforcer devaluation may instead influence the temporally-entrained responses that are more proximal to reinforcement (Smedley & Smith, 2018).

In summary, the implementation of the timing-with-opportunity-cost procedure in rats replicated (a) typical findings in the peak-interval procedure, and (b) key findings from implementing the timing-with-opportunity-cost procedure in pigeons (Sanabria et al., 2009). These replications support the notion that timing performance is not only a function of the operation of a timing mechanism, but also reflects a competition between scheduled and contextual reinforcement for such control. Isolating these sources of variance in timing performance requires controlling contextual rates of reinforcement, which is rarely done. Such exercise, implemented in the present study, suggests that reducing motivation slows down the internal clock in some subjects, but selectively

affects the frequency of trial initiation in others. The mechanisms that govern the interaction between motivational and timing processes thus appear to vary at the individual subject level. Further research is needed to characterize biological and procedural factors that may predict these differences.

CHAPTER 5

WHAT TIME SHOULD WE START? DORSAL HIPPOCAMPUS INACTIVATION DELAYS ENTRAINMENT OF TEMPORAL RESPONDING

Introduction

A key insight from timing research is that well-defined biological mechanisms govern the timing of behavior in the sub-second range (Buhusi & Meck, 2005; Fraisse, 1929; Hinton & Meck, 1997) and in the circadian range (Oprisan, Buhusi, et al., 2018; Reppert & Weaver, 2002). Intermediate to these ranges are intervals of enormous psychological interest: seconds, minutes, and perhaps a few hours. This is the temporal range within which key behaviors unfold, from conversations to foraging. Relative to other ranges, the biological mechanisms that support timing in the seconds-to-minutes range (*interval timing*) are still poorly understood. Nonetheless, research has unveiled important behavioral regularities in interval timing. An early and particularly critical finding is that the temporal dispersion of reinforced behavior is proportional to the interval separating reinforcers. This regularity, known as *scalar invariance*, is the expression of Weber's law in the temporal dimension, and has motivated a substantial amount of research (Oprisan & Buhusi, 2014; Simen et al., 2013).

Scalar invariance suggests that a single behavioral mechanism—and its biological substrate—governs interval timing. However, various findings support the notion that distinct mechanisms support behavioral control by shorter and longer times within the interval timing range. Whereas control by shorter intervals appears to depend on the activity of cortico-striatal circuits, increasing evidence suggests that control by longer intervals requires a functional hippocampus (HPC; Gupta et al., 2019; Lee et al., 2020;

Mau et al., 2018; Palombo et al., 2016; Palombo & Verfaellie, 2017). Notably, Patient HM, whose HPC was ablated to treat intractable seizures, accurately timed short intervals, but underestimated those longer than 40 s (Richards, 1973). Additionally, several studies have shown that inactivation of the dorsal HPC (dHPC), in particular, increase the prevalence of premature responses when timing long intervals (Jackson et al., 1998; Yin & Meck, 2014).

The dependency of hippocampal effects on interval length is reflected in a range of experimental data. Sabariego et al. (2020) showed, for instance, that dHPC lesions reduced temporal discrimination performance (10 vs. 20 s) to chance level, but although lesioned rats gradually improved their performance on 10-s trials, performance on 20-s trials did not rise above chance. Patients with medial temporal lobe damage are impaired in temporal estimation for long, but not short intervals (Palombo et al., 2016). Our lab has also demonstrated that dHPC lesions induce premature food-seeking responses, but whereas this effect is transient when food is scheduled after 16 s, it appears to be more long-lasting when food is scheduled after 32 s (Gupta et al., 2019).

Evaluating this length-dependent effect, however, is complicated by the observable link between timing behavior and motivation (Galtress et al., 2012). Because timing tasks inherently impose a delay to reward, increasingly long intervals can induce schedule strain and reduce motivation to engage in the task, indexed by decreased frequency of trial initiation and increased latency to initiate (LTI) trials (Daniels & Sanabria, 2017; Fox & Kyonka, 2015). In response-initiated timing tasks, LTIs are selectively sensitive to motivational manipulations (Daniels & Sanabria, 2019; Mazur et al., 2014; Rojas-Leguizamón et al., 2018; Watterson et al., 2015), including rate of

reinforcement, which necessarily declines when timing longer intervals (Holter et al., 2019). Because HPC suppresses task-irrelevant motivations and enhance motor readiness (Jarrard, 1993; Tracy et al., 2001), its inactivation may interfere with motivation to engage in a reinforced behavior. Additionally, observed responses in timing tasks appear to be controlled by a mixture of timing and non-timing processes (Daniels, Fox, et al., 2015; Daniels et al., 2018; Watterson et al., 2015). The proportion of non-timed responses increases as a function of interval requirement (Daniels, Fox, et al., 2015; Watterson et al., 2015), indicating that an increase in the to-be-timed interval brings with it a decrease in schedule control. It is thus possible that the engagement of the HPC in long-interval timing depends on non-timing factors such as the background richness of reinforcement or changes in motivation which accompany an increase in interval length. Although evidence points at HPC involvement in interval timing, the involvement of other non-timing processes in mediating this involvement has not been ruled out. Further, the investigation of the HPC involvement in interval timing has largely focused on steady-state temporal responding (Lee et al., 2020; Meck et al., 2013; Palombo et al., 2016; Yin & Troger, 2011), rather than on the encoding of novel temporal intervals, for which dHPC appears to be particularly critical (Gupta et al., 2019; Jackson et al., 1998; Tam et al., 2013).

It is thus possible that HPC lesion and inactivation effects on long-interval timing have been misinterpreted: they may reflect the involvement of HPC function not in tracking long intervals, but in engaging a timing task with a relatively low payoff. The current study aims at assessing the role of dHPC in acquisition of novel temporal intervals, while isolating and tracking motivational effects.

Daniels and Sanabria (2017) demonstrated that some operant responses in fixed-interval (FI) schedules of reinforcement are under temporal (i.e., schedule) control, but many others are not. They also showed that reducing motivation (via pre-feeding) increases the prevalence and flattens the temporal distribution of non-timing responses. In a follow-up study, Daniels and Sanabria (2019) demonstrated that motivational effects can be isolated and tracked if subjects are required to initiate each FI trial with a topographically distinct response. The introduction of this requirement makes schedule performance robust to motivational manipulations, which are now reflected in the latency to initiate trials. The first step, then, to control for potential motivational effects is to require subjects to initiate trials, a requirement that is absent so far in studies that examine the role of HPC in long-interval timing.

Incorporating self-paced trials in a long-interval timing task is particularly challenging, because reinforcement of trial-initiating responses is necessarily delayed, so subjects may stop initiating trials altogether. Sanabria et al. (2009) introduced a procedure that may circumvent this challenge. In this procedure, the FI schedule is programmed concurrently with a random-ratio RR schedule, in which each response has a constant low probability of reinforcement. Because the RR reinforcers may be more immediate than FI reinforcers, the former may maintain trial initiation when the latter alone cannot.

A concurrent RR schedule has another important utility in timing tasks. Sanabria and colleagues (2009) demonstrated that, on richer RR trials, pigeons start responding on the FI component later and at more consistent times, and stop responding on the FI component earlier and also at more consistent times. In other words, timing performance

improved as alternative activities were reinforced at a higher rate. This implies that indices of timing precision and accuracy such as start and stop times are sensitive to the value of alternative sources of reinforcement. Thus, in the absence of a concurrent schedule of reinforcement, the effect of HPC lesions and inactivation may reflect changes in the relative efficacy of implicit sources of reinforcement, such as resting, grooming, and exploratory behavior. By implementing a concurrent schedule of reinforcement and defining start and stop times in terms of changeovers between components, these measures of precision and accuracy are effectively isolated from changes in the relative efficacy of alternative sources of reinforcement.

The current study assessed acquisition of novel temporal intervals under dHPC inactivation, using the response-initiated *timing-with-opportunity-cost* task, which facilitates procedural dissociation of motivation and timing, ad also provides a concurrent, non-timed, source of reinforcement. Rats were first trained on a self-paced concurrent *variable*-interval (VI) RR schedule of reinforcement, with mean VI requirement of 24 s. Once performance stabilized, baclofen/muscimol (GABA A/B agonist cocktail that induces neuronal inactivation) or vehicle was administered in the dHPC of all rats while performing pre-treatment training, except that the VI component was be substituted with either a FI 12-s schedule (short-interval condition) or a FI 48-s schedule (long-interval condition). Acquisition was then assessed by characterizing changes in parameters of temporal responding across testing.

Methods

Subjects

Thirty-eight Long-Evans rats (18 female, 20 male) bred at Arizona State University served as subjects. Rats were single-housed on post-natal day (PND) 60 on a 12:12 h light cycle, with dawn at 1900 h. All behavioral training and treatments were conducted during the dark phase of the light cycle. Training and food restriction were implemented shortly after single-housing. Access to food was reduced daily from 24, to 18, 12, and finally 1 h/day. Food was placed in home cages of rats during the dark phase of the light. During behavioral training, food was provided 30 min after the end of each training session, such that at the beginning of the next session weights were, on average, 75% of the mean ad libitum weights estimated from growth data collected during the breeding process. During dHPC guide cannula surgery and recovery, rats were taken off of food restriction, which was gradually reintroduced over the last 4 days of recovery. Water was always available in home cages. All animal handling, training, and surgical procedures in this study followed National Institutes of Health guidelines and were approved by the Arizona State University Institutional Animal Care and Use Committee.

Apparatus

All testing was conducted in 6 MED Associates (St. Albans, VT, USA) modular test chambers (305mm long, 241 mm wide, 210 mm high). Each chamber was enclosed in a sound- and light-attenuating cabinet equipped with a ventilation fan that provides approximately 60 dB of masking noise. The front and back walls and the ceiling of the chambers were made of Plexiglas, and the front wall was hinged and served as the door to the chamber. The two side panels were made of aluminum. The floor consisted of thin

metal bars positioned above a catch pan. On the right-side panel, the reinforcement port was a square opening (51-mm sides) located 15 mm above the floor and centered on the test panel. The port provided access to a dipper (MED Associates, ENV-202M-S) fitted with a cup (MED Associates, ENV 202-C) that can hold 0.01 cc of a liquid reinforcer (33% sweetened condensed milk diluted in tap water; Kroger, Cincinnati, OH). The port was furnished with a head entry detector (MED Associates, ENV-254-CB). A multiple tone generator (MED Associates, ENV-223) was used to produce 1- to 20-kHz tones at approximately 75 dB through a speaker (MED Associates, ENV-224 AM) centered on the top of the left side panel and 240 mm above the floor of the chamber. Two retractable levers (MED Associates, ENV-112CM) flanked the reinforcement port. Lever presses were recorded when a force of approximately 0.2 N is applied at the end of the lever. The opposite side panel was furnished with an illuminated nose-poke device (MED Associate, ENV-114BM) at the bottom panel. A house light on this side panel could dimly illuminate test chambers. Experimental events were arranged via a MED PC ® interface connected to a PC controlled by MED-PC IV ® software.

Procedure

All sessions were conducted 6 days/week for 90 min each, unless noted otherwise. All sessions began with a 3-min warmup period, during which no manipulanda or stimuli were active.

Pretraining

Reinforcer Consumption Training. Prior to training on the switch-timing task, all rats were trained to consume the reinforcer (sweetened condensed milk) from the liquid dipper in the reinforcement port. Following the 3-min acclimation period, a reinforcer

was made available at the liquid dipper. All subsequent reinforcers were made available at variable intervals, with a mean inter-trial interval (ITI) of 45 s. During the ITI, no stimuli or manipulanda were activated. When a reinforcer was delivered, the house light turned on and the liquid dipper was activated. Head entries into the reinforcement port activated a 15-kHz tone. The dipper and tone were deactivated 2.5 s after the head entry. Reinforcer consumption training continued for 7 days, until rats retrieved at least 100 reinforcers per session and the median time of retrieval was 4 s of less, which took two sessions.

Manipulandum Shaping. Lever-pressing and nose-poking were shaped, using a Pavlovian conditioned approach (auto-shaping) procedure. After the 3-min acclimation period, a single reinforcer was delivered, and reinforcer delivery was followed by a 7.5-s ITI. At the end of each ITI thereafter, a single manipulandum (left lever extended, right lever extended, or nose-poke device illuminated) was pseudo-randomly selected from a list, such that no manipulandum could be selected consecutively in more than six trials. Following the activation of the selected manipulandum, reinforcement was delivered (as described in reinforcer consumption training) and the manipulandum was deactivated either after a response was made on the manipulandum (lever press, nose poke), or after 8 s had elapsed, whichever happened first. This phase continued until all rats responded on the active manipulandum per session, which took 5 sessions.

Lever and Nose Poke Training. The lever and nose poke shaping procedure was modified such that reinforcement was only delivered following a single response on the active manipulandum, as described. This continued until all rats were reliably lever

pressing and responding on the nose-poke device and were completing at least 100 trials per session, which took 3 sessions.

Baseline Training

Concurrent Random-Ratio (RR) Variable-Interval (VI) Training. An overview of the experimental design is displayed in Figure 1. Once rats were consistently responding to the active manipulandum, they were trained on a concurrent RR VI 24 s schedule of reinforcement (*baseline training*), as pretraining for the terminal concurrent RR FI testing (see Sanabria et al., 2009). The RR and VI components of the schedule were each assigned to a different lever. Following trial initiation by nose-poke, the houselight turned on and both levers were extended. Either a VI-active or RR-active trial was pseudo-randomly sampled from a 16-item list such that neither trial type occurred consecutively in more than 8 trials; the active schedule was not signaled.

On RR-active trials, each press on the RR lever was reinforced with probability 1/*x*, where *x* is the RR requirement, and pressing the VI lever was extinguished. On VI-active trials, the first press on the VI lever after an interval of average length *t*, which was randomly sampled without replacement from a 12-item Fleshler-Hoffman list (Fleshler & Hoffman, 1962) elapsed; presses on the RR lever were extinguished. Mean interval *t* was set to 24 s for all rats during this phase of training. Across baseline sessions, *x* was adjusted individually and progressively until the median RR-active trial length was approximately 96 s, which took 22 sessions. Because of between-subject variability in response rate, the terminal *x* requirements ranged between 112 and 125 responses.

Dorsal Hippocampus Guide Cannula Surgery and Infusions

Following baseline training, rats underwent stereotaxic surgery to implant bilateral guide cannulae at dHPC (AP -3.5; ML +/-3; DV 2.8). Under aseptic conditions, rats were anesthetized using isoflurane and placed in a stereotaxic frame with non-puncture ear bars. Sterile lubricant was applied to the eyes, a 1-cm circular incision was made, and the skin and tissue were excised. The head was leveled, and cannula coordinates were measured and marked with a permanent marker. Holes for three anchoring screws (two in front of cannula coordinates and one behind) were drilled and screws were placed. Holes for the cannula were then drilled according to the markings, and the bilateral stainless steel guide cannulas (23 gauge, 4 mm with 1-mm projection; In Vivo Preclinical, Roanoke, VA) were lowered, aiming at dHPC (2.8 mm DV). Implanted cannulas and screws were then cemented to the skull. Dummy cannulas (1-mm projection) were inserted into the implanted cannulas to maintain patency. Figure 2 depicts cannula placement coordinates as well as representative placement images.

Testing

Intracranial Infusions and Test (Timing-with-Opportunity Cost) Procedure. Following 10 days of surgical recovery, each rat was assigned to one of four groups, which varied on two levels: FI Length (Short: 12 s, Long: 48 s) and drug treatment [Vehicle (VEH), Baclofen-Muscimol (BM)]. Groups were assigned such that the mean number of trials completed across the last five baseline sessions, mean ratio of RR to VI responses, and mean time to first response on the VI lever were approximately equal between groups.

One hour prior to testing, rats received intracranial infusions of baclofen-muscimol (0.1/1.0 mM, respectively) or vehicle (phosphate buffered saline). A volume of 0.5 µl was infused on each side of the bilateral cannula, one side at a time, in awake rats at a rate of 0.25 µl/min using a microsyringe pump (Harvard Apparatus 11 Plus model, Holliston, MA), using 30-gauge injectors that extended 1 mm past the end of the guide cannula. After the infusion was complete, injectors were left in place for 1 min to allow for drug diffusion. Rats were then returned to their home cages for a 30-min period. Rats were then tested in the response-initiated *timing-with-opportunity cost* (Sanabria et al., 2009) procedure, to assess temporal entrainment of behavior. This procedure was similar to the baseline training procedure, except that *t* was a fixed interval (FI), either 12 s or 48 s. Infusions and timing-with-opportunity cost testing proceeded for 7 sessions.

One rat died during surgical recovery, prior to the start of timing-with-opportunity cost testing, and eight additional rats died during the course of testing. Data from these rats were included in analyses insofar as inclusion criteria were met (described in Data Analysis).

Tissue Collection

Following timing-with-opportunity cost testing, a subset of 9 subjects were euthanized using isoflurane and rapidly decapitated. Their brains were extracted and frozen, and later sliced using a cryostat (50 µm thickness), and stained (cresyl violet) to confirm cannula tip placements

Data Analysis

Inclusion Criteria

Unless otherwise stated, assessment of temporal responding was restricted to presses on the FI lever during *long RR-active trials*, in which the RR schedule was active and lasted at least twice the length of the assigned FI (2*t*). Distribution of the number of long RR-active trials is displayed in Figure 3**.** Rats who completed fewer than 20 long RR-active trials across the 7 testing sessions were excluded from analysis, such that final sample sizes in each group were VEH – 12 s, n = 7, VEH – 48 s, n = 5, BM – 12 s, n = 5, and BM – 48 s, n = 6.

Measures of Temporal Responding

The analysis of test performance assumed that temporal control is expressed in each trial as a sequence of three response states, in which rats transition from an initial low rate of responding to a high rate of responding at a *start* time, then maintain this higher rate of responding for a period (*width*) that typically encapsulates the criterial interval *t*, and then transitions back to a low rate of responding at a *stop* time (K. Cheng & Westwood, 1993; Church et al., 1994; Sanabria et al., 2009). Start and stop times were estimated from FI responses during long RR-active trials pooled across all 7 session testing sessions.

Estimates of start and stop times were obtained using the method described by Church and colleagues (1994). Briefly, in each long RR-active trial, every possible combination of two responses on the FI schedule was tested as potentially occurring at the start time (s_1, the earlier of the two responses) and at the stop time (s_2, the later of the two responses). Times s_1 and s_2 were estimated as those that maximized the expression

$$s_1(r - r_1) + (s_2 - s_1)(r_2 - r) + (d - s_2)(r - r_3), \quad (5.1)$$

where r is the response rate computed over the whole trial, r_1 is the (low) response rate computed over the interval between trial onset and s_1, r_2 is the (high) response rate computed over the interval between s_1 and s_2, and r_3 is the (low) response rate computed over the interval between s_2 and the end of the trial (d). This estimation procedure was implemented in Wolfram Systems Mathematica v12. Estimates of widths and midpoints were derived from the estimates of s_1 and s_2; namely, *width* = $s_2 - s_1$ and *midpoint* = $(s_1 + s_2)/2$.

Latency to Initiate Trials (LTIs)

The latency to initiate a trial (LTI) was defined as the intervals between the illumination of the nose-poke device and the subsequent trial-initiating nose poke. When the end of the session truncated this interval, the LTI was coded as greater than the truncated interval and included in the computation of median LTI. LTIs from all trial types, including FI trials, were calculated and included in analysis.

Estimation of Acquisition Parameters

Because baseline training did not entrain behavior to a specific interval, entrainment of temporal responding to the FI was assessed by analyzing the change in the central tendency and dispersion of timing parameters (s_1, s_2, midpoint, width) across trials. More specifically, on each trial n, each timing parameter θ was assumed to be sampled from a distribution whose mean [$\mu_\theta(n)$] and variance [$\sigma^2_\theta(n)$] varied between

trials. Estimates of $\mu_\theta(n)$ and $\sigma^2_\theta(n)$ were obtained from successive pairs of long-RR trials (n and n+1)[1]:

$$\mu_\theta(n) = [\theta(n) + \theta(n+1)] / 2, \quad (5.2)$$

$$\sigma^2_\theta(n) = [\theta(n) - \theta(n+1)]^2 / 2. \quad (5.3)$$

Acquisition of temporal responding was assessed by fitting a generalized logistic function (Richard's Curve; Richards, 1959) to start times, stop times, midpoints, and widths. The function was of the form

$$y\,(\theta, n) = b_\theta + \frac{a_\theta}{\left(1+T_\theta e^{-k_\theta(n-m_\theta)}\right)^{1/T_\theta}}, \quad (5.4)$$

where $y(n,\theta)$ is the statistic (μ or σ^2) of the distribution of a timing parameter θ sampled on trial n, b_θ is the left asymptote of the function, $a_\theta + b_\theta$ is the right asymptote of the function, k_θ is the growth rate, T_θ fixes the point of inflection (m_θ), which is the trial in which maximum change occurs. A generalized logistic was chosen in place of a standard 4-parameter logistic function because it more readily accounts for the variable shape of acquisition curves (Gallistel et al., 2004), and because its parameters facilitate analysis of both the rate and time of acquisition. Functions were fit using maximum likelihood estimation implemented in Wolfram Systems Mathematica. In order to assess

[1]To identify a successive pair of long-RR trials, other types of trials and inter-session intervals were ignored. Estimates of $\mu_\theta(n)$ and $\sigma^2_\theta(n)$ were not obtained for the last long-RR trial.

changes in motivation across testing, the generalized logistic function was also fit to LTIs as a function of trial. A 2 × 2 (dHPC inactivation: BM vs.VEH × FI Length: 12 vs. 48 s) Bayesian ANOVA was conducted on higher-order temporal acquisition parameters (k_θ, m_θ, a_θ, b_θ, T_θ) of $s1$, $s2$, midpoints, and widths, as well as those obtained from LTIs.

Results

Bayesian ANOVA on higher-order temporal acquisition parameters obtained from midpoints revealed strong evidence for an effect of FI ($LogBF_{10} = 1.345$), where the right asymptote ($a + b$) of midpoints was higher when tested on FI 48-s than when tested on FI 12-s, the high-response state was later for rats tested on the longer schedule. Analysis of parameters obtained from logistic fits to LTIs revealed strong evidence for an effect of FI on right asymptotes of LTIs ($LogBF_{10} = 1.209$) and substantial evidence for an effect of FI on left asymptotes of LTIs ($LogBF_{10} = 0.967$), where both parameters were increased for rats tested on FI 48 s thaLin for those tested on FI 12 s.

Higher-order temporal acquisition parameters of start and stop times are detailed in Table 1, and predicted vs. obtained start times are depicted in Figure 5.4. In general, start times increased across trials (see estimates of k_{START} in Table 1), and Bayesian ANOVA revealed strong evidence ($LogBF_{10} = 1.821$) for an effect of dHPC inactivation on their inflection point (m_{START}), where m_{START} was increased for BM rats compared to VEH rats, which suggests a dHPC inactivation-induced delay in the temporal entrainment of start times on the FI lever (Table 1; Figure 5a). Figure 5b illustrates the expression of changes m_{START} on the generalized logistic function.

Discussion

The function of the dHPC in entraining behavior to periodic reinforcement was studied by implementing a response-initiated version of the timing-with-opportunity-cost task in rats (Sanabria et al., 2009). All subjects were initially trained on a concurrent RR VI 24 s schedule of reinforcement, such that their behavior did not entrain to a specific interval. Following stable performance, all rats were implanted with bilateral guide cannulas in dHPC. Initial temporal entrainment of behavior to either a 12 or 48 s interval was then assessed in a timing-with-opportunity-cost task (concurrent RR FI schedule) following daily intracranial administration of vehicle (VEH) or baclofen-muscimol (BM).

In the present experiment, the effect of dHPC inactivation on initial temporal entrainment of behavior was assessed by characterizing the dynamics of the transition from baseline training on a VI schedule to testing on a FI schedule. Timing studies in rats have demonstrated that novel target durations can be encoded after just a few trials (Lejeune et al., 1997; Sanabria & Oldenburg, 2014), and that, in acquisition of peak responding, the start time of the high-response rate is the first component of the peak function to garner temporal control (Balci et al., 2009). Further, the onset of this control appears to be abrupt, rather than gradual (Balci et al., 2009; MacDonald et al., 2012). Although the present study utilized a baseline VI schedule rather than a baseline FI schedule, some of these dynamics were still observed. In general, start times on the FI lever increased as a function of trial, as indexed by estimates of a and $a + b$ (Table 1).

In RR FI performance, dHPC inactivation did not systematically affect the asymptotes or slope of measures of temporal responding (start times, stop times, midpoints, and widths) across trials. Rather, dHPC inactivation selectively delayed the

trial at which maximum change in start time occurred (m_{START}; Figure 4), indicating that temporal entrainment of the initiation of responding was impaired. Importantly, the observed variability in cannula placements (Figure 5.2) did not appear to systematically alter the direction of the observed effect of dHPC inactivation on inflection points. For example, one subject with cannula placement near the dentate gyrus (Figure 5.2; magenta circles; BM group) had an m_{START} inflection point of 5.45, close to the group mean.

This selective effect of dHPC inactivation on temporal entrainment of start times reflects an established dissociation between the neurobiological substrates of the initiation and termination of timed responding (K. Cheng & Westwood, 1993; MacDonald et al., 2012; K. M. Taylor et al., 2007; Yin & Meck, 2014) and aligns with previous findings from our laboratory (Gupta et al., 2019), where dHPC lesions resulted in earlier start times when encoding a novel long interval, while leaving stop (i.e., departure) times relatively unaffected.

Implementation of the timing-with-opportunity-cost task also serves to further affirm that dHPC involvement in temporal responding is timing-specific. Importantly, delayed entrainment of start times in following dHPC inactivation was observed in the absence of an effect of dHPC inactivation on motivation to engage in timing across testing (LTIs). Additionally, presence of the concurrent RR schedule, which has been shown to focus temporal responding closer to the time of reinforcement (Sanabria et al., 2009), reduces the likelihood that the effect of dHPC inactivation on start times reflects changes in the behaviors not under procedural control (grooming, resting, etc.)

Post-hoc analysis of the effect of dHPC inactivation and FI length on temporal regulation of start times.

In order to characterize the observed increase in inflection points of start times in terms of the sub-processes nested within interval timing, inferences on the effect of dHPC inactivation on temporal entrainment of start times were based on a modified pacemaker-accumulator model of interval timing (Gibbon, 1977; Simen et al., 2013), in which timed responses are modeled as a weighted mixture of time (gamma-distributed) and non-timed (exponentially-distributed) responses (Daniels & Sanabria, 2017). The parameters of the pacemaker-accumulator model are the mean inter-pulse interval (c; its reciprocal, $1/c$, is the mean speed of the internal clock), a response threshold, and a reference memory of pulse count in the interval (Daniels & Sanabria, 2017; Daniels et al., 2015). The product of the threshold and memory constitute the criterion pulse count for responding (ε).

Performance in interval timing tasks, however, is often contaminated by responses that are not governed by the time of reinforcement (Daniels, Fox, et al., 2015; Daniels, Watterson, et al., 2015; Sanabria & Killeen, 2008). To account for this noisy performance, the pacemaker-accumulator model is amended such that, with probability q, rats enter a timing state in which initiation of responding is sensitive to time, and, with complementary probability $1 - q$, rats enter a non-timing state in which temporal judgments are emitted randomly. Whereas the timing state produces gamma-distributed judgements, the non-timing state produces exponentially-distributed start times with mean K. Thus, according to this model, start times on the FI lever in the timing-with-opportunity cost are distributed according to the function:

$$P(\text{start time} \leq \tau) = q\,\Gamma(\tau; \varepsilon; c) + (1 - q)(1 - e^{-\tau/K}), \quad (5.5)$$

where Γ is a gamma cumulative distribution with shape parameter ε and scale parameter *c*.

Parameters of Equation 5 were estimated for each individual rat by fitting it to the start times from all long RR-active trials, pooled across all 7 testing sessions. All models were fit using maximum likelihood estimation in Wolfram Systems Mathematica.

Effects of dHPC inactivation and assigned FI length on temporal start times was then assessed by analyzing estimated parameters (q, τ, ε, c, K) from Equation 5 to start times using 2 × 2 (dHPC inactivation: BM vs.VEH × FI Length: 12 vs. 48 s) Bayesian ANOVAs. Parameters were log-transformed prior to analysis. Analysis of parameters of the temporal regulation model fit to start times revealed moderate evidence for an interaction of FI and dHPC inactivation ($LogBF_{10} = 0.749$) on ε of start times (Figure 6), where BM reduced the criterion pulse count ε in the FI 48-s test ($LogBF_{10} = 0.679$), but not in the FI 12-s test ($LogBF_{10} = -0.459$).

Because the criterion pulse count is the product of the response threshold and pulse-count memory, its reduction may reflect a lower threshold for responding or a biased memory that favors the recollection of shorter intervals. Interpretation of this effect is also limited by the dynamic nature of the acquisition data collected in the current experiment. Nonetheless, the delayed adjustment of start times (Figure 6) combined with the reduced criterion pulse count, suggest that dHPC inactivation delayed the upward adjustment of either the response threshold or of the start times encoded in memory.

Meck and colleagues (1984) suggest that initial adjustments in measures of temporal responding during acquisition of novel reinforcement times reflect changes in response threshold, prior to any change in the temporal criterion itself. Because data in

the current experiment captured only initial temporal acquisition, this account suggests that dHPC inactivation delays the adjustment of the response threshold to a novel interval. However, a threshold-driven effect would predict a similar effect of dHPC inactivation on stop times (Fuat Balci et al., 2009; MacDonald et al., 2012; Yin & Meck, 2014). Accordingly, results more closely support a memory-based account, where dHPC inactivation delayed the process of updating memory with higher pulse counts when the FI was introduced, expressed behaviorally as a deferral of start time adjustment. Additionally, whereas underestimation of timed intervals following HPC lesion in transitions from a short FI schedule to a longer FI schedule may reflect proactive interference from the criterion value in a previous condition (Gupta et al., 2019; Lejeune et al., 1997; Meck et al., 1984), start times in the current experiment reflect changes into temporal entrainment from non-temporally-contingent responding, and are thus unlikely to be under control of the previously trained VI schedule.

It is particularly notable that the effect of dHPC inactivation on the shape of the gamma distribution was specific to rats tested on the longer (FI 48 s) schedule. Although entrainment of behavior to long temporal intervals is concomitant with changes in non-temporal HPC-mediated functions, including reward value and reduced task-related motivation (Tracy et al., 2001; Wagner et al., 2014), we observed no effect of dHPC inactivation on motivation to engage in timing, and this effect is thus more likely to reflect timing processes. A model of topological organization of temporal information in HPC (Oprisan, Aft, et al., 2018; Oprisan, Buhusi, et al., 2018) suggests that the long-interval specificity is driven by the distribution of duration representations in HPC when criterion times are learned, where shorter and longer durations garner greater

representation in vHPC and dHPC, respectively. Additionally, the pacemaker accumulator model posits that a longer interval would require more trials to update memory with accurate pulse counts (Simen et al., 2013). Thus, the expression of reduced criterion pulse count as delayed inflection of start times suggests that, for rats tested on the longer schedule, dHPC inactivation induced a reduced (or slowed) capacity to encode accurate pulse counts in memory.

There are important caveats to the proposed interpretation of the data. First, although there is robust statistical support for the effect of dHPC inactivation on inflection point of start times, testing data were only collected over seven sessions, and thus, did not capture the entire progression to stable FI responding. Analyses were also limited to long RR-active trials, which comprise only about 25% of the total trials completed, and thus limit the resolution of the analytical approach utilized in the current experiment. Further, although the dynamics of FI-to-FI and FI-to-peak responding transitions are well characterized by extant data (Fuat Balci et al., 2009; Buhusi & Meck, 2009; Lejeune et al., 1997; Meck & Church, 2003; Meck et al., 1984), to our knowledge, such dynamics from VI to FI performance have not been rigorously assessed. Thus, further study is needed to characterize dynamics of acquisition of temporal responding from previously non-entrained behaviors, and to understand the role of the dHPC in acquisition of timed behavior across longer testing durations.

CHAPTER 6

GENERAL DISCUSSION

Summary of Background

The present dissertation sought to identify factors which tune the involvement of the hippocampus (HPC) in temporally-entrained behavior. According to the pacemaker-accumulator (PA) family of theoretical interval timing models (Figure 1.1; Gibbon, 1977; Simen et al., 2013), entrainment of behavior to the temporal periodicity of the environment is facilitated by the interaction of three dynamic sub-components: a clock which emits pulses to an accumulator, a memory component which stores previously reinforced times, and a decision process which compares values sampled from memory to the pulse count in the accumulator and responds when they are sufficiently similar according to some threshold (Church et al., 1994; Gibbon, 1977; Treisman, 1963). Across repeated trials of reinforcement and non-reinforcement, memory updates with accurate pulse counts, and temporal responding comes to approximate reinforced times. This model accounts for a key property of interval timing behavior: the adherence of temporal estimates to Weber's law (Fechner, 1912), where the variance of estimates increases with proportionally with the length of the to-be-timed interval (Gibbon, 1977; Kacelnik & Brunner, 2002; Simen et al., 2013; but see Bizo et al., 2006). This regularity of temporal estimates drives the intuition that interval timing behavior is facilitated by some unified neurocomputational mechanism, but such a system has not been precisely identified.

Accurate interval timing capacity appears to be at least partially dependent on intact HPC function, where dorsal HPC (dHPC) lesions systematically induce underestimation of intervals (Jackson et al., 1998; MacDonald et al., 2014; Meck et al.,

2013; Meck et al., 1984; Palombo et al., 2016; Yin & Meck, 2014). This underestimation is attributable to a range of hippocampal functions which covary with time, including the presence hippocampal time cells which track the time between relevant events (Buzsáki & Tingley, 2018; Eichenbaum, 2014; Lusk et al., 2016) and graded representation of durations in HPC by interval length (Buhusi et al., 2018; Oprisan, Buhusi, et al., 2018). Unlike medial prefrontal cortex and dorsal striatum, which display consistent neuronal population clock activity across measures of temporal responding and task contingencies (Bakhurin et al., 2017; Emmons et al., 2017; Kim et al., 2013), HPC-dependence of interval timing appears to increase as the length the of the to-be-timed interval increases, concurrent with an increase in the presence of motivational effects on emitted behavior and increased prevalence of non-timed responses.

Summary of Major Findings

In this dissertation, function of the HPC in interval timing and its associated processes (motivation, non-temporal behavior) were evaluated in four studies. In Chapter 2, rats were trained on a switch-timing procedure (Figure 2.1), and tested in reacquisition of the previously trained interval and acquisition of a novel (longer) interval following dHPC lesion. dHPC lesions systematically shortened the start times (switches; Figure 2.4) or responding, while leaving stop time (departures) and response variability (CQVs) similar to pre-lesion responses, reflecting an effect of dHPC lesion on the memory component of PA model processes. Importantly, shortening of start times was enhanced and sustained when encoding a novel interval, compared to when retrieving a previously trained interval (Figure 2.4), suggesting that HPC is critical for adjustment of temporal responding to the reinforced criterion.

Chapter 3 assessed effects of chronic variable stress, which reliably induces HPC dendritic retraction (Conrad, 2006; Kleen et al., 2006a), on interval timing. This was done by training rats on a prospective (switch-timing) or retrospective (temporal bisection) timing task, and then tested subjects on these tasks during chronic variable stress. In order to control for well-established effects of stress on motivation and increase the temporal signal of the data, both tasks were *response-initiated*, such that any effects of stress on motivation would be indexed by the frequency of trial initiation. This revealed task-dependent effects, where stress induced a transient decrease in motivation to engage in switch-timing (Figure 3.3), whereas temporal responding remained similar to baseline. Contrastingly, stress-induced a transient increase in the variability of temporal judgments in temporal bisection (Figure 3.5), without any effect on motivation. Decreased motivation in switch-timing aligns with effects of stress on motivation and reward value (Loas, 1996; Rygula et al., 2005; Xu et al., 2017), but stress-induced increased in variability of temporal judgments in temporal bisection suggests an effect on the memory component of temporal responding in the PA framework, where stress resulted in temporary noisiness of pulse-counts sampled from memory, or temporary failure to retrieve reinforced samples from memory.

Having observed disparate HPC involvement in components of temporal responding and their concomitant motivational indices, Chapter 4 validated a response-initiated *timing-with-opportunity-cost* task (Figure 4.2; Sanabria et al., 2009) in rats. In this task, availability of a concurrent random ratio schedule imposes a cost on fixed-interval responding, thus bringing some proportion of non-temporal behavior under schedule control. Motivation to engage in the task scaled with the FI requirement (Figure

4.5). Further, consistent with findings in pigeons, removal of the RR component of the schedule yielded earlier start times on the FI component, indicating that opportunity-cost increases precision of timed responses (Figure 4.9). Pre-feeding revealed heterogenous effects on timing and motivation, where for some rats, pre-feeding reduced the number of trials initiated without affecting timing performance; for other rats, pre-feeding delayed responding on the FI component but had a weaker effect on trial initiation (Figure 4.8).

In Chapter 5, the role of dHPC in initial temporal entrainment of behavior was evaluated by first training rats on a response-initiated concurrent RR VI 24 s baseline condition. Rats were tested for 7 sessions on the response-initiated timing-with-opportunity-cost task (FI 12 s or 48 s) following intracranial infusions targeted at dHPC of a vehicle or baclofen-muscimol, which prevents excitation of the target structure while onboard. dHPC inactivation delayed the inflection point of start times on the FI lever, indicating slowed entrainment of behavior to the temporal contingency (Figure 5.5). Further, temporal regulation modeling revealed a dHPC inactivation-induced reduction in the criterion pulse count for responding for rats tested on FI 48 s (Figure 5.6). Taken together, these results suggest that for entrainment to the longer interval, prolonged stability of start times following dHPC inactivation may have reflected slowed updating of memory with accurate pulse counts.

Hippocampal Contributions to Temporal Responding are Largely Timing-Specific

The present dissertation sought to deconvolve components of behavior entrained to timed intervals, and thereby increase the temporal signal of behavior under any hippocampal manipulations. This is especially critical given the observation that HPC-dependence of interval timing increases at longer intervals (Jacobs et al., 2013; Palombo

et al., 2016; Sabariego et al., 2020), which are subject to enhanced interference from motivational aspects of behavior, as well as non-timing components of operant responding (Daniels & Sanabria, 2017; Watterson et al., 2015). Data from the current dissertation provides some support for a timing-specific function of the hippocampus in temporal responding, independent of these other processes.

When motivation and timing were procedurally dissociated by implementation of response-initiation, increased variability in temporal bisection responding following chronic variable stress (Figure 3.5) was observed in the absence of a stress-induced effect on motivation. In subsequent chapters (4, 5) availability of a concurrent probabilistic schedule of reinforcement further placed non-temporal responding and implicit sources of reinforcement under schedule control (Figure 4.2). Under these conditions, which facilitate enhanced temporal entrainment of start times on the timed schedule (Figure 4.9), dHPC inactivation delayed entrainment of temporal responding (Figure 5.5) without any synchronous changes in motivation. Importantly, this motivation-independent effect was observed in testing on both a short (12 s) and long (48 s) fixed-interval. Further, temporal regulation analysis (Figure 5.6) revealed no systematic effect of dHPC inactivation on the proportion of non-timed responses embedded in start times (parameter *q*), indicating that delayed temporal entrainment is not attributable to more frequent entry into a non-timing state.

This timing-specific account of HPC function in interval timing is somewhat opposed by evidence for a stress-induced decrease in motivation to engage in switch-timing, with no observed effect on measures of temporal responding (Figure 3.2, Figure 3.3). Although the basis of this discrepancy remains unclear, it implies two potential

deterministic factors of HPC recruitment in timing behavior. The first of these is task contingencies: the relatively higher response requirement of switch-timing, a prospective timing task, may have occluded effects of stress on interval timing or relegated these effects to the more reward-distal initiating response. This interpretation is bolstered by observed individual-subject differences in effects of pre-feeding on concurrent RR FI performance in Chapter 4, where effects of reward devaluation were expressed as either changes in measures of temporal responding *or* as reduced motivation to initiate trials (Figure 4.8). The second potential mediator of this discrepancy is pre-treatment experience with the test schedule. Experiments in Chapter 3 assessed stress-induced influences on temporal responding in previously entrained schedules (retrieval), as opposed to entrainment of novel schedules (encoding), for which HPC function appears to be more critical (Figure 2.4, 5.5). Together with the heterogenous neurobiological effects induced by stress (Bangasser et al., 2019; Bondi et al., 2008; Koolhaas et al., 2011), this experimental setting may have occluded HPC effects on prospective interval timing. Collectively, this configuration of findings suggests that, absent a significant motivational challenge, the function of HPC in interval timing is not merely incidental, and is not systematically related to interference from motivation or the presence of non-timed sources of reinforcement.

Dorsal Hippocampus Contributions to Timing Reflect the Memory Component of Theoretical Models of Interval Timing

The systematic shift to earlier temporal responding observed following dHPC lesions and pharmacological inactivation (MacDonald, 2014; Meck et al., 1984; Olton et al., 1987; Oprisan, Aft, et al., 2018; Yin & Meck, 2014) has been attributed to its function

in two candidate components of the PA model of interval timing. The first of these is as a mediator feedback control between clock and working memory, where dHPC tunes temporal expectancy over repeated trials of reinforcement and non-reinforcement (Meck, 1988; Yin & Troger, 2011). Alternatively, dHPC may function in the temporal decision process, such that earlier responding following dHPC lesions reflects a reduction in the response threshold (Meck, 2005; Wearden, 2004).

Observations from the present dissertation favor a specific memory-based account of dHPC function in temporal responding. In prospective interval timing tasks (switch-timing, Chapter 2; timing-with-opportunity-cost, Chapter 5) effects of dHPC lesion and deactivation were exclusively expressed as changes in the *start* of temporal responding, while other measures of temporal responding (stop time, midpoint, and width), remained relatively unaffected by interruptions in dHPC function. Whereas effects on clock speed and decision threshold are expected to be expressed in the totality of temporal responding, such that both start and stop times are earlier (Church et al., 1994; MacDonald, 2014; MacDonald et al., 2012; Yin & Troger, 2011), the selective effect of dHPC manipulations on start times can be interpreted in terms of specific memory processes within temporal entrainment of behavior (Jacobs et al., 2013; Meck, 1988; Meck & Church, 2003).

In PA models, at the point of any given evaluation of *A*, a response is made (or not made) on the basis of a comparison between A and the criterion pulse count (threshold multiplied by memory; θm). According to some instantiations of PA models, (Gibbon, 1977; Killeen & Fetterman, 1988), θ is constant in a given experimental setting or across a given trial. Thus, given a reduction in θ, any given response would be a linear

function of the value sampled from memory, and be reflected in both start times and stop times. In this way, a threshold-based account of HPC effects on timing does not account for the observed selectivity of HPC lesion (Chapter 2) and deactivation (Chapter 5) effects on start times. This selectivity can, on the other hand, be explained by an effect on memory. For any given value of *A*, which changes across time, the value sampled from memory (*m*) could be comparatively too low or too high, which could be expressed differently across the interval and across different responses. For example, a decreased *m* could result in a premature *start time* relative to the reinforced time for a given evaluation of *A*, but as *A* updates, subsequent responses relative to *m* (e.g the *stop time*) could more accurately approximated the to-be-timed interval. Further, values sampled from memory are necessarily a function of values *encoded* in memory, and thus, effects of dHPC lesions on either encoding or retrieval could be expressed as the observed selectivity to start times.

As stated, there are two distinct memory processes implied by the PA framework: *encoding* of reinforced intervals from working memory to reference memory and *retrieving* of previously reinforced times from reference memory (see Figure 1.1; Gibbon, 1977; Gibbon et al., 1984; Rakitin et al., 1998). There is at least partial evidence in the current dissertation of HPC involvement in both of these memory functions. In Chapter 2, dHPC lesions induced transient shortening of start times (LTSs) on a previously entrained schedule (FI 16 s), indicative of a temporary failure in retrieval (Figure 2.4). In a subsequent condition, dHPC lesions induced more sustained underestimation of start times when learning a novel longer interval (FI 32 s). This effect supports the involvement of dHPC in both PA model memory processes: earlier start

times during acquisition of the novel schedule could index biased retrieval of short intervals from memory, but could also be explained by impaired updating of memory with accurate pulse counts.

To a limited degree, the findings in Chapter 5 serve to clarify the discrepancies between encoding and retrieval-based interpretations of findings from Chapter 2. Following pretraining on a concurrent RR VI schedule, which did not entrain responding to any specific interval, dHPC inactivation induced delayed entrainment of start times in FI schedules (Figure 5.5). Importantly, this was observed in the absence of any potential proactive interference from previously entrained responding, suggesting that dHPC inactivation slowed updating of temporal memory. Accordingly, we may speculate that rather than reflecting retrieval of insufficiently long intervals from reference memory, the sustained underestimation of start times during acquisition of a longer FI schedule in Chapter 2 is more readily explained by the apparent function of dHPC in updating memory to new temporal contingencies (encoding). Thus, data from the present dissertation support a memory-based account of dHPC function within the theoretical models of interval timing (Buhusi & Meck, 2005; Gibbon, 1977; Gibbon et al., 1984), which is expressed both by both the selectivity of effects to the start of timed responding, and by differential effects during encoding and retrieval of temporal contingencies.

Dorsal Hippocampus Function is Critical for Updating Memory to Novel Temporal Contingencies

Perhaps the most critical development in characterizing hippocampal contributions to temporal dynamics of behavior made by the present dissertation is the

observed support for the role of dHPC in updating memory to novel temporal contingencies. Prior demonstrations of HPC lesion-induced changes in temporal responding have arrived at three primary conclusions. First, that HPC-lesions interfere with feedback control between the clock and working memory (Meck, 1988; Olton et al., 1987), such that temporal expectancy of reinforcement is not accurately maintained across the duration of an interval. Second, that HPC-lesions bias the content of temporal reference memory, expressed as underestimation of learned intervals (Meck et al., 2013; Olton et al., 1987; Yin & Meck, 2014; Yin & Troger, 2011), and third, that HPC is not critical to acquisition of new temporal criteria (Meck, 1988; Meck et al., 2013; Meck et al., 1984). As discussed, the current dissertation found variable support for the first two of these assertions, but critically, counterevidence for the third.

Taken together, evidence from the present dissertation asserts that dHPC is indeed involved in *encoding* intervals in memory. Across several experiments, especially when compared to effects on encoding of new intervals (Figure 2.4, Figure 2.5, Figure 5.5), effects on retrieval were relatively slight and transient (Figure 2.5, Figure 3.5). Most notably, in Chapter 5, timing-with-opportunity cost testing under dHPC inactivation was the *first* experimental condition that imposed a temporal contingency for reinforcement. Importantly, systematic effects reflecting memory encoding processes were observed across a diverse range of HPC manipulations, including lesion (Chapter 2), stress (Chapter 3), and pharmacological inactivation (Chapter 5). Even in the absence of previously learned temporal contingencies, dHPC inactivation systematically delayed entrainment of start times (Figure 5.5). The specificity of this effect to start times during acquisition reflects the general dynamics of temporal entrainment, during which start

times appear to be the first component of temporal responding to come under schedule control (Balci et al., 2009; Lejeune et al., 1997). This concordance between the established progression of acquisition in interval timing and the expression of dHPC inactivation in temporal entrainment of behavior suggests that dHPC function is critical to initial entrainment between the behavior of an organism and the temporal periodicity of its environment.

Limitations and Future Directions

There are several limitations to the proposed interpretation of the collective data in this dissertation, independent of the limitations recognized in each chapter. Notably, manipulations of HPC function were highly variable between experiments (lesions in Chapter 2, chronic stress in Chapter 3, pharmacological deactivation in Chapter 4). Particularly in the case of chronic variable stress, which has reliable morphological effects in the prefrontal cortex (Bondi et al., 2008; Mika et al., 2013) and striatum (Dias-Ferreira et al., 2009; Taylor et al., 2014), specific attribution of observed timing effects to hippocampal function were assumed through the PA model, and not confirmed by any analysis of dendritic morphology.

Further, in line with the variable effects of pre-feeding observed in Chapter 4 , it is evident that timing and motivation are only partially dissociable through implementation of response-initiation (Daniels & Sanabria, 2019) and presence of concurrent sources of reinforcement (Figure 4.8). Further, even when behavior is under procedural control of multiple rich schedules of reinforcement, adjunctive behaviors and implicit sources of reinforcement (grooming, resting, etc.) are invariably present in operant responding (Killeen & Fetterman, 1988), and may indeed be operants in their

own right (Killeen & Pellón, 2013). Such behaviors were not monitored in the current dissertation, and thus their modulatory role HPC function in interval timing cannot be ruled out.

Third, the present dissertation assumed strong adherence of interval timing to the processes implied by the PA framework, and interpreted all observed effects in this context. However, it is possible that the observed role of HPC in the memory sub-processes of timing could be more parsimoniously explained by other generative mechanisms. Most notably, the multiple-time-scales theory (MTS; Staddon, 2005; Staddon & Higa, 1999) posits that the learned signal in interval timing is reinforced and non-reinforced values of memory (memory trace strengths) for time-marking stimuli, and lacks a separate clock component. Assuming this structure, the ostensible role of HPC in acquisition of temporal contingencies can simply index comprised encoding of these memory trace strengths, without any need for a clock-memory feedback mechanism (Staddon, 2005).

In order to properly adjudicate between such models, future research should work to further characterize the evident role of the HPC in tuning behavior to temporal criteria. Notably, to our knowledge, Chapter 5 of this dissertation was the first investigation of the function of HPC in the initial temporal entrainment of behavior, without any temporal contingencies imposed by pretraining. However, data from Chapter 5 were limited to 7 days of testing, and did not capture the full progression from acquisition to stable responding. Thus, future studies should implement designs to further delineate the role of HPC in the progression from non-temporal responding to stable temporal entrainment,

and through computational modeling, map this progression to dynamic changes in covert timing parameters across training.

Conclusion

In order for an organism to recognize a change in experience, it must be apart from that change. The index for this distance between changes is *time*. Accordingly, through experience with the temporal periodicity of their environment, behavior of organisms comes to covary with and anticipate these changes. The present dissertation concludes that hippocampal involvement in this covariation is not simply incidental, but rather, is specific to timing processes, mediated by effects on memory, and critical to encoding novel temporal intervals. Thus, if time is the index for change, it appears that the hippocampus is critical to the lability of behavior that facilitates efficient behavioral adjustment to those changes.

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APPENDIX A

BOOTSTRAP ANALYSIS OF PRE-FEEDING EFFECTS

The procedure described in the Data Analysis section (*Pre-feeding effects*) yields bootstrap distributions of medians and CQVs of stable parameter estimates (s_1, s_2, midpoint, width) prior to pre-feeding. Let θ_B represent any of these statistics (e.g., the sampled CQVs of s_1), so $\mu(\theta_B)$ represents its mean and $\sigma(\theta_B)$ its standard deviation. Let θ_P represent the corresponding pre-feeding statistic θ. The size of the pre-feeding effect on statistic θ was computed as

$$d(\theta) = [\theta_P - \mu(\theta_B)] / \sigma(\theta_B). \quad \text{(A1)}$$

$d(\theta) > 2$ was indicative of a significant pre-feeding-induced increase in parameter θ; $d(\theta) < -2$ was indicative of a significant pre-feeding-induced reduction in parameter θ.

APPENDIX B

TABLES

Table 3.1
Description of Dependent Measures in Switch Timing

Dependent Measure	Definition
Latency-to-Switch (LTS)	Time to respond on Long FI lever after trial initiation
Latency-to-Depart (LTD)	Time to last response on Short FI lever after trial initiation
Latency-to-Initiate (LTI)	Time to initiate trial
Start Ratio (SR)	Proportion of trials initiated on the Short FI lever
Persistence Ratio (PR)	Proportion of trials in which LTS was longer than LTD

Table 4.1
Details of Experimental Conditions

FI	Subject	# of RR Adjustment Sessions	Total # of BL Sessions	Total # of Trials Initiated	# of Stable Long RR-Active Trials BL (PF) [No RR]	Final RR Requirement (x)
t = 12-s	1	15	53	5734	1210 (46)	65
	2	15	53	3571	892 (15)	74
	3	15	53	4768	1146 (95)	70
	4	15	53	3721	589 (13)	72
	5	15	53	1225	296 (4)	67
	6	15	53	2883	790 (2)	77
t = 24-s	1	38	53	2300	634 (13)	122
	2	38	53	608	161 (1)	130
	3	38	53	813	264 (7)	126
	4	38	53	1295	246 (0)	130
	5	38	53	2464	519 (0)	120
	6	38	53	810	141 (4)	136
t = 48-s	1	32	70	2408	134 (2) [110]	225
	2	32	70	1663	165 (9) [125]	229
	3	32	70	1239	117 (11) [20]	225
	4	32	70	2060	77 (0) [73]	231
	5	32	70	2082	133 (5) [54]	225
	6	32	70	3554	117 (10) [80]	235

Table 4.2 – Summary of Bootstrap Analysis of Pre-feeding

Weighted Mean $d(\theta)$	FI	Start Time	Stop Time	Midpoint	Width
Median	FI 12-s	0.946829	-0.67073	0.756403	-0.76136
	FI 24-s	1.344522	1.940308	2.870678	0.470231
	FI 48-s	0.895746	0.652057	0.543764	0.78572
CQV	FI 12-s	0.806235	-1.50014	-0.26712	-1.14313
	FI 24-s	1.458136	1.554853	1.995856	0.945438
	FI 48-s	0.372338	-0.16462	-0.3412	0.671652

Table 5.1
Summary of Generalized Logistic Parameter Estimates

	μ_{START} (mean ± SEM)		μ_{STOP} (mean ± SEM)	
	Left asymptote (b_{START})		Left asymptote (b_{STOP})	
	12 s	48 s	12 s	48 s
VEH	2.68 ± 0.52	1.93 ± 0.38	73.31 ± 12.28	90.08 ± 14.50
BM	2.51 ± 0.36	3.25 ± 0.57	71.05 ± 19.86	154.04 ± 24.92
	Right asymptote (a_{START} + b_{START})		Right asymptote (a_{STOP} + b_{STOP})	
VEH	2.93 ± 0.46	7.05 ± 3.04	60.44 ± 14.02	116.16 ± 14.99
BM	2.77 ± 0.70	12.34 ± 7.23	56.85 ± 10.98	128.27 ± 41.86
	Growth rate (k_{START})		Growth rate (k_{STOP})	
VEH	6.92 ± 3.29	0.54 ± 0.20	-10.91 ± 11.18	0.27 ± 0.15
BM	0.86 ± 0.55	3.31 ± 1.60	-0.015 ± 0.25	-0.0022 ± 0.12
	Inflection point (m_{START})		Inflection point (m_{STOP})	
VEH	2.94 ± 0.90	3.94 ± 1.61	2.32 ± 1.70	2.28 ± 1.02
BM	8.84 ± 2.68	6.27 ± 0.85	6.78 ± 4.62	0.44 ± 0.18

APPENDIX C

FIGURES

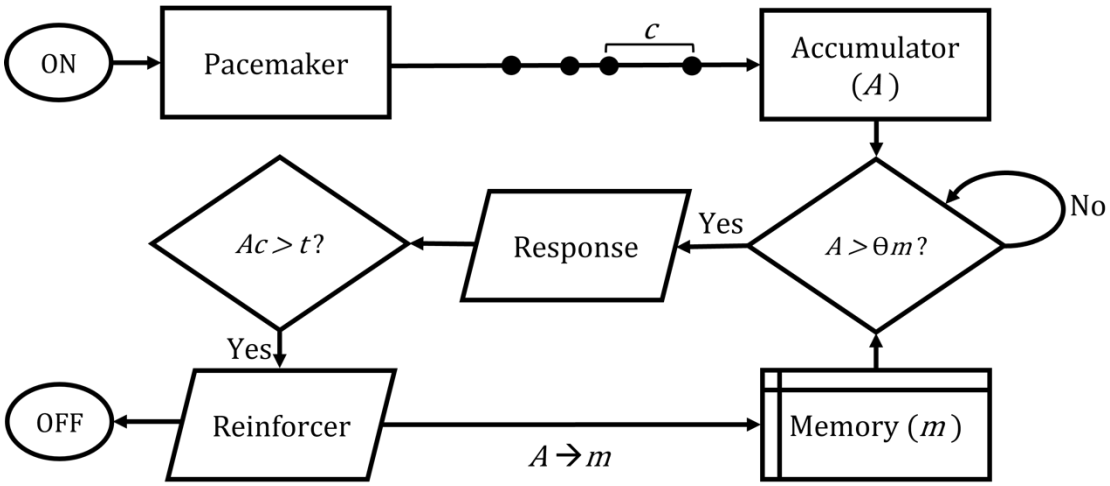

Figure 1.1. Schematic of the components of the pacemaker-accumulator model of interval timing.

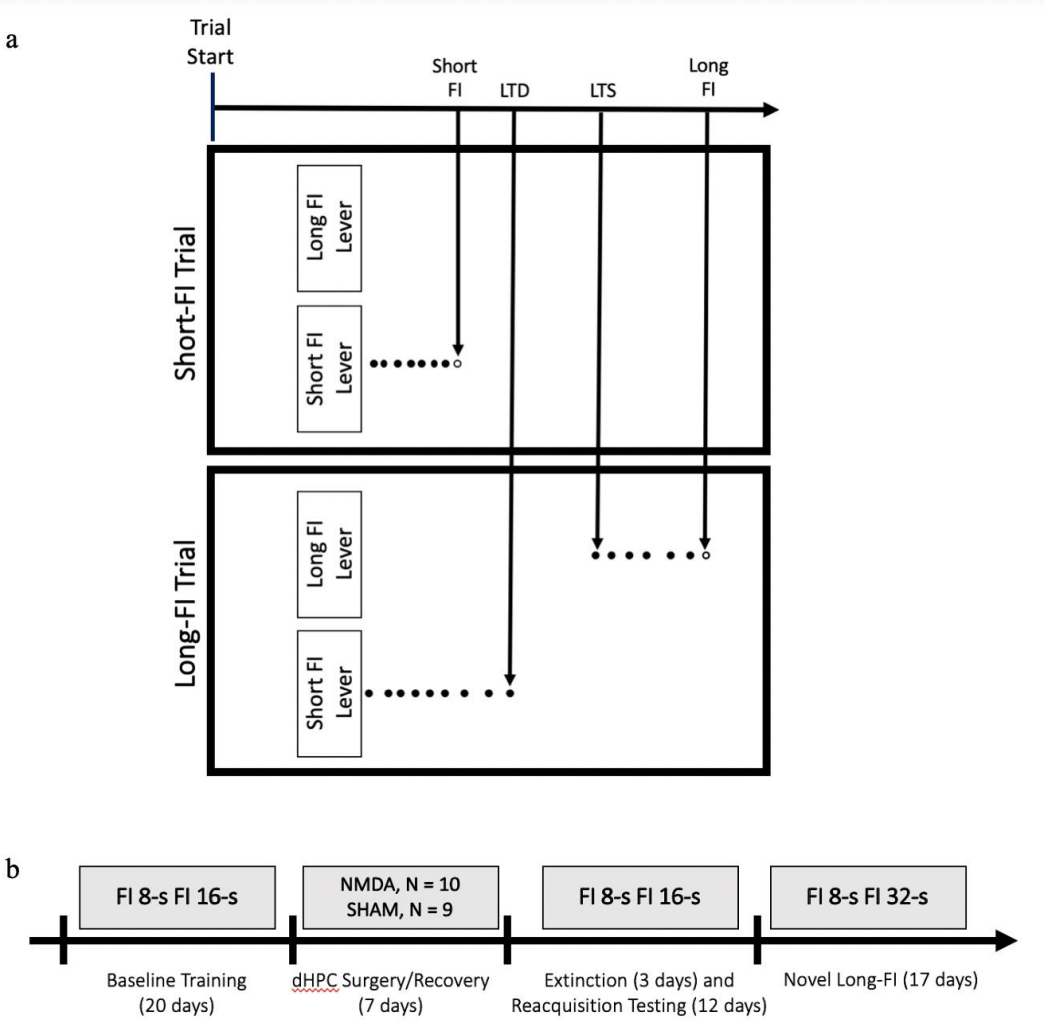

Figure 2.1 (a) Schematic of switch-timing task. Filled circles represent lever presses and open circles represent reinforcer delivery. In long-FI trials, as the FI progresses and the subjective probability of reinforcement on the short-FI lever decreases, well-trained animals are expected to depart from the short-FI lever (latency-to-depart; LTD) and switch to responding on the long-FI lever (latency-to-switch, LTS). (b) Timeline of experimental events. Following 20 days of baseline testing, animals underwent either NMDA lesion or sham dHPC surgery, followed by 3 days of testing in extinction, and then 12 days of reacquisition of the FI 8-s FI 16-s schedule with reinforcement. The long-FI was then increased to 32-s for the remaining 17 days of testing.

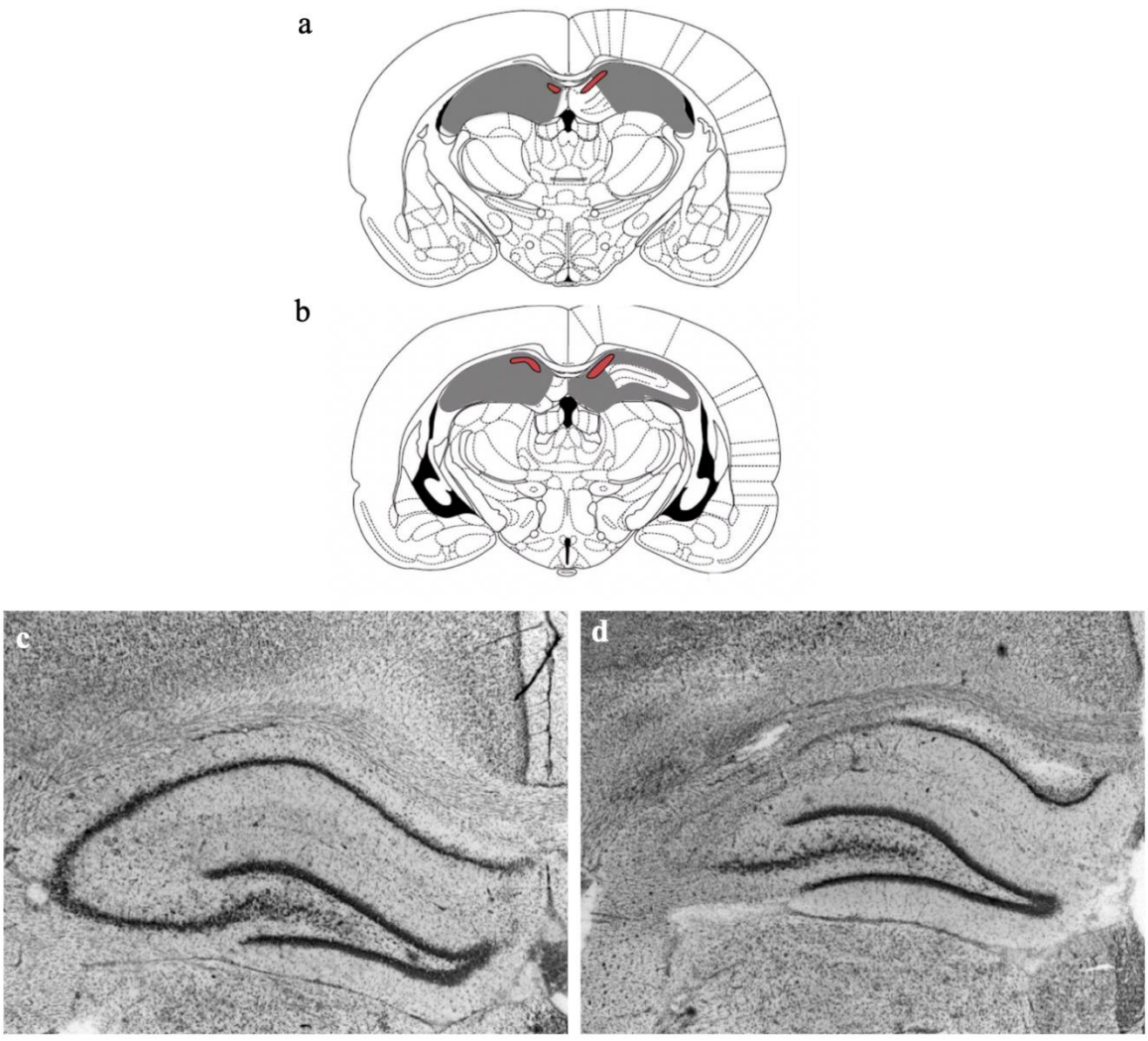

Figure 2.2. Rostral (a) and caudal (b) hippocampus sections (images taken at A/P, -2.8) following dHPC NMDA lesion surgery. Dark and light gray areas indicate the largest and smallest lesions, respectively. Representative images from SHAM (c) and NMDA-infused (d) lesions following dHPC surgery. The section from an NMDA-infused dHPC shows signs of cell death in the dorsal hippocampus, denoted by a lack of staining in the CA1 and CA3 cell body layer.

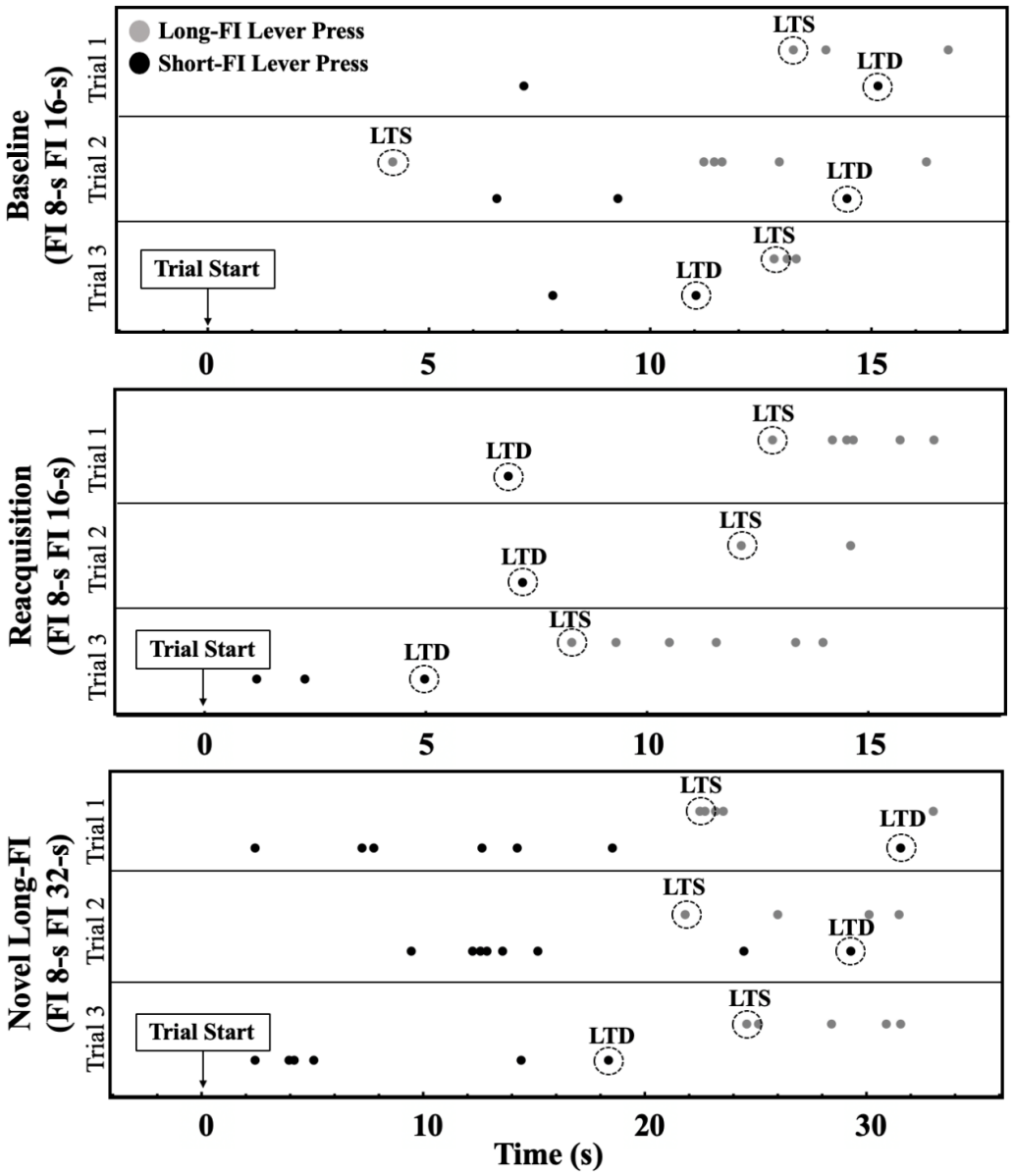

Figure 2.3. Plot of short-FI (black circles) and long-FI (gray dots) lever presses in individual baseline, reacquisition, and novel long-FI trials of a representative rat. This rat was chosen because it had the median asymptotic LTS in both the reacquisition and novel long-FI testing. The first three long-FI trials of the first asymptotic session in each phase are displayed. The circled lever presses indicate the trial LTD (last response on the short-FI lever) and LTS (first response on the long-FI lever).

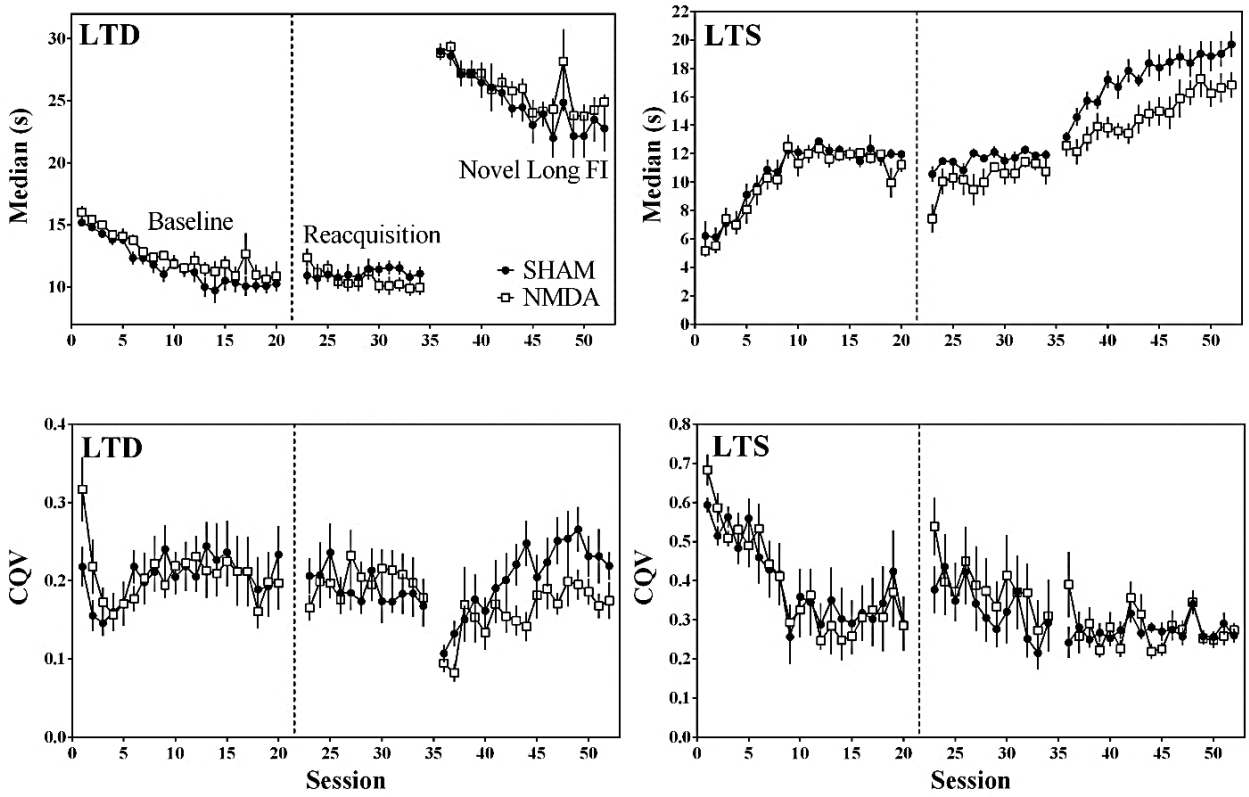

Figure 2.4. Median LTD (top left), Median LTS (top right), LTD CQVs (bottom left), and LTS CQVs (bottom right) for each session of Baseline, Reacquisition, and Novel Long-FI training (untransformed data). Between Reacquisition and Novel Long-FI training, median LTDs and LTSs increased significantly and persistently, and the LTD CQVs and LTS CQVs initially declined but stabilized at similar levels. Initial median LTSs, but not LTDs, were significantly shorter for NMDA rats across Reacquisition and Novel Long-FI training. Asymptotic median LTSs were also shorter for NMDA rats across testing conditions, but only marginally so. No significant effect of NMDA was observed on the LTD CQVs and LTS CQVs. Error bars indicate the standard error of the mean for each data point.

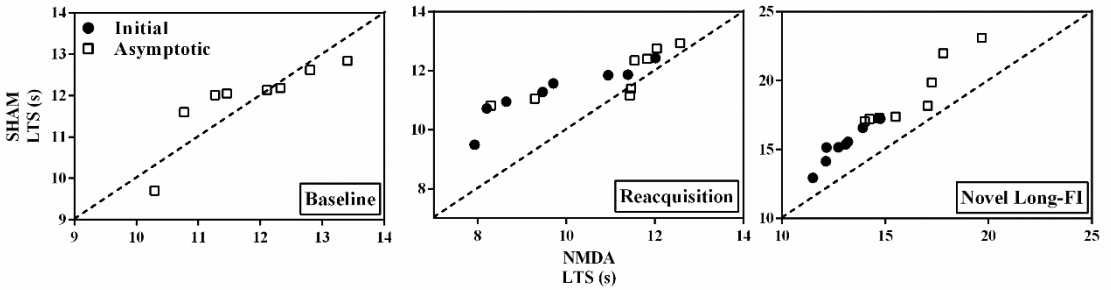

Figure 2.5. P-P plots displaying rank-ordered individual-subject median LTSs for NMDA (x-axis) and SHAM (y-axis) rats as ordered pairs in initial (filled circles) and asymptotic (open squares) performance for the Baseline, Reacquisition, and Novel Long-FI conditions, plotted against an identity line (y = x). In order to account for unequal groups sizes (N_{NMDA} = 10, N_{SHAM} = 8), the minimum and maximum values from the NMDA group were not included in these plots. Data points in both initial and asymptotic performance in Reacquisition and Novel Long-FI testing fall above the identity line, indicating that NMDA LTSs (x-axis values) were consistently lower than SHAM LTSs (y-axis values).

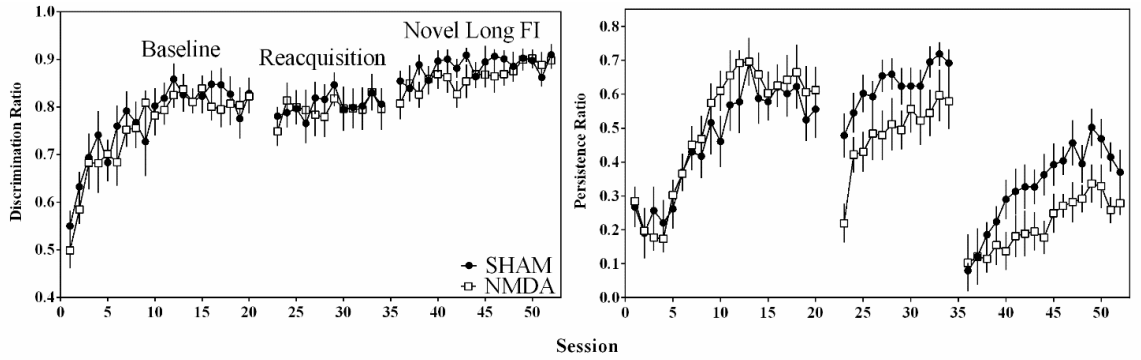

Figure 2.6. Mean discrimination ratios (DRs; left) and persistence ratios (PRs; right) for each session of Baseline, Reacquisition, and Novel Long-FI training (untransformed data). Error bars indicate the standard error of the mean for each data point.

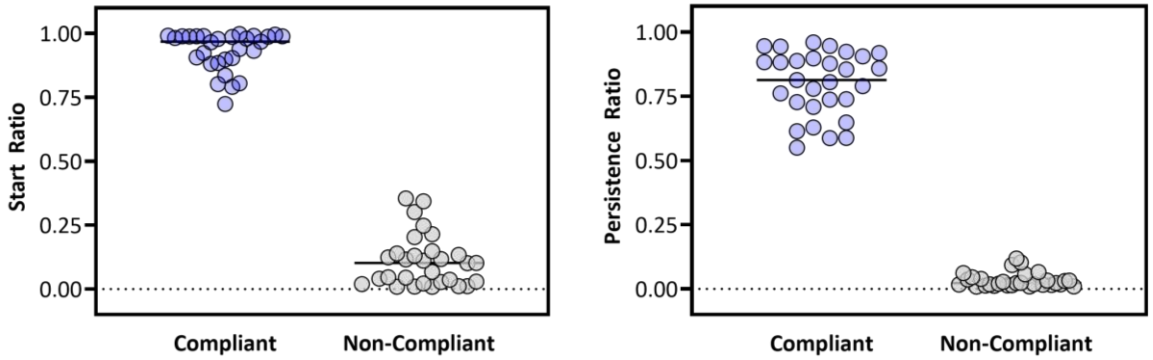

Figure 3.1. Individual start ratios (left) and persistence ratios (right) for Compliant and Non-Compliant rats in response-initiated switch-timing.

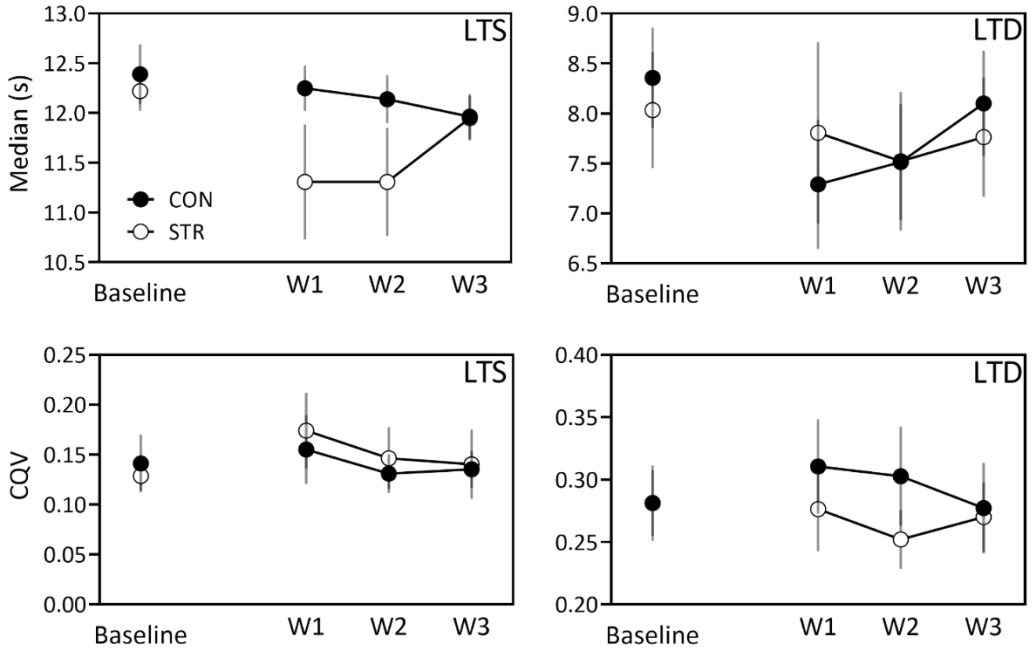

Figure 3.2. Median LTS (top left), Median LTD (top right), LTS CQVs (bottom left), and LTD CQVs (bottom right) for Baseline performance and Weeks 1, 2, and 3 of CVS. There was no evidence for an effect of Treatment or Sex on these measures.

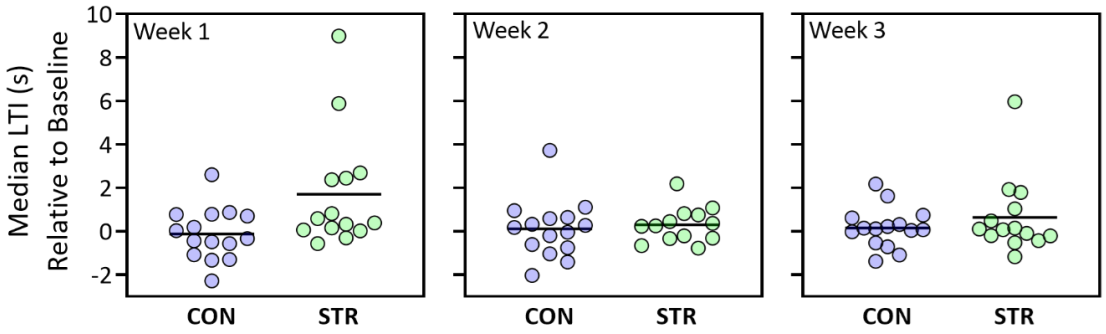

Figure 3.3 Individual median LTIs (relative to Baseline) for CON (blue) and STR rats (green) as ordered pairs in Baseline testing and Weeks 1, 2, and 3 of CVS. Horizontal lines denote the mean of each dataset

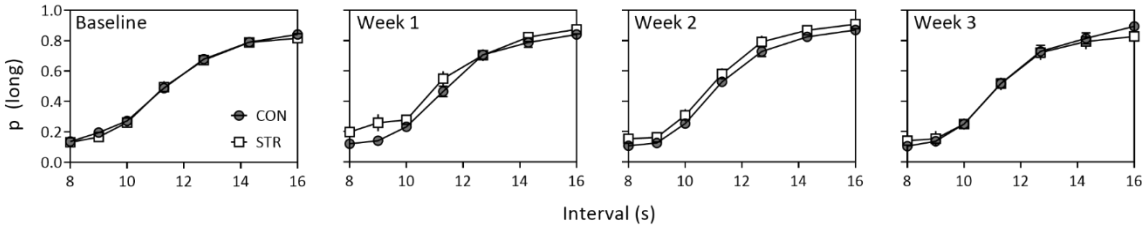

Figure 3.4. Psychophysical functions obtained from temporal bisection probe testing in Baseline and Weeks 1, 2, and 3 of CVS.

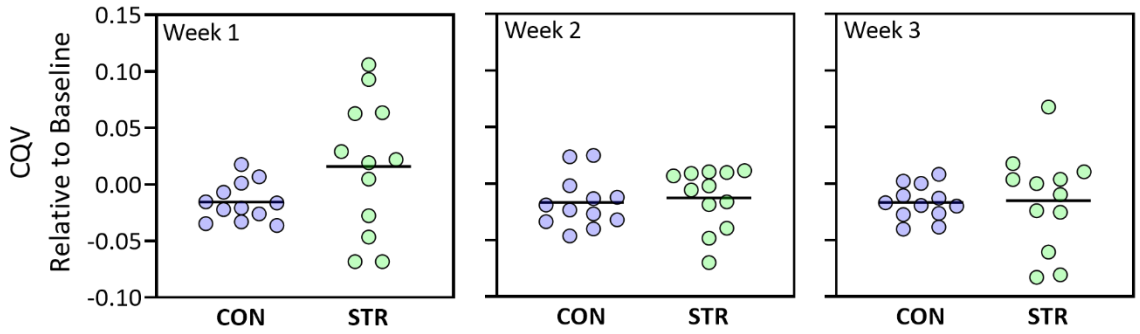

Figure 3.5. Individual median CQVs (relative to Baseline) for CON (blue) and STR (green) rats as ordered pairs in Baseline testing and Weeks 1, 2, and 3 of CVS. Horizontal lines denote the mean of each dataset.

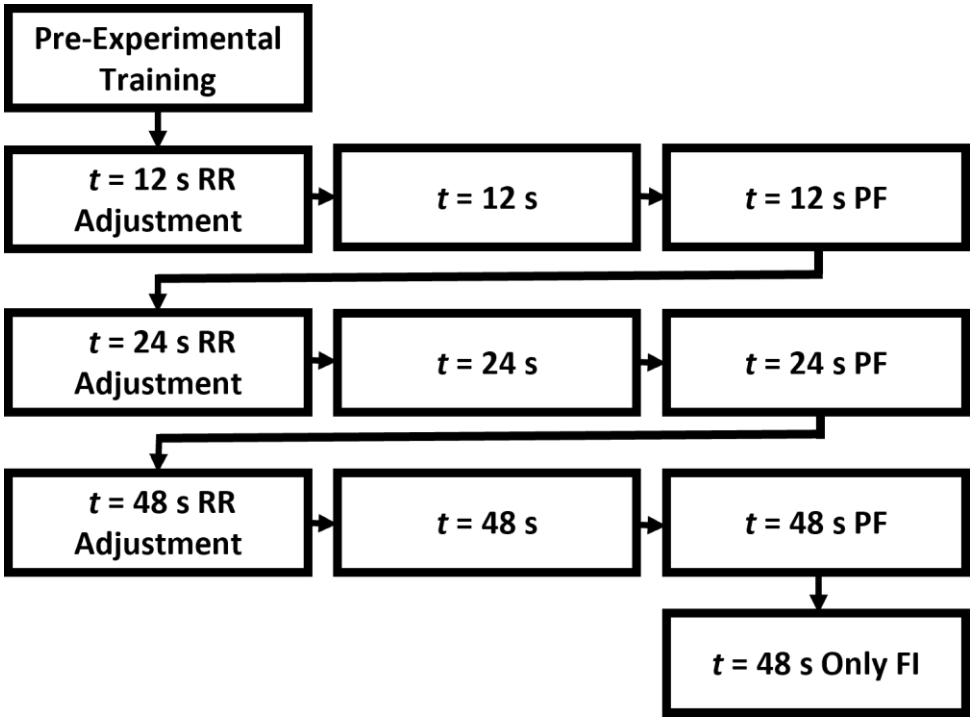

Figure 4.1. Order of training conditions. Following pre-experimental training, fixed-interval (FI) requirement *t* was increased to 12, 24, and 48 s. Within each FI requirement, the random-ratio (RR) requirement *x* was adjusted. Baseline (BL) training was conducted under a stable *x*, with 1-h extra-session feeding 30 min after each session. Pre-feeding training (PF) training was also conducted under a stable *x*, with 1-h extra-session feeding immediately before each session. Training ended with the removal of the RR component.

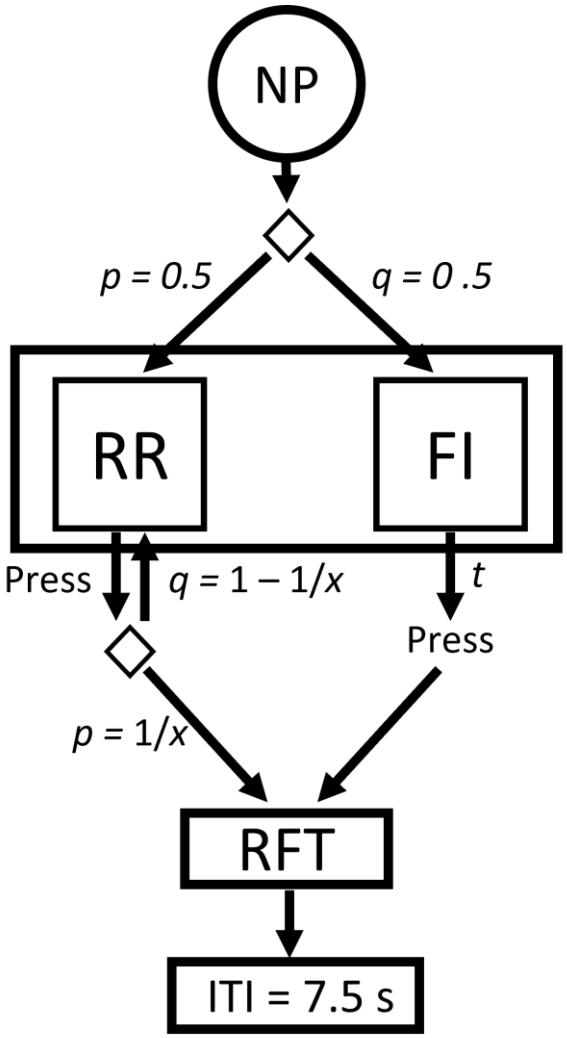

Figure 4.2. Diagram of response-initiated timing-with-opportunity cost trials. This procedure consists of a dependent concurrent random-ratio (RR) fixed-interval (FI) schedule of reinforcement. The opportunity cost of timing was manipulated by changing *x*, the RR schedule requirement. Following response initiation via nosepoke (NP), RR-active or FI-active trials are assigned with equal probability. Trials end when the active schedule is reinforced (RFT), followed by a 7.5-s inter-trial interval (ITI). Diamonds indicate selection between actions with probabilities p and $q = 1 - p$.

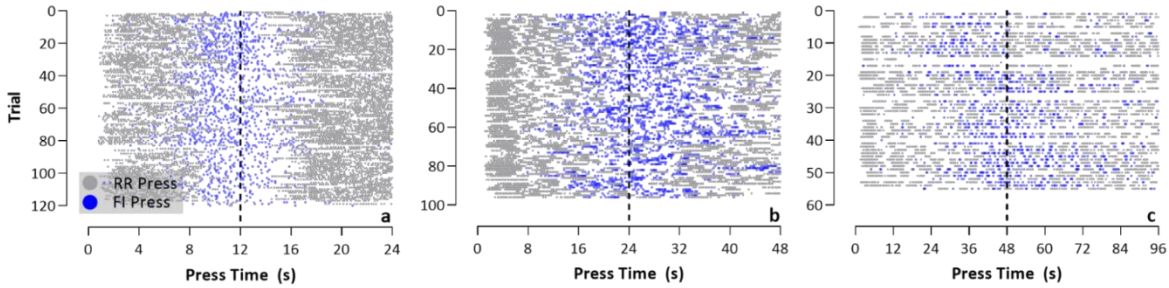

Figure 4.3. Illustrative raster plots of FI (blue) and RR (gray) lever press times in long RR-active trials with FI requirement t = 12 s, 24 s, and 48 s from a representative subject (subject 4). Vertical dashed lines denote t.

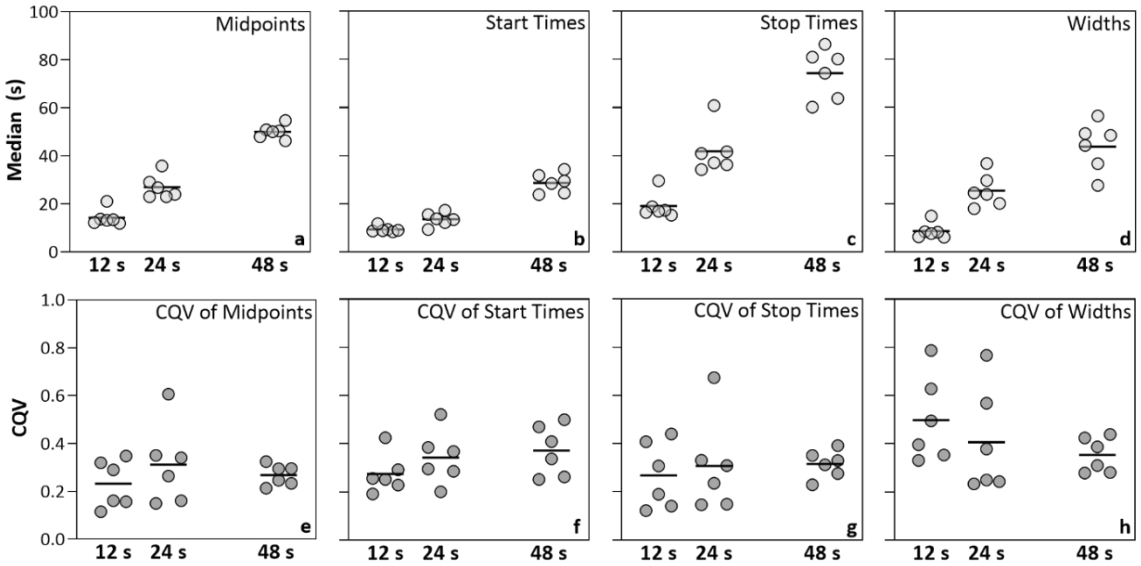

Figure 4.4. Effects of FI requirement on central tendency (top row) and dispersion (bottom) of FI lever responding during stable long RR-active trials. Individual median midpoints (a), start times (b), stop times (c) and widths (d) and their respective coefficients of quartile variation (CQVs; e, f, g, h) for t =12 s, t = 24 s, and t = 48 s. Horizontal lines denote the mean in each condition

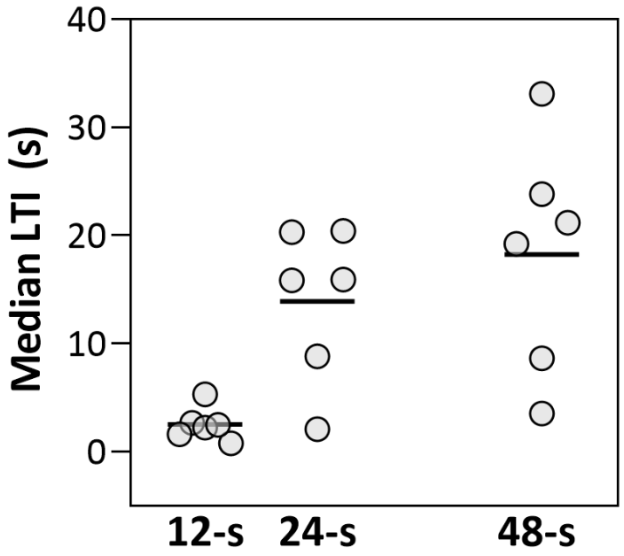

Figure 4.5. Effects of FI requirement on median latency to initiate trials (LTI) for *t* =12 s, *t* = 24 s, and *t* = 48 s. Horizontal lines denote the mean in each condition

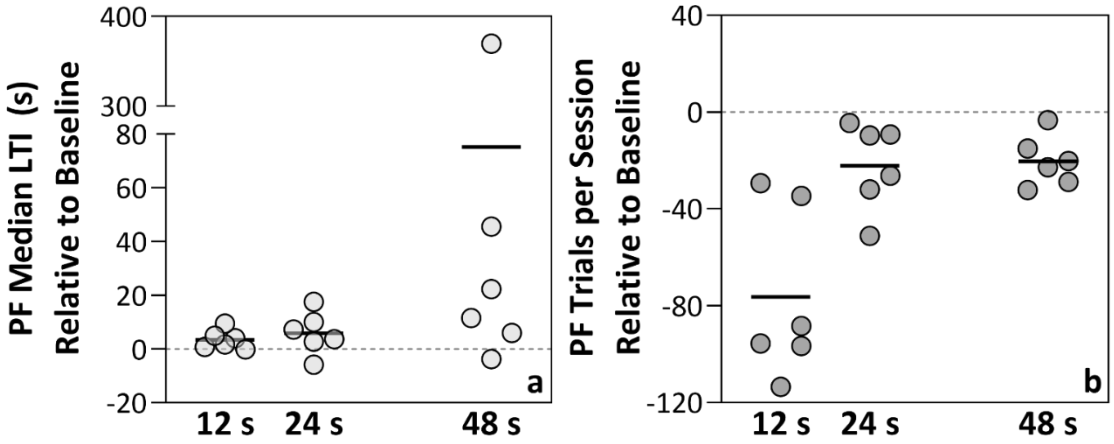

Figure 4.6. Individual median LTIs (a) and trials initiated per session (b) during pre-feeding as differences relative to Baseline for *t* =12 s, *t* = 24 s, and *t* = 48 s. Horizontal solid lines indicate the mean difference from Baseline. Dashed lines at *y* = 0 indicate no change from Baseline.

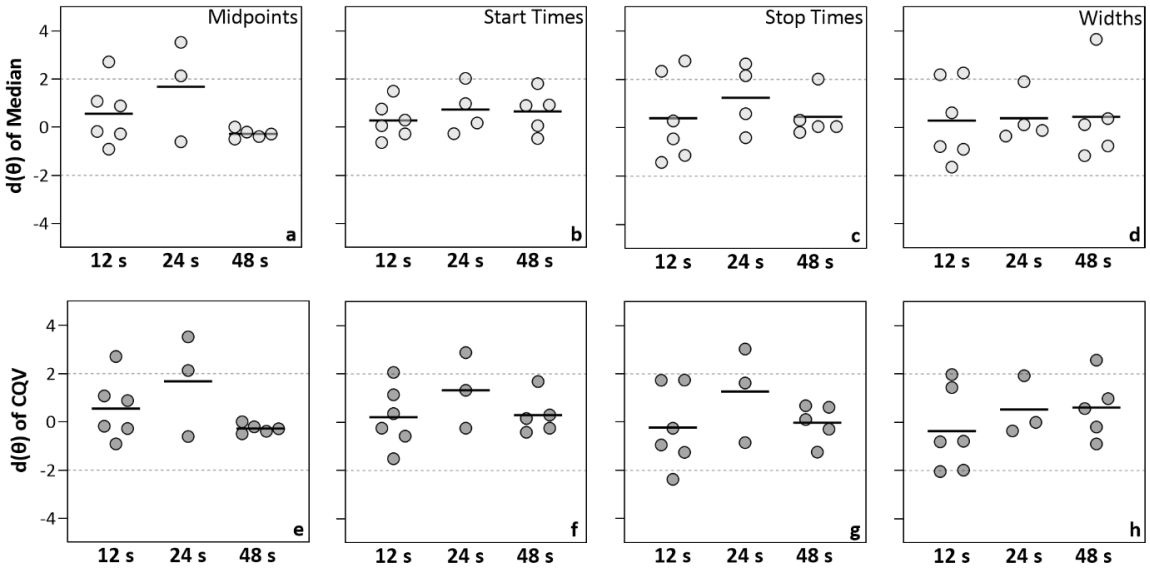

Figure 4.7. Individual *d*(θ) values for median midpoints, start times, stop times, and widths (a, b, c, d) and their respective CQVs (e, f, g, h) . Horizontal solid lines indicate the mean d(θ) for each measure. Dashed lines at $y = 2$ and $y = -2$ denote, respectively, the threshold for a significant increase and decrease for that parameter under pre-feeding.

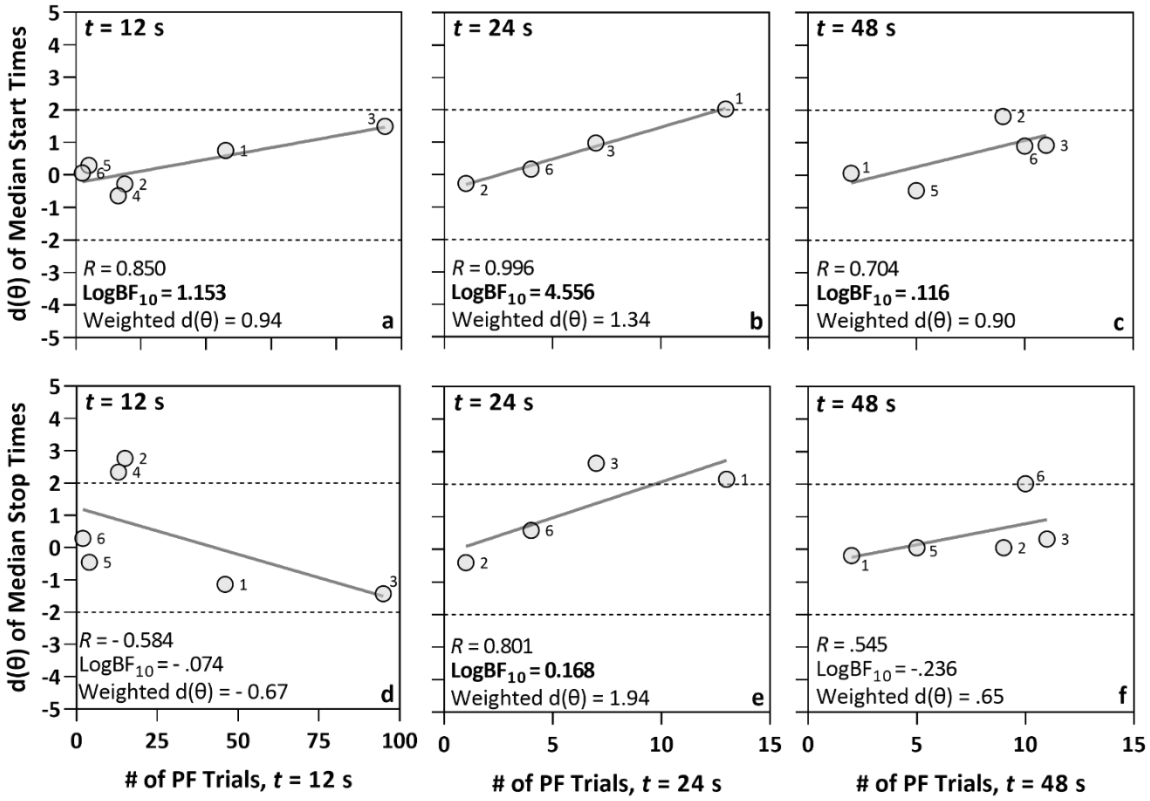

Figure 4.8. Correlations (with simple linear regression lines) between the number of trials completed in pre-feeding (*x*-axis) and individual *d*(θ) values (*y*-axis*;* labeled with subject number; see Table 1) for median start times (a, b, c) and stop times (d, e, f). Bayes factors with evidence for H_1 are bolded.

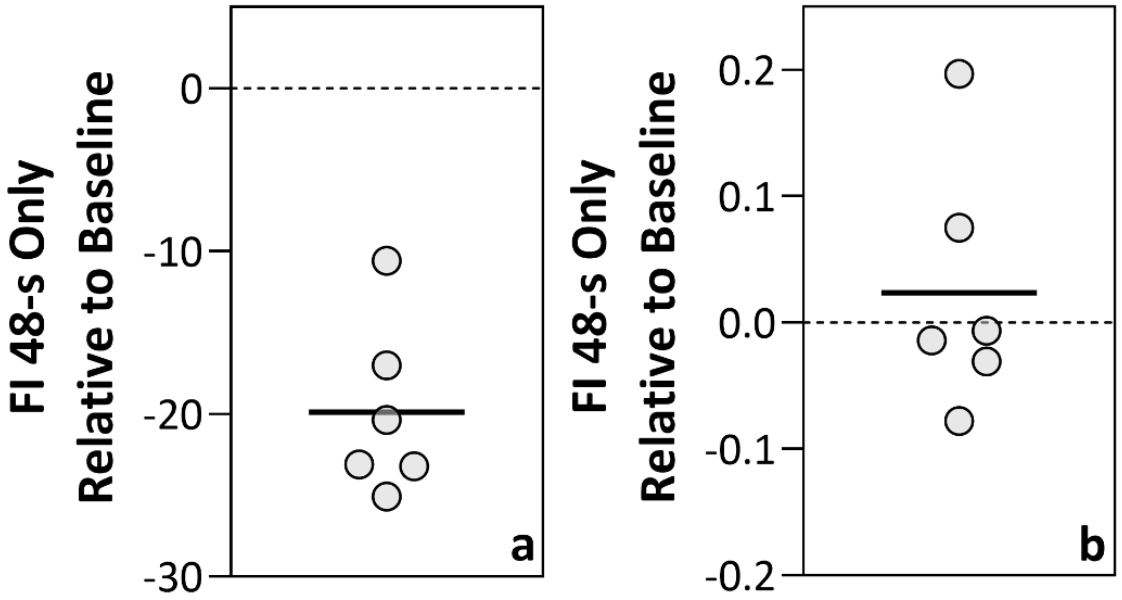

Figure 4.9. Individual median start times (a) and CQV of start times (b) in the FI 48-s Only condition, reported as differences relative to Baseline. Horizontal solid lines indicate the mean difference from Baseline. Dashed lines at $y = 0$ indicate no change from Baseline.

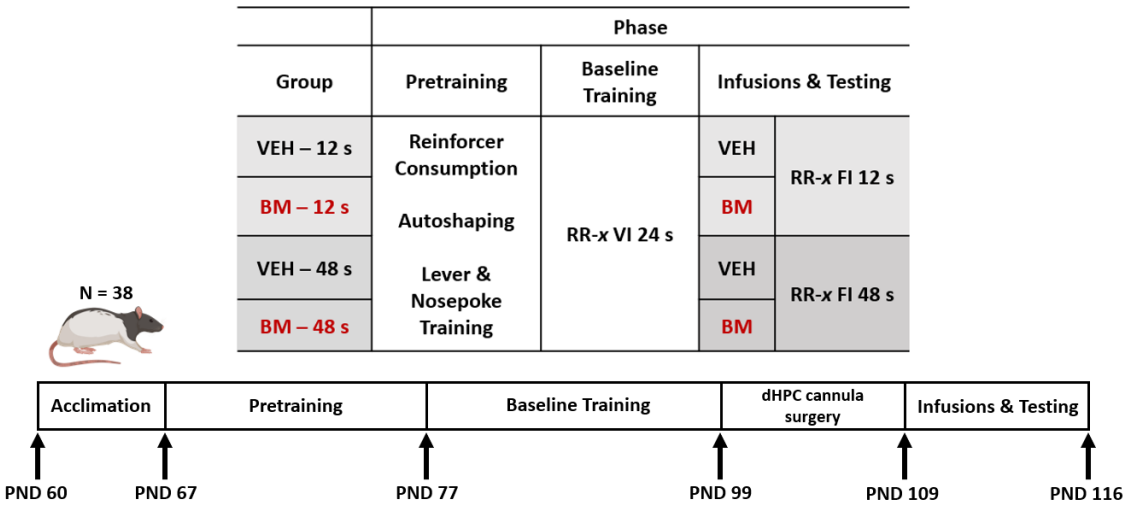

Figure 5.1. Overview of experimental groups and events.

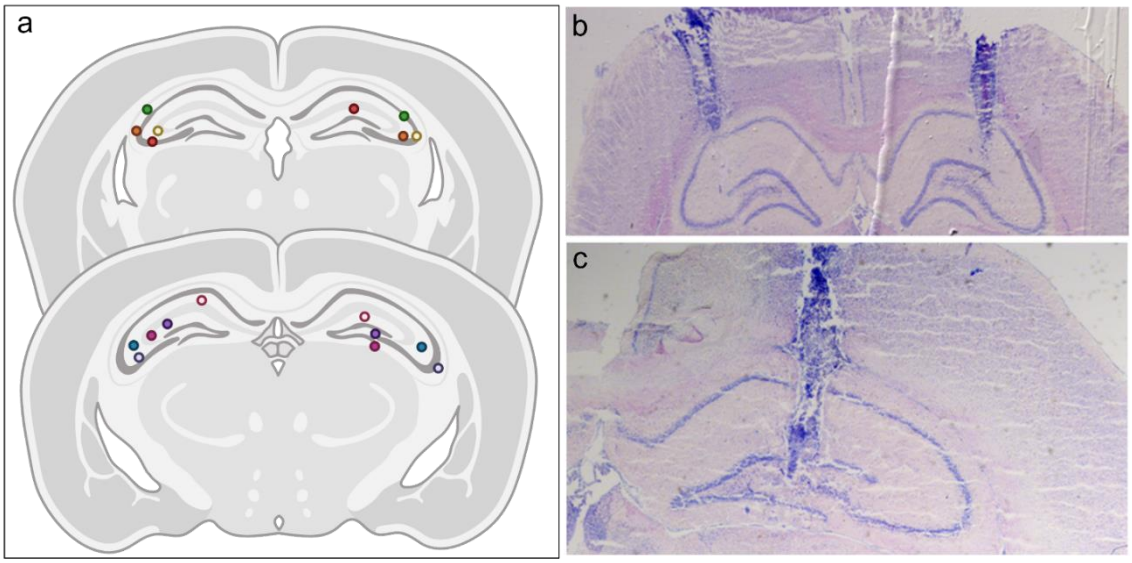

Figure 5.2. a) Caudal (top) and rostral (bottom) hippocampus sections with marked cannula placements following. Each pair of colored dots denotes a different subject. b, c) Representative cresyl violet stained coronal sections in the dorsal hippocampus (images taken at A/P – 3.5). Darkly stained regions indicate the injector track.

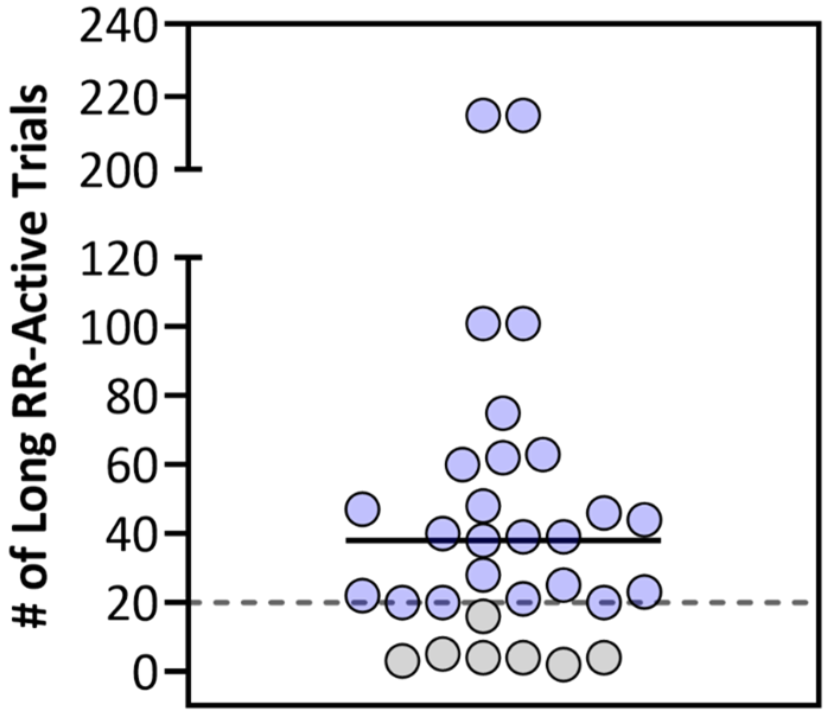

Figure 5.3. Number of long RR-active trials completed by each subject. Subjects below the 20-trial (dashed line) threshold were excluded from analysis. Note: six subjects, who completed zero long RR-active trials, are not shown.

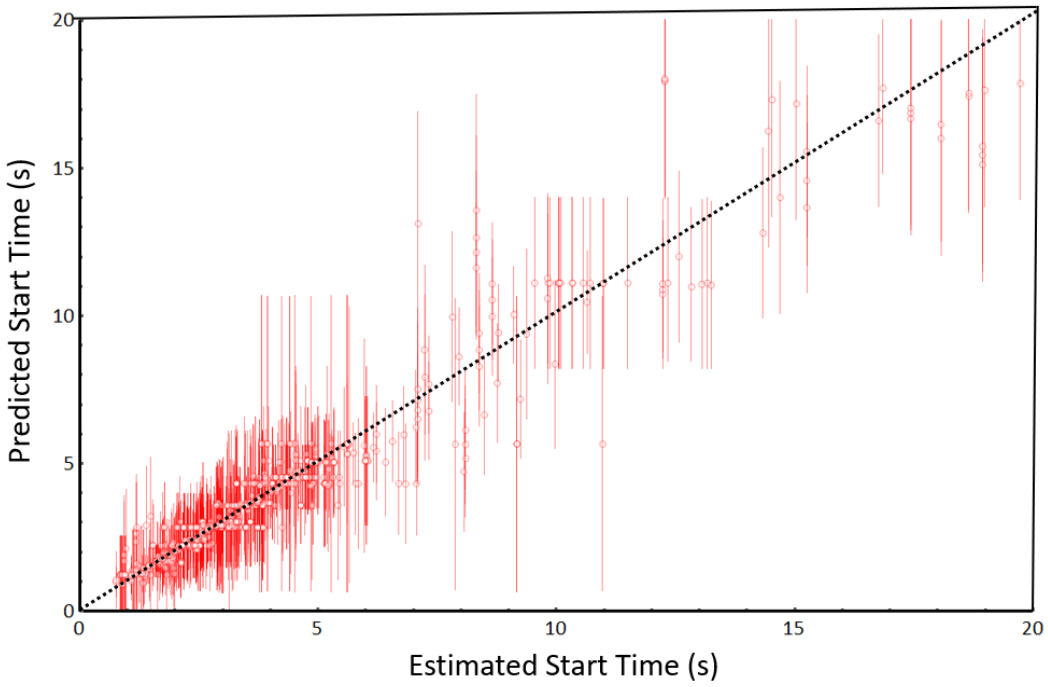

Figure 5.4. Estimated empirical start times (x – axis) and predicted start time for each subject on each trial (y – axis) +/- σ^2 against an identity line ($y = x$). Goodness-of-fit of the generalized logistic to μ_{START} is characterized by ranges of σ^2 which envelop the identity line.

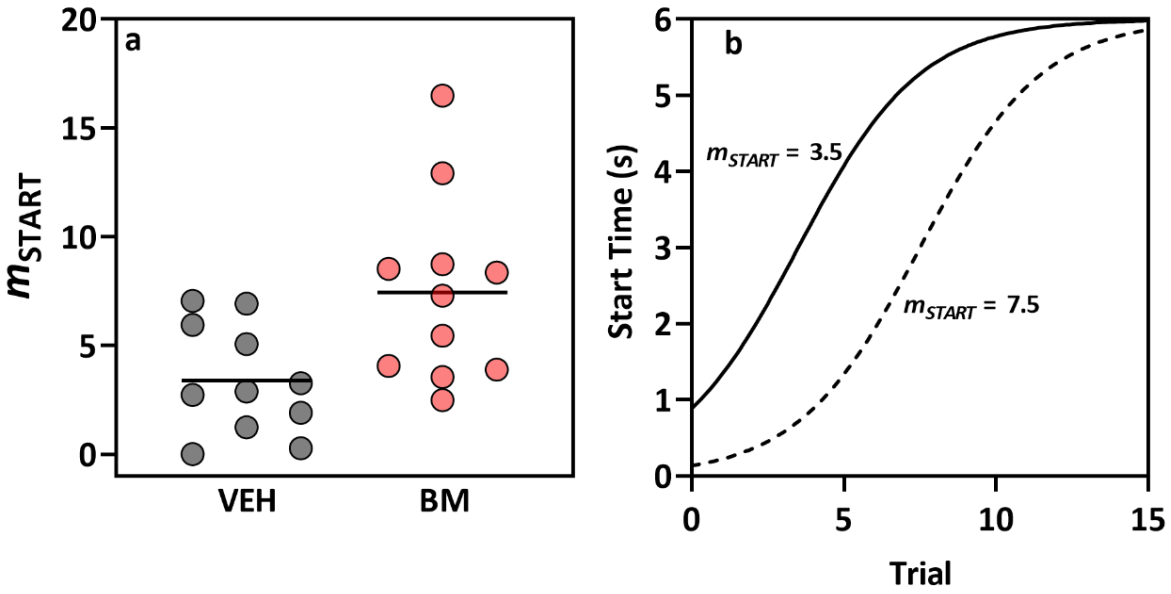

Figure 5.5. a) Individual inflection points (m_{START}) of μ_0 of start times across trials for VEH and BM rats. Horizontal lines denote the mean in each condition. b) Effect of change in m_{START} parameter on illustrative generalized logistic functions, using approximately the average m_{START} for VEH (solid line) and BM (dashed line) rats. For the remaining parameters, the median across groups was used. A reduction in m_{START} shifts the function leftward, such that the inflection point is at an earlier trial.

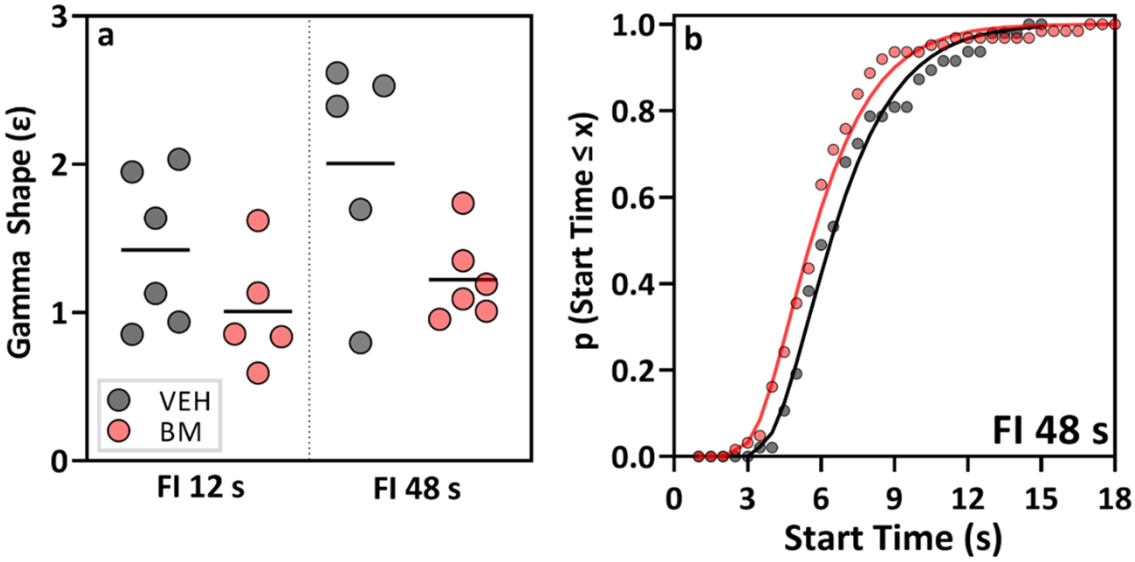

Figure 5.6. a) Individual gamma shape (ε) parameter estimates obtained from gamma-exponential fits to start times for VEH and BM rats tested on FI 12 s and FI 48 s. Horizontal lines denote the mean in each condition. b) Mean cumulative distribution functions of gamma-exponential fits (lines) to start times and their mean empirical cumulative distributions (circles) for VEH (black) and BM (red) rats tested on FI 48 s.