Flexible Temporal Processing and Its Neural Bases

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Abstract

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Temporal information-processing is critical for adaptive behavior and goal-directed action. It has a crucial role in various cognitive and behavioral processes that are of vital biological significance. Therefore, it is important to characterize the behavioral and neural mechanisms of adaptive timing behavior. To this end, in this dissertation we first demonstrate the behavioral properties of flexible timing ability in humans and mice, and then investigate the neural substrates of temporal processing. After providing a brief introduction to theoretical and methodological approaches to interval timing in Chapter 1, we first examined the nature of temporal representations in Chapter 2, with the aim of understanding how the temporal distance between behaviorally relevant events is encoded to guide behavior. Our findings demonstrate that mice can represent experienced durations both as having a certain magnitude (absolute representation) and as being shorter or longer of the two durations (an ordinal relation to other cue durations), with relational control having a more enduring influence in temporal discriminations. After showing that animals are capable of representing event durations in various ways (i.e., in relation to one another or in absolute time units), in Chapter 3 we investigated if humans can mentally manipulate time intervals and perform arithmetic operations on durations. Our results indicated that participants' time estimates were highly accurate and similar across conditions that required them estimate either single durations or the sum of two durations, further providing evidence for the flexible timing ability. After presenting two behavioral studies on adaptive timing behavior in humans and mice, we then investigated the

neural bases of adaptive temporal processing, with a particular focus on the serotonergic and dopaminergic systems. We first examined the effects of serotonin (5-HT) 2A receptor activation on temporal discriminations by systemic administration of a 5-HT_{2A} agonist, DOI, prior to testing in a duration discrimination task in Chapter 4. Our findings consistently revealed that a higher dose of DOI led to the relative shortening of longer durations, possibly due to parallel changes in attention and memory processes which are highly related to temporal cognition. Finally, in Chapter 5, we focused on the dopaminergic modulation of timed actions and investigated the effects of chemogenetic inactivation of dopamine D1 and D2 receptor-expressing striatal neurons in a waiting task. Our results shed light on the distinct contributions of direct and indirect pathways to timing, and provide evidence for a significant role of the striatopallidal pathway in modulating time-dependent behaviors. Taken together, these studies not only help elucidate how organisms represent and mentally manipulate time intervals, but also illustrate the modulation of flexible timing behavior by serotonergic and dopaminergic systems.

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Dedication

This dissertation is dedicated to my family.

Chapter 1: Introduction

1.1 General Overview

Interval timing is defined as the ability to perceive and remember time intervals in the seconds-to-minutes range (Buhusi & Meck, 2005). This central cognitive function forms an indispensable component of natural life. For instance, our ability to assess if we should hit the brakes as traffic light turns yellow or whether the food order we placed online is running late indicates that individuals are able to monitor time intervals and make adaptive time-based decisions. In addition to these conditions which necessitate the use and processing of temporal information, timing function has a crucial role in various cognitive and behavioral processes that are of vital biological significance. For instance, associative learning models posit that time intervals have high relevance to the ability to learn associations (e.g., Matzel et al., 1988; Miller & Barnet, 1993). Timing function has also been implicated in organisms' ability to compute reward rates and adopt reward maximizing response strategies (e.g., Gallistel et al., 2007). Collectively, these studies underscore the importance of intact timing ability in various human and nonhuman functions.

It is well-established that individuals of different species can utilize temporal information in an efficient manner and with similar psychophysical properties (Coull et al., 2011; Gibbon, 1977; Gibbon et al., 1997). Numerous vertebrates including humans can time intervals with, on average, high accuracy, but also with limited precision. For instance, when individuals are prompted to provide repeated temporal estimates regarding a time interval (e.g., 5 s) via a motor response (e.g., holding down a key on the computer keyboard), their time judgments would form a distribution whose mean would be equivalent to the timed interval, suggesting that time estimates would increase linearly with increasing target intervals and exhibit considerable trial-

to-trial variability. In addition to this monotonic relationship between time estimates and target durations, the standard deviation of the distribution of temporal responses is expected to be proportional to the mean of this distribution, a statistical property which is denoted as scalar variability that suggests that the coefficient of variation (CV; the ratio between the standard deviation and mean of temporal responses) is constant across various target durations within an individual (Gibbon, 1977).

1.2 Interval Timing Procedures

1.2.1 Temporal Discrimination Task

A common procedure for studying timing performance in different species is the temporal discrimination task (e.g., Church & Deluty, 1977). In this task, participants are trained to discriminate a set of cue durations as *short* or *long* by responding on two different response keys. After learning to respond differentially to a short (e.g., 2 s) or a long (e.g., 8 s) reference duration, participants are also tested with intermediate durations. Their task is to categorize all stimulus durations as short or long based on their subjective similarity to previously learned reference durations. In order to capture the perceptual aspects of temporal processing, only the correct categorizations of the reference durations are reinforced.

Temporal bisection performance can be evaluated by forming the psychophysical function which illustrates the proportion of *long* responses as a function of stimulus durations. Analysis of the psychophysical curve yields various measures to make inferences about key elements of temporal judgments such as the accuracy and precision of time estimates. For instance, the stimulus duration that is classified as long in 50% of trials indexes the point of subjective equality (PSE), whereas the slope of the psychophysical function reflects temporal sensitivity.

Previous work suggests that the PSE is sensitive to a number of experimental manipulations including stimulus spacing (Wearden & Ferrara, 1995), the ratio between short and long reference durations (Allan & Gibbon, 1991; Wearden & Ferrara, 1996), payoff structures (Akdoğan & Balcı, 2016b; Avlar et al., 2015; Cambraia et al., 2019), and probability manipulations (Akdoğan & Balcı, 2016a; Cambraia et al., 2021). Form of the psychometric functions has also been shown to differ across species (Balcı & Gallistel, 2006; Church & Deluty 1977; Wearden, 1991). Therefore, the conceptualization of timing and decision processes in the temporal bisection task requires the consideration of such methodological factors. In addition to short and long choice proportions, the analysis of response times associated with those choices also enables researchers to make inferences about the latent processing dynamics that underlie duration discrimination performance (e.g., Balci & Simen, 2014).

1.2.2 Peak-Interval Procedure

Another very common interval timing procedure used particularly in nonhuman animal timing research is the peak-interval (PI) procedure (Bitterman, 1964; Catania, 1970; Roberts, 1981). In this procedure, subjects are initially trained on fixed-interval (FI) trials in which only the first response (e.g., lever/ key press) after a fixed duration has elapsed since trial onset is reinforced. Here, trial onsets are signaled by the presentation of visual or auditory stimuli such as tones or cue lights. After the subjects learn to anticipate rewards after the FI period in an accurate and stable manner, they are then tested on "peak" (probe) trials. Similar to an FI trial, a typical peak trial also starts with the cue presentation. However, responses after the target time do not result in any food rewards, and trials last 2-3 times longer than FI trials.

When averaged across trials, response time distributions are well-represented by a Gaussian distribution function with a slight rightward skew. Furthermore, peak time of

responding usually matches the target interval and increases linearly with longer durations. The spread of response distributions indicates an organism's precision in their temporal estimates and changes proportionally to the timed duration. A closer look at response times on individual trials reveals that smooth peak functions are an artifact of averaging across trials. Specifically, individual trials are characterized by a break-run-break response pattern where animals start responding at a low rate and then abruptly switch to a phase of high rates of responding and later return to a low rate of responding (Cheng &Westwood, 1993; Church et al., 1994; Gibbon & Church, 1990). Single-trial analyses enable researchers to identify the start time, stop time, spread, and midpoint of responding, which then allows for a more thorough and complete assessment of temporally-controlled behaviors (Balci et al., 2009).

1.2.3 Other Common Timing Procedures

Some of the other timing tasks mainly used in humans include the *temporal reproduction*, *temporal production*, and *time estimation* procedures. In the temporal reproduction task, participants are first presented with a target time interval cued by a visual stimulus (e.g., square) or an auditory tone, and then prompted to reproduce the same duration as accurately as possible by making a motor response. Similar to the reproduction task, participants in the temporal production task are also asked to produce a target duration by a specific motor response. However, the target duration is only specified in temporal units by the experimenter instead of being cued to the participants with an external sensory stimulus. Finally, the time estimation task requires participants to verbally estimate the length of a target duration that is either presented via a visual stimulus or demarcated by two auditory tones.

As in other interval timing paradigms, time estimates obtained from these three tasks are also evaluated to determine the accuracy and precision of subjects' timing behavior. The ratio

between the mean time estimates and target durations is widely used as an accuracy index. The closer the normalized time estimates are to 1, the more accurate are the temporal judgments. Further, the ratio between the standard deviation and the mean of time estimates, coefficient of variation (CV), is one of the measures to assess the trial-to-trial variability of subjects' temporal judgments. Lower CVs indicate more precise time estimates, whereas higher CV values indicate more variability in repeated temporal judgments (Gibbon et al., 1997).

Here, it is important to note that experimenters should be cautious in interpreting these accuracy and precision indices of timing behavior. It is well-established that how organisms form temporal representations and report their temporal judgments can differ both within and across interval timing tasks due to the nature of the experimental tasks. Specifically, the execution of different motor responses while reporting temporal judgments (e.g., Droit-Volet, 2010; Shi et al., 2013; Wearden, 2003), providing performance feedback on timing behavior (e.g., Acerbi et al., 2012; Franssen & Vandierendonck, 2002; Ryan & Robey, 2002), or the involvement of linguistic processes either during the presentation or estimation phases of target intervals (Buhusi & Meck, 2005; Wearden, 2003) are some of the experimental factors that have been shown to affect different measures of timing behavior. Therefore, it is crucial to consider such cognitive, perceptual, and motor processes while assessing interval timing ability and designing experimental protocols.

1.3 Theoretical Models of Timing

1.3.1 Scalar Timing Model

One prominent theoretical model of interval timing that steered the discussion on the psychophysical properties of interval timing is the Scalar Timing Model (Gibbon et al., 1994). It assumes that timing function is governed by an *internal clock* mechanism consisting of a

pacemaker, switch, accumulator, reference memory, and comparator. The pacemaker generates pulses according to a Poisson process, and upon stimulus onset (signaling the timed duration) a switch closes, which then transmits the pulses to an accumulator until stimulus offset. The number of pulses integrated in the accumulator is then transferred to the reference memory. During this transfer process, the accumulated clock readings are multiplied by a random memory variable drawn from a variability source forming a Gaussian distribution with a mean of 1. Consequently, this *consolidation* process leads to scalar property in memory representations of target durations. When the same target duration is experienced on subsequent trials, a randomly selected value from the temporal representations stored in the reference memory is then compared to the clock reading in the accumulator. When the ratio between the accumulator value and random reference memory representation exceeds a certain decision threshold, time-based anticipation results in the production of a time judgment (e.g., emitting a *short* vs. *long* response).

1.3.2 Behavioral Theory of Timing

Another behavioral model of interval timing is the Behavioral Theory of Timing (BeT; Killeen & Fetterman, 1988). This model assumes that animals' different behavioral states mediate their temporal discriminations. The transitions between states are caused by pulses from a pacemaker whose speed depends on and is proportional to the reinforcement rate. Therefore, as the rate of reinforcement changes, so does the speed of the internal pacemaker, which then produces changes in temporal choices. During the choice stage of a temporal discrimination trial, an animal will report *short* if it is in the behavioral state that was associated with the short cue. Similarly, on a long trial, the animal will report *long* if it is in the state that it had associated with reinforcement after long cues. Further, since the pacemaker speed depends on the rate of

reinforcement, the animal might progress through its behavioral states at a faster (or slower) speed with higher (or lower) rates of reinforcement. Manipulating the inter-reinforcement interval by varying the inter-trial intervals, for instance, would then affect the horizontal placement of points of subjective equality on the psychometric functions (Killeen & Fetterman, 1988).

1.3.3 Drift-Diffusion Model of Timing

The diffusion model is widely used to examine choices and associated response times in various two-choice decision tasks (e.g., Balci et al., 2011; McKoon & Ratcliff, 2012; Palmer et al., 2005; Ratcliff, 1978; Ratcliff & Starns, 2013). It assumes that decisions are made by a noisy evidence accumulation process over time that moves from a starting point towards one of the two response boundaries, after which a response is initiated. Diffusion models have been shown to account for different aspects of two-choice data including accuracy, average response times for correct and incorrect responses, as well as the full response time distributions on correct and incorrect trials (Ratcliff & McKoon, 2008).

Recently, this diffusion model architecture has been adapted to explain timed responses, temporal discriminations, and associated response times, while preserving timescale invariance (Balcı & Simen, 2014; Simen et al., 2011). For instance, in a temporal discrimination task, some of the main assumptions include: (1) the rate of evidence accumulation (drift rate) changes linearly as a function of stimulus durations, (2) the two response thresholds correspond to short and long choices, and (3) the starting point of evidence accumulation should be closer to the *short* threshold and move towards the *long* threshold with elapsed time. These adaptations of drift diffusion models have been successfully used to model interval timing performance on a

trial-by-trial basis in various timing tasks (e.g., Akdoğan & Balcı, 2016a; De Kock et al., 2021; Luzardo et al., 2017; Tipples, 2015).

1.4 Goals of This Dissertation

The main goal of this dissertation is to investigate the behavioral and neural mechanisms of adaptive timing behavior in humans and mice. Following chapters have been written as independent manuscripts. Consequently, there is some redundancy across chapters, but this is done to ensure that key theoretical and experimental implications are discussed in-context. In Chapter 1, we will start with exploring the nature of temporal representations. Specifically, we will test mice on different temporal discriminations to understand how they use absolute and relative temporal codes to guide their adaptive behavior. After showing that animals are capable of representing event durations in various ways (i.e., in relation to one another or in absolute time units), in Chapter 3 we will investigate if humans can mentally manipulate time intervals and perform arithmetic operations on durations. These two studies will form the basis of our investigation of different forms of flexible timing behavior. In Chapters 4 and 5, we will focus more on the neural basis of temporal processing. Specifically, in Chapter 4 we will investigate the effects of a serotonergic hallucinogen on temporal discriminations and show that the acute activation of the serotonin 2A receptor leads subjective shortening of perceived durations, possibly due to changes in attention and memory processes that are closely related to timing behavior. Finally, in Chapter 5, we will focus on the dopaminergic modulation of timed actions and investigate the role of dopamine D1 and D2 receptor-expressing striatal neurons in temporal cognition. Taken together, these studies will not only help elucidate how organisms represent and mentally manipulate time intervals, but also illustrate the modulation of flexible timing behavior by serotonergic and dopaminergic systems.

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Chapter 2: Absolute and Relative Temporal Representations Guide Timing Behavior

2.1 Introduction

Temporal information-processing is critical for adaptive behavior and goal-directed action. Our ability to assess if we should hit the brakes as traffic light turns yellow or whether the food order we placed online is running late indicates that we are able to process the passage of time and utilize this information to guide our decisions. Despite the great extent of experimental and theoretical approaches examining the psychophysical characteristics of timing behavior across species, there is not yet a consensus about how organisms represent durations. Due to the presumed similarities in the perception of time, space, and quantity (e.g., Meck & Church, 1983; Walsh, 2003), it might well be the case that time is represented in a similar way to the spatial and numerical properties of stimuli. A study on numerical cognition by Davis (1984), for instance, reported number sensitivity in animals and showed that they could correctly select a set containing three objects from alternatives containing lower or higher number of stimuli, suggesting the use of an absolute responding rule (see also Taniuchi et al., 2016). In contrast, Brannon and Terrace (1998; 2000) showed that animals trained to discriminate between different sets of numerosities can transfer previously learned ordinal information to new numerosities. A similar use of relative relationships has been also found in several spatial cognition studies investigating the transfer of previously learned landmark-to-goal distances to novel inter-landmark distances (e.g., Kamil & Jones, 2000; Spetch et al., 2003). These findings indicate that numerical and spatial information might be encoded in either absolute or relative terms or both of these two types of representations. The relation between these types of representations is not symmetric in the sense that a relative representation of quantities cannot exist without absolute

representations. To know that one thing is twice as large as another or even that one thing is greater than the other requires that the absolute quantities first be encoded. Thus, the use of a relative representation implies that an absolute representation of quantity must be used to create a relative one.

In the present studies, we examined how these kinds of representations interact in the temporal domain. Although this question of how the passage of time is represented is not a novel one, previous literature has provided mixed evidence on whether and how relative and absolute temporal representations guide behavior, and studies typically argue for one type of temporal code over the other. Several theories of timing, including some of the neurobiologically-oriented ones, explicitly or implicitly assume that the brain encodes durations as disparate objects (e.g., Gershman & Uchida, 2019; Hardy & Buonomano, 2018; Karmarkar & Buonomano, 2007; Matell et al., 2003; Petter et al., 2018). Attributing a categorical representation to a duration would imply that a 2-s duration is coded as one object category, whereas a 6-s duration is coded as another, different category. Such nominal representations would limit the brain's ability to perform basic operations on a set of durations and not allow a response associated with the fact that 6 s is longer than 2 s or shorter than 18 s (which would constitute a third object category). By contrast, other timing models (Church, 1984; Gibbon, 1977; Gibbon et al., 1984) assume that duration experiences are metrically encoded, suggesting that the brain can apply basic arithmetic operations and decide that an 18-s duration is not only greater than 6-s duration but that it is 3 times as long. From a measurement theory perspective (Stevens, 1946), if time is encoded on a ratio scale then other representations can be computed. In this view, multiple temporal representations might guide behavior including, categorical (nominal), relative (ordinal), and even metric (interval and ratio) representations of time.

There is much evidence absolute representations of time guide performance in duration discriminations. For example, Carvalho and Machado (2012) and Carvalho et al. (2016) initially trained pigeons in duration discrimination tasks with two time intervals and then altered one of the durations so the unchanged duration had a change in ordinal position. In the focal groups for example, an animal might have been trained to peck the green response key after a 6-s duration and peck the red response key after an 18-s duration. In a transfer test, the animal might still peck the green key after the 6-s cue but now has to peck the red key after a 2-s cue. In this way the mapping of the 6-s time to a particular response is preserved. However, what was previously the shorter cue (6 vs. 18s) has now become the longer one (6 vs. 2s). If the encoding of absolute time was guiding performance in the original discrimination then one would predict strong positive transfer to the second discrimination. Perhaps, nearly perfect transfer to the 6-s cue while the animal has to learn what to do to a 2-s cue. In the case of the 2-s cue, there might even be some negative transfer as the 2-s cue is closer to the 6-s than the 18-s cue of the original discrimination. This might result in an initial tendency to peck the 6-s key following both cues. If the animals had encoded the original discrimination with a relative code, then there ought to be negative transfer to the 6-s cue. This cue switched its relative identity from being the shorter cue in the first discrimination (6 vs. 18 s) to being the longer cue in the second discrimination (6 vs. 2 s). If the animal had learned to peck the green key to the shorter cue and the red key to the longer one, then once the animal discovered that 6 s was now the longer cue the performance ought to deteriorate as the animals starts to choose the red key based on the new relative duration of the 6-s cue. In the aforementioned studies from the Machado lab, subjects in general had high accuracy following the duration that was associated with the same response key in both training and transfer phases (i.e., preserved absolute mapping). In different experimental conditions

where short and long durations were associated with same response keys in both phases (i.e., preserved relational mapping), subjects had low accuracy following the duration that was preserved from the training phase, for which they had to reverse the classification response.

However, a closer look at individual subjects' discrimination accuracy after transfer in the Carvalho and Machado (2012) study reveals that most subjects in one of the experimental conditions (Phase B, Upshift) did not benefit from the preserved absolute mapping. Furthermore, in both Carvalho and Machado (2012) and Carvalho et al. (2016) studies, although preserving the response location of a duration seemed to have helped with initial transfer in most cases, individual acquisition curves frequently followed a U-shaped pattern during the transition phase. That is, the discrimination accuracy for the cue duration with preserved response location started at a high level, but then dropped down significantly before the animals learned the new task rules. While the initial transfer is consistent with an absolute account of temporal control, the subsequent deterioration is not. Similarly, other studies employing temporal discrimination tasks have also yielded support for the hypothesis that at least some of what is encoded has to do with the absolute duration of events (e.g., Russell & Kirkpatrick, 2007; Spinola et al., 2013), though perhaps not to the exclusion of a relative encoding.

There is also considerable behavioral evidence suggesting that organisms rely on relative temporal representations (e.g., Church & Deluty, 1977; Fetterman et al., 1989, 1993; Wan et al., 2010). For example, in the Church and Deluty (1977) study, transfer to conditions that preserved the relative mappings were learned faster than those that did not. However, performance was aggregated across long and short stimuli, so specific ways in which absolute durations might have affected the transfer were not presented. In a clever test of the absolute and relative accounts, Zentall et al. (2004) trained two groups of pigeons on a temporal discrimination task

where they first learned to discriminate a set of two time intervals (2 vs. 8s or 4 vs. 16s). They were then tested on an additional temporal discrimination in which one of the durations was the geometric mean of the durations used in the first discrimination. Specifically, the shorter duration from the 4 vs. 16s discrimination represented the geometric mean of the previous, 2 vs. 8s discrimination in one group. In the other group, the longer duration from the 2 vs. 8s discrimination represented the geometric mean of the previous, 4 vs. 16s discrimination. These intermediate durations were expected to produce indifferent choices based on the absolute encoding, as it is not obvious how an absolute account could predict positive or negative transfer to a value that is midway between the previous short and long durations. Moreover, from an associative strength point of view (e.g., Spence, 1938), the generalized excitatory tendencies to call the middle value long or short ought to be equal. Any generalized inhibition of the alternate response also ought to be equal. Thus, there is no basis for positive or negative transfer to the intermediate cue. Subjects ought to perform at chance levels when presented with intermediate durations. The results, however, indicated that the stimulus durations at the mean were grouped with the other duration based on their relative relationships (i.e., as being shorter or longer than the alternative) during training, demonstrating an example of relational learning (see also Molet & Zentall, 2008 for similar findings in humans). When Maia and Machado (2009) tested pigeons in a duration bisection experiment similar to the Zentall et al. (2004) study, however, they found no evidence for the relative classification of durations.

Even though the exact reasons for these discrepancies in previous findings are not clear, there is a considerable amount of difference in training procedures, learning criteria, and analysis methods across studies. Aside from these methodological differences, however, the lack of consensus on the description of how time is encoded in different timing procedures might also

indicate that animals form both absolute and relative temporal representations, not either one or the other. Previous research on how temporal information is utilized in associative learning indeed shows that animals are capable of both encoding the length of time intervals and comparing the durations of behaviorally relevant events to one another to guide behavior. It should be noted that even though the order in which the absolute and relative categorizations are formed is not known, one possibility is that animals first encode the absolute duration of events and then compare them while learning new temporal contingencies (e.g., Balsam & Gallistel, 2009; Balsam et al., 2006).

To further probe the question of how time is encoded, we first trained mice to categorize the length of two time intervals as *short* or *long* in a temporal discrimination task and then examined how they transferred this discriminative behavior to novel cue durations and response locations in two different experiments (Table 2.1). Specifically, after training mice to correctly discriminate a duration pair, we either increased or decreased the length of one of the initial time intervals by a factor of 3 (Experiment 1 and Experiment 2, respectively) and/or switched the associated response locations (left or right lever).

Table 2.1

Cue Durations and Corresponding Response Locations in All Experimental Phases

	Experiment 1 (Upshift)		Experiment 2 (Downshift)	
Phases and conditions	Left Lever	Right Lever	Left Lever	Right Lever
Training (Phase 1)	2 s	6 s	6 s	18 s
Transfer (Phase 2)				
Control	2 s	6 s	6 s	18 s
Reversal	6 s	2 s	18 s	6 s
Relative	6 s	18 s	2 s	6 s
Absolute	18 s	6 s	6 s	2 s
Transfer (Phase 3)			4 s	12 s

Note. Opposite duration-lever assignment was used for half of the animals. Note that Experiment 1 consisted of only one transfer phase.

In Experiment 1 (Upshift), we started with a short duration pair and trained mice on 2 vs. 6 s discriminations. After they reached a high discrimination accuracy level, we divided the animals into four groups. In two of these groups (*absolute* and *relative* groups), we increased the short duration from 2 s to 6 s and the long duration from 6 s to 18 s, thus preserving one of the cue durations (6 s) between both experimental phases. In the *absolute* group, the specific response location (left or right lever) associated with this unchanged, 6-s cue duration remained constant, such that if the mice were trained with the "2s/Left, 6s/Right" mapping, they were then tested with the "18s/Left, 6s/Right" mapping in the transfer phase. Because of the accompanying change in the alternate duration, the lever associated with the consistent mapping of absolute time (6 s) also experienced a reversal of relative mappings. That is, the preserved response location associated with the long cue of the training pair was now associated with the short cue of the transfer pair. Consequently, if the experienced durations are only coded in an absolute way (e.g., categorically), animals should be little affected by the shift in durations and response

locations and still perform with high accuracy after transfer. However, if they rely on relative representations there should be negative transfer in this group, as the identity of the short and long levers during testing is reversed from what it was during training.

The *relative* group in Experiment 1 (Upshift), on the other hand, was tested with the same relative lever-duration mapping after transfer (e.g., short cue/left lever, long cue/right lever). As in the absolute group, the 6-s duration was preserved from the training to the transfer phase, such that after being initially trained with the "2s/Left; 6s/Right" mapping, animals were tested with the "6s/Left, 18s/Right" mapping in the transfer phase. Since the identity of the correct short and long levers was preserved and the 6-s cue became the short cue of the transfer pair, animals had to change the classification response for the unchanged duration. Thus, if animals rely primarily on absolute representations, we should see negative transfer in the relative group. However, if the relative ordering of remembered durations is a property of temporal encoding, maintaining the relative mapping might facilitate the transfer to the new duration pair.

In the remaining two groups of Experiment 1, we did not change cue durations. In the *reversal* group, we only switched response locations in the transfer phase. If the old mapping was "2s/Left, 6s/Right" in Phase 1, then the new mappings were "6s/Left, 2s/Right" in Phase 2. Since, both the relative and absolute mappings had to be relearned in the reversal group, we thought this might make it the most difficult of the transfer conditions if both types of representations guide behavior. Finally, a *control* group was tested with same duration pairs and response locations in both training and transfer phases (e.g., 2s/Left, 6s/Right in both phases). We ran this last group of animals as a very conservative control to demonstrate that performance does not systematically change as a function of the amount of training.

In another experiment with a separate group of mice, we replicated the design of Experiment 1 (Upshift), except that in Experiment 2 (Downshift), we tested the animals in the reverse transfer order. Specifically, we initially trained all subjects with the "6s/Left, 18s/Right" mapping, and then divided animals into four groups and randomly assigned them to absolute, relative, reversal, and control groups as outlined above. In the relative and absolute groups, we preserved one of the cue durations (6 s) and changed the other cue duration by decreasing it by a factor of 3 (short cue: 2 s, long cue: 6 s), whereas the control and reversal groups continued to be tested with the 6 vs. 18 s duration pair. To address how relative and absolute information is utilized both during initial transfer and later as animals adjust to the transfer task, we analyzed animals' duration discriminations over the course of the transfer tests in both Upshift and Downshift experiments.

2.2 Experiment 1

Method

Subjects

Thirty-one naive male C57BL/6j mice (The Jackson Laboratory, Bar Harbor, ME, USA) were used in this experiment. Mice were approximately 9 weeks-old upon arrival. They were housed in groups of four or five and kept with 12:12h light–dark cycle. Experimental sessions were conducted during the light phase. The mice were maintained at 85-90% of their free-feeding weight. Water was available ad libitum in home cages. The experiments reported in this article were in accordance with the Columbia University and New York State Psychiatric Institute Institutional Animal Care and Use Committees and Animal Welfare regulations.

Apparatus

Sixteen operant chambers (ENV-307W; Med-Associates, St. Albans, VT) placed inside sound and light attenuating boxes were used in the experiment. A feeder trough was centered on one wall of the chamber and used to deliver liquid reward of evaporated milk (0.01 ml). Two retractable levers (ENV-312-2W; 2.2 cm above the grid floor and 11 cm apart) were mounted on either side of the feeder trough. An audio speaker (ENV-324W) was used to deliver a tone (90 dB, 2500 Hz) stimulus. A house light (ENV-315W) was located on the opposite wall, at the top of the chamber. A cooling fan was activated in each chamber at the start of an experimental session. The experimental protocol was controlled via Med-PC computer interface and Med-PC IV software located in an adjacent room. Behavioral events were recorded with a temporal resolution of 10 ms.

Procedure

Magazine and lever press training. Lever press training consisted of two phases. On the first day of the first phase, animals were trained to consume the milk reward from the raised dipper which was lowered 9.5 s after the first head entry. A variable inter-trial interval (ITI) selected from a list of durations with a mean of 30 s was presented after reward delivery. The session ended either after 30 min or 20 dipper presentations, whichever came first. On the subsequent day, animals were trained with the same protocol except this time the dipper was presented for 8 s. All mice were able to collect 20 rewards in both sessions. In the second phase, animals were trained to press a lever to earn the reward. A lever press during the first 6 s of lever insertion resulted in immediate reward for 5 s (*Fixed-ratio* 1 [FR1]). The milk reward was also presented when the lever has been extended for 6 s without a press (*Fixed-time* 6 s [FT6s]). In both cases, the lever was retracted the moment the dipper was raised, and then a random ITI

selected from an exponential distribution with a mean of 45 s was presented after 5 s of food delivery. Sessions ended when mice earned 60 reinforcements or after 60 min elapsed, whichever occurred first. Mice continued receiving sessions like this for a maximum of five sessions until they earned 30 rewards in two consecutive sessions. If they could not attain the performance criterion, they were then switched to FR1 trials in which there was no reward delivery independent of lever pressing. That is, the animals were required to press a lever to earn the reward. Otherwise, the levers stayed extended until the session ended after 60 minutes. The ITI and performance criterion were the same as in FR1&FT6s trials. On average, lever press training lasted 8.32 sessions (*SD* = 1.80, *range* = 7 - 14). Sessions occurred once a day, seven days per week both during this and all other experimental phases.

Duration Discrimination Training, Phase 1. Following lever press training, mice were trained on a duration discrimination task in which they learned to correctly report an experienced duration as *short* or *long* to earn milk rewards. Specifically, mice were required to press either the left or the right lever after the presentation of an auditory tone for one of the two randomly selected durations (short: 2 s, long: 6 s). Half of the mice (n = 16) were trained to press the right lever after the long tone and the left lever after the short tone presentation, whereas the remaining mice (n = 15) were trained with the reverse lever and duration mapping. Duration discrimination training consisted of six phases with increasing complexity from the presentation of only a single duration and the corresponding lever to a standard discrimination procedure to facilitate the acquisition of temporally controlled behavior. We mainly followed the training protocol described in Ward et al. (2009; Experiment 2), with slight modifications. On Day 1 of the *single-lever, single-duration* program, after the presentation of the short duration (2 s) only the lever associated with a correct short categorization was extended. A press on this lever within

10 s led to the retraction of the lever and was rewarded with 5-s of reinforcement delivery. Late responses resulted in trial termination. On Day 2, only the long duration (6 s) and the corresponding lever were presented. Duration-lever mapping was counterbalanced across subjects. On Days 3-6, a *single lever, two-duration* program was in effect, where only one lever was extended after the presentation of one of the two, randomly selected durations (2 or 6 s). If the cue duration corresponded to the presented lever, presses were reinforced. If the cue duration did not correspond to the presented lever, responses did not lead to reward delivery. Lever identity (left or right) was constant within a session and was counterbalanced across days.

On Days 7-8, the mice completed the *interspersed single-lever, single-duration* program in which each of the two durations were randomly presented with their respective corresponding levers. The mice then completed 3 sessions of *50% choice response trials* (50% single-lever and single-duration trials), 2 sessions of *75% choice response trials* (25% single-lever and single-duration trials), and 5 sessions of *100% choice response trials* programs which had an increasing presentation probability of trials that involved the extension of both levers after tone presentation and required animals to choose the lever that was associated with a cue duration to earn a reward. On choice trials, short and long durations were randomly presented, and both levers were retracted upon a lever press. Moreover, an incorrect categorization was followed by a correction trial in which the house light was illuminated for 1 min, and following an ITI (with a mean of 45 s) another choice response trial with the same cue duration initiated. The house light was illuminated only during correction trials. The correction procedure was then removed, and five more sessions of 100% choice response trials were completed. Sessions ended when mice earned 40 reinforcements or after 70 min elapsed, whichever occurred first. Mice continued the duration discrimination training until their discrimination accuracy, quantified by the proportion of correct

responses, for each reference duration was at least 75% for two consecutive sessions. On average, the mice required 2.94 more sessions to reach criterion (*SD* = 1.90, *range* = 2 – 11), and the whole duration discrimination training lasted between 24 and 34 sessions.

Duration Discrimination Upshift Transfer Test, Phase 2. After successfully completing the duration discrimination training, mice were randomly divided into four groups and transferred to conditions in which target intervals and corresponding response locations were systematically manipulated. In Phase 2, we switched the response locations and/or durations of cues in three of the four groups (Table 1). In the first group (*control*, *n* = 8), neither the durations nor the response locations were altered. The second group (*reversal*, *n* = 7) was tested with the original target intervals (2 vs. 6 s), but the lever-duration mapping was reversed. Consequently, if animals were originally trained to press the left lever following the short, 2-s tone and the right lever following the long, 6-s tone, they now had to press the right lever after the short cue and the left lever after the long cue presentation. For this group, both the absolute and relative mappings are disrupted. In the remaining two conditions, the short cue duration was increased from 2 s to 6 s (former long duration) and long cue duration was increased from 6 s to 18 s. These shifts in durations ensured that the ratio between short (S) and long (L) cues was kept constant at 1:3 in both training and transfer phases. The *relative* group (*n* = 8) was required to utilize the original lever assignment for the relative duration judgment, such that they had to press the same short-lever after the short (now 6 s) cue presentation and press the same long-lever following the 18-s duration. In the absolute group (*n* = 8), on the other hand, mice had to press the same lever following the 6-s cue in both experimental phases. This meant that the lever that was formerly associated with the longer duration was the correct response for the new short duration. During the transfer phase, all mice completed 10 sessions and then continued until they

achieved at least 75% discrimination accuracy in two consecutive sessions (resulting in the completion of a minimum of 12 sessions by all animals).

Data analysis

Changes in temporal categorizations in response to the manipulations of cue durations and response locations were analyzed with respect to duration discrimination accuracy, quantified by the proportion of correct responses, and days to criterion. When necessary, Holm-Bonferroni corrections were used to adjust for all multiple comparisons. An alpha level of .05 (two-tailed) was used for all statistical tests. Data analyses were run in MATLAB (Mathworks, Natick, MA) and JASP (Version 0.12.2).

Results

There was no significant difference in discrimination accuracy between groups during the initial training prior to transfer, One-way ANOVA: $F(3, 27) = 0.42$, $p = .74$. To investigate the effects of altering cue durations and response locations on duration discriminations, we analyzed the discrimination performance across the first 12 post-transfer sessions (Figure 2.1). Our results indicated that the proportion of correct responses in all groups, except the controls, decreased dramatically in the first session after transfer. Reversal of the cue duration-lever assignment had a particularly detrimental effect on average accuracy. Recovery of discrimination accuracy in the relative groups occurred very rapidly and was more complete than in the absolute and reversal groups even after 12 days of training. To examine these changes in discrimination accuracy and learning speeds more in detail, we first compared the proportion of correct responses in the first session of the transfer phase and found evidence for positive transfer based on both absolute and relative representations of time. As can be visualized in Figure 2.1 insets, the overall average proportion of correct trials in the absolute and relative groups dropped from approximately .90

pre-transfer to .50 in the first post-transfer session. Discrimination performance of the reversal group deteriorated even further, and the average accuracy dropped approximately to .20. As expected, the control groups' classification accuracy did not change. One-way ANOVAs supported these findings and revealed an overall difference in the proportion of correct choices across groups, $F(3, 27) = 90.30$, $p < .001$, $MSE = 0.58$, $n^2 = 0.91$. Holm-Bonferroni corrected post hoc comparisons showed that all groups differed from one another except for the relative and absolute groups ($p = .49$, all other ps $< .001$).

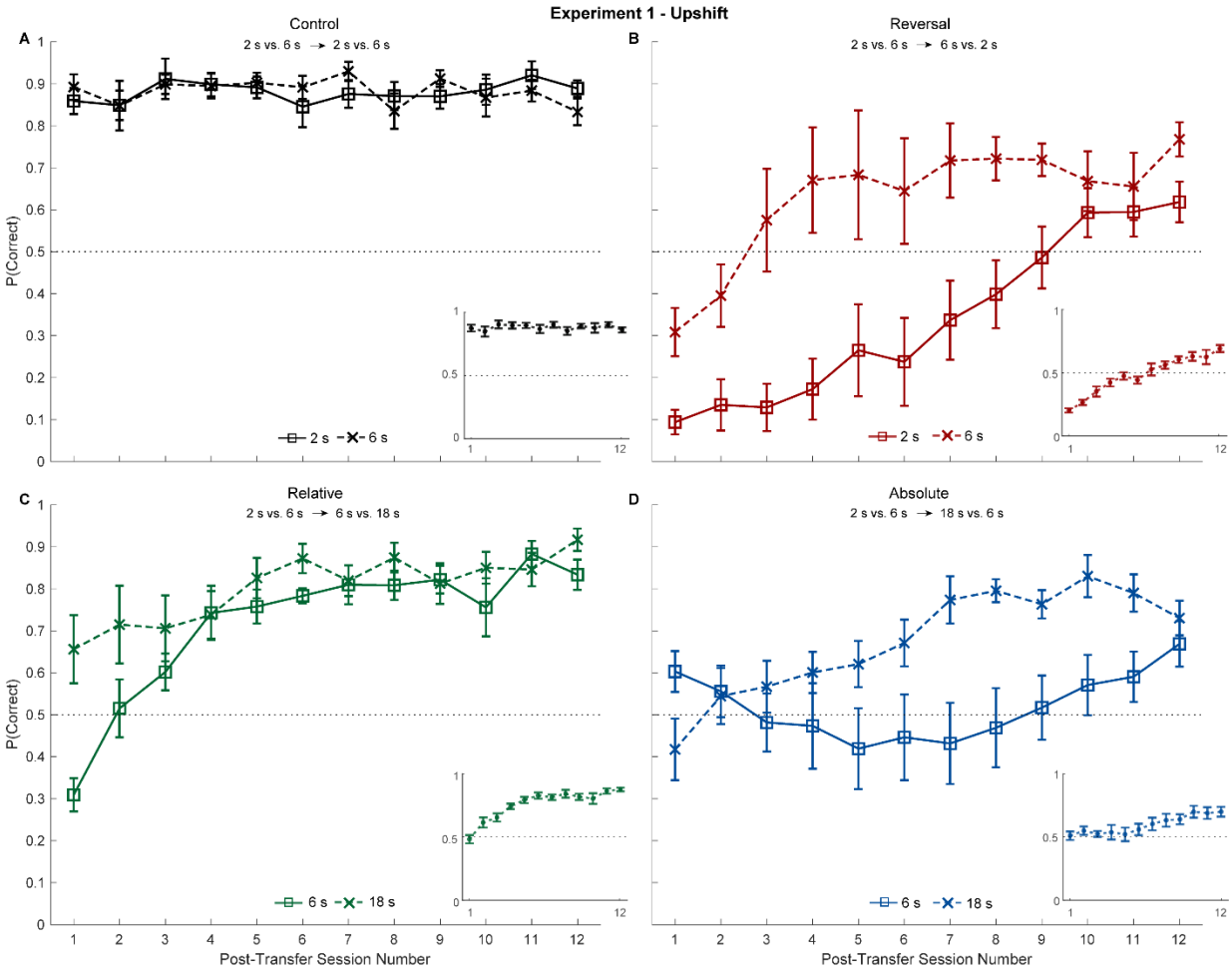

Figure 2.1. Discrimination accuracy in Phase 2 of Experiment 1. Proportion of correct responses on short and long trials after transfer are shown separately for each experimental group. The insets show the average proportion of correct responses for all trials. Error bars show *SEM*. Note that we only included the first 12 post-transfer sessions in this graph, as some animals completed Phase 2 in 12 sessions, whereas others required more prolonged training.

The overall group averages conceal systematic differences in the proportion of correct choices on short and long trials (Figure 2.1). Also, averaging across subjects within groups conceals theoretically interesting between-subject differences in responses to the same change. To better address these issues, we first broke down the averages by cue durations in the post-transfer sessions (Figure 2.1). Reversal of the cue duration-correct lever mapping drove average

accuracy below chance following both 2- and 6-s cues (Figure 2.1B). In the relative group (Figure 2.1C), by contrast, the first post-transfer session performance following the novel, 18-s cue duration was on average significantly above chance. On the other hand, when subjects in the relative group were presented with the old, already familiar cue duration (6 s) with a new mapping to the correct lever, the initial performance was on average below chance. We observed the reverse pattern in the absolute group and found that the proportion of correct trials was above chance following the presentation of the 6-s cue, while performance dropped slightly below chance following the 18-s cue duration (Figure 2.1D).

To evaluate the consistency of these observations about the first transfer session, we examined the proportion of correct responses only for the 6-s cue which was the common duration across all four groups, and found significant differences (One-way ANOVA; $F(3, 27) = 39.93$, $p < .001$, $MSE = 0.61$, $n^2 = 0.82$). Pairwise comparisons revealed that all groups performed worse than the control group ($M_{control} = 0.89$, $SD_{control} = 0.09$; all ps $< .001$). Importantly, even though the absolute group's average proportion correct was only at .60 ($SD = 0.14$), they still performed better than the relative and reversal groups whose accuracy levels were well below chance (both ps $< .001$) and did not differ from one another ($M_{relative} = 0.31$, $SD_{relative} = 0.11$; $M_{reversal} = 0.31$, $SD_{reversal} = 0.15$; $p = 0.99$). These differences in discrimination accuracy indicate that the absolute group benefited from the preserved absolute mapping of the 6-s cue during initial transfer.

We then analyzed the absolute and relative groups' discrimination accuracy following the novel, 18-s cue duration in the first post-transfer session (Figure 2.1). The findings revealed that the relative group performed well above chance, whereas the proportion of correct responses in the absolute condition was below chance following the 18-s cue ($M_{relative} = 0.66$, $SD_{relative} = 0.23$;

$M_{absolute}$ = 0.42, $SD_{absolute}$ = 0.21; independent samples *t*-tests; $t(14) = -2.18$, $p = .047$, $d = -1.09$). These findings suggest that even though both groups of mice were tested with a novel duration in Phase 2, maintaining the relative duration-lever mapping before and after the shift resulted in an immediate and appropriate shift in performance following the novel, 18-s cue duration.

As can be inferred from Figure 2.1, altering the mapping of relative and absolute durations to response locations also affected how fast the new task was learned in Phase 2. The relative group's discrimination accuracy increased and reached asymptote only in a few sessions after transfer, whereas the reversal and absolute groups required many more sessions to re-establish baseline accuracy. Since we tested animals (including those in the control group) for a minimum of 10 days before starting to assess their discrimination accuracy, we included those 10 days in our first analysis of learning speeds. As expected, our one-way ANOVA on the number of sessions the animals took to achieve at least 75% overall discrimination accuracy for two consecutive sessions after completing at least 10 sessions indicated that relearning speeds indeed differed across groups, $F(3, 27) = 7.10$, $p = .001$, $MSE = 86.21$, $n^2 = 0.44$. Specifically, the absolute and reversal groups ($M_{absolute}$ = 18.38, $SD_{absolute}$ = 5.83; $M_{reversal}$ = 19.00, $SD_{reversal}$ = 2.94; $p = 1.00$) were slower than the relative and control groups ($M_{relative}$ = 13.38, $SD_{relative}$ = 2.13; $M_{control}$ = 12.50, $SD_{control}$ = 0.93; $p = 1.00$) to achieve high duration discrimination accuracy (all other $ps \leq 0.02$).

Although we tested animals for at least 10 sessions before assessing their performance, visual inspection of individual acquisition curves (see Figures 2.S1 and 2.S2 in the supplementary text) indicates that some animals did not require such lengthy training. Furthermore, we also found that the change in discrimination accuracy for the 6-s cue that had the preserved response location pre- and post-transfer followed a U-shaped pattern for most

subjects in the absolute condition (see also Figure 2.1D), whereas the accuracy levels for both short and long duration discriminations increased monotonically in other experimental groups. Consequently, in the next set of analysis we aimed to better characterize the differences in relearning speeds both within and across groups. To this end, we calculated the number of training days that each animal needed to achieve at least 75% accuracy in their categorizations of short and long durations separately for two consecutive sessions (Figure 2.2).

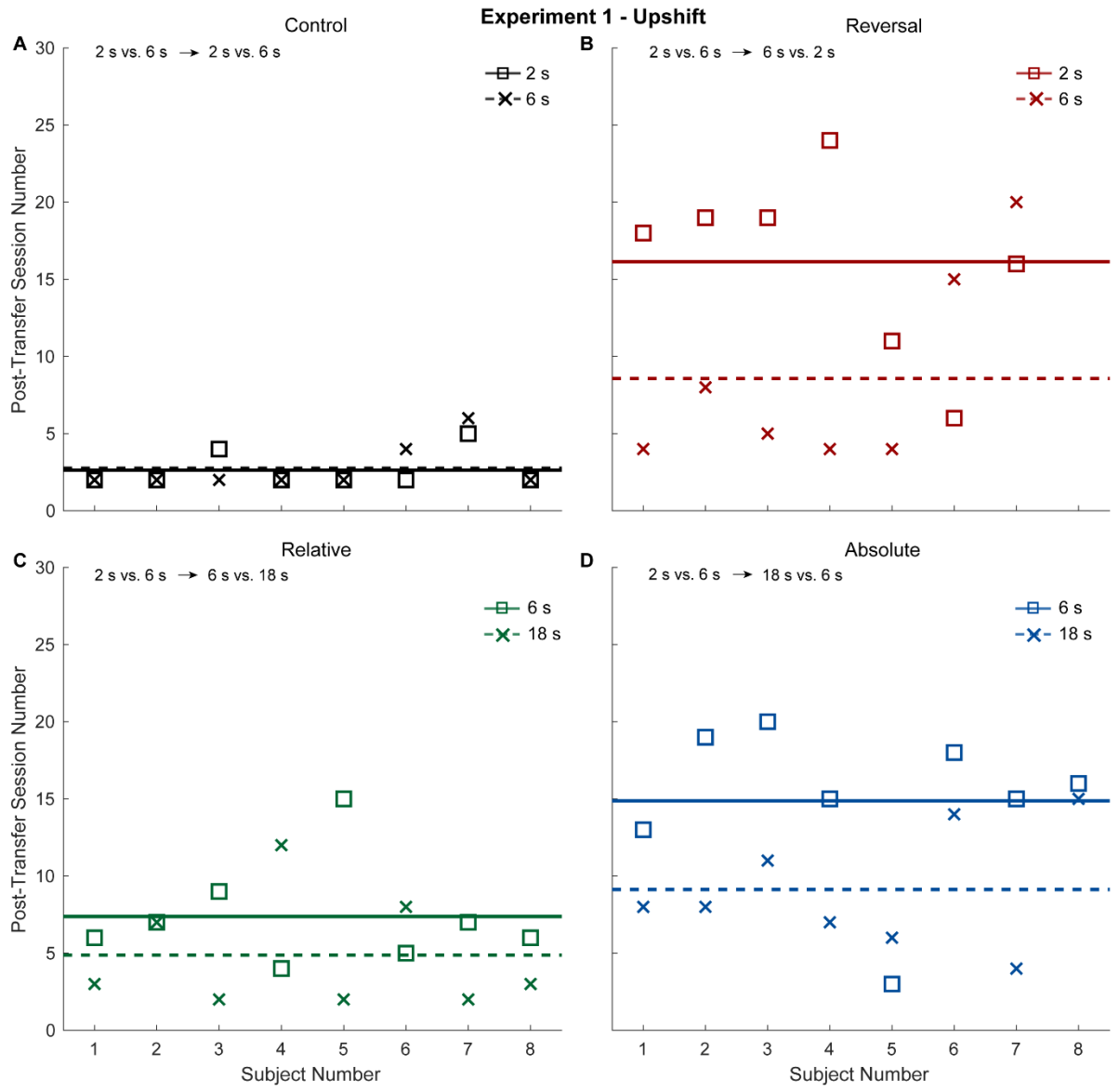

Figure 2.2. Relearning speed in Experiment 1. Number of sessions required to perform at ≥ 75% accuracy for two consecutive sessions shown separately for short and long cue durations in Phase 2 transfer test. Lines (solid: short duration, dotted: long duration) show the mean session numbers and symbols (square: short duration, cross: long duration) illustrate individual subjects' data.

Our mixed ANOVA number of training days that each animal needed to achieve at least 75% accuracy revealed significant main effects of Duration, $F(1, 27) = 11.43$, $p = .002$, $MSE =$

237.88, n_p^2 = 0.30, and Group, $F(3, 27) = 22.88$, $p < .001$, $MSE = 342.66$, $n_p^2 = 0.72$, as well as a non-significant interaction, $F(3, 27) = 2.14$, $p > .05$, $MSE = 44.46$, $n_p^2 = 0.19$. Pairwise comparisons conducted for the main effects of Group and Duration revealed that the reversal and absolute groups required similar amount of training ($p = .80$) and were slower to achieve 75% correct choices than the other two groups (all ps < .001). The difference between the relative and control groups was small, though statistically significant ($p = .04$). Further, long durations were learned faster than short durations in the Upshift experiment ($p = .004$). Although the groups followed these general trends, it is worth noting that Sub #5 in the absolute group had a very quick recovery of correct responding to both short and long cue durations, which was highly comparable to the performance of the relative groups.

Discussion

Our findings indicate that both absolute and relative representations can be used by mice in this temporal discrimination task. When the mapping between relative duration and response was preserved, even when there was an initial decrement in performance, subjects rapidly recovered high levels of accuracy. When the mapping between absolute duration and response was maintained, there was an initial positive transfer to new conditions. However, over the course of next several sessions, performance to the preserved cue duration deteriorated before the animals in the absolute group re-established baseline discrimination accuracy. The deterioration of performance suggests that when both representations are available and put in conflict, preserving the relative identity of the short and long levers has a greater long-term impact on the animals' ability to adapt to new conditions than preserving the mapping between an absolute duration and the associated response. Finally, preventing them from relying on either type of temporal representation by reversing the duration and lever assignments, as in the case of the

reversal group, resulted in below chance accuracy for both short and long durations as well as a slow recovery in performance. Taken together, these findings illustrate that animals encode event durations in both absolute (e.g., 2 s vs. 6 s) and relative terms (shorter or longer) in a temporal discrimination task.

2.3 Experiment 2

To further investigate the nature of temporal representations, we tested a new set of 32 adult mice in the duration discrimination tasks with the reverse order of Experiment 1 (Upshift) and different sets of durations across three experimental phases. Specifically, we initially trained all subjects with the "6s/Left, 18s/Right" mapping, and then divided them into four groups in Phase 2. In the relative and absolute groups, we preserved one of the cue durations (6 s) and changed the other cue duration by decreasing it by a factor of 3, whereas the control and reversal groups continued to be tested with the 6 vs. 18 s duration pair. To further investigate absolute and relative temporal representations, we then tested all animals in another experimental phase with two novel durations (4 vs. 12 s) that had the same relative duration-lever mapping as in Phase 2. These new durations in Phase 3 were selected such that one of them was the arithmetic mean of a previously learned duration pair in Phase 2. Based on absolute temporal control, we expected that the novel, intermediate duration would create ambiguity and lead to a deterioration in discrimination accuracy (Molet & Zentall, 2008; Zentall et al., 2004) following the 12-s cue in the control and reversal groups of Phase 2, and following the 4-s cue duration in the relative and absolute groups of Phase 2. Based on relative temporal control, however, these changes in absolute time intervals were expected not to lead to any significant changes in discrimination accuracy and the speed of acquisition, as the short and long durations were associated with the same levers.

Method

Subjects and apparatus

Thirty-two naive male C57BL/6j mice (The Jackson Laboratory, Bar Harbor, ME, USA) were used in this experiment. The equipment used in this experiment was identical to that in Experiment 1.

Procedure

Lever Press Training. Procedural details were the same as in Experiment 1. On average, lever press training lasted 9.22 sessions (*SD* = 1.83, *range* = 4 - 14).

Duration Discrimination Training, Phase 1. The training protocol was the same as in Experiment 1 except that the target intervals were 6 and 18 s in Experiment 2. Mice completed the last phase of duration discrimination training in an average of 16.13 sessions (*SD* = 5.27, *range* = 12 - 29).

Duration Discrimination Downshift Transfer Test, Phase 2. As in Experiment 1, animals were equally divided into four groups, each tested with a different temporal and/or spatial contingency. Transfer in Experiment 2 was tested in the opposite direction of Experiment 1, such that instead of upshifting the absolute and relative groups' cue durations, in Experiment 2 the cue durations went from 6 and 18 s during initial training to 2 and 6 s during the transfer test. Control and reversal mice were tested with 6 and 18 s in both phases. Please refer to Table 1 for a complete description of transfer conditions. Performance criterion was the same as in Experiment 1 in this and subsequent phases. One mouse in the absolute group did not meet the performance criterion, and for that mouse transfer training was terminated after 35 sessions.

Duration Discrimination Transfer Test, Phase 3. Following Phase 2 an additional transfer test was conducted. To examine transfer to a new set of absolute times while preserving

the mapping of relative times to responses, all subjects were tested with a new set of durations: 4 and 12 s. These values were the arithmetic means of the duration pairs used in Phase 2. Specifically, the 12-s cue was the arithmetic mean of the 6- and 18-s cue durations learned by the control and reversal mice (hereafter referred to as $Group_{MeanLong}$, $n = 16$), and the 4-s cue was the arithmetic mean of the 2- and 6-s cues that were used for the relative and absolute mice (referred to as $Group_{MeanShort}$, $n = 16$). The relative mapping was the same as in the previous phase. Since the discrimination performance stabilized earlier than 12 sessions in the relative group of Experiment 1 (Figure 2.1C), the mice completed a minimum of only seven sessions instead of 12 sessions.

Results

There was no significant difference in discrimination accuracy between groups during the initial training prior to transfer, One-way ANOVA, $F(3, 28) = 1.81$, $p = .17$. Our analysis of the average discrimination performance across the first 12 post-transfer sessions in Experiment 2 also revealed a deterioration in duration discrimination performance following the shifts in cue durations and/or response locations (Figure 2.3 insets). We again found that the reversal group's average discrimination accuracy was below chance level, whereas the absolute and relative groups' overall discrimination accuracy dropped down only to chance level, as initial performance in the latter two groups was a mixture of positive transfer to one cue and negative transfer to the other. One-way ANOVAs supported these findings and revealed an overall difference in the proportion of correct choices across groups in the first post-transfer session, $F(3, 28) = 214.30$, $p < .001$, $MSE = 0.50$, $n^2 = 0.96$. Follow-up comparisons showed that all groups differed from one another except for the relative and absolute groups ($p = .15$, all other ps $< .001$).

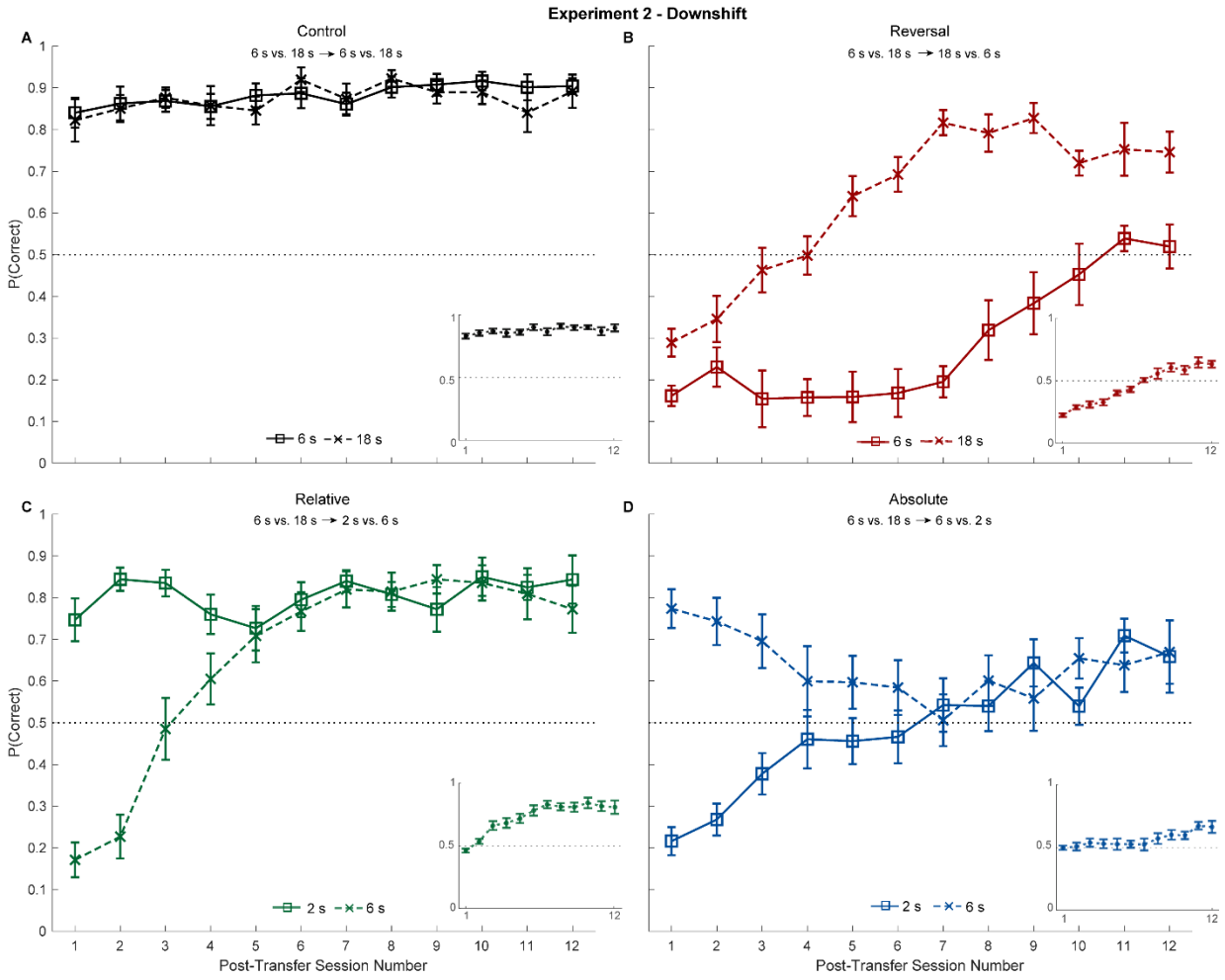

Figure 2.3. Discrimination accuracy in Phase 2 of Experiment 2. Proportion of correct responses on short and long trials after transfer are shown separately for each experimental group. The insets show the average proportion of correct responses for all trials. Error bars show *SEM*. Note that we only included the first 12 post-transfer sessions in this graph, as some animals completed Phase 2 in 12 sessions, whereas others required more prolonged training.

As in Experiment 1, when we broke down the averages by cue durations (Figure 2.3), we found that reversing the cue duration-correct lever mapping drove average accuracy below chance following both the 6- and 18-s cues in the first post-transfer session. In the relative group,

by contrast, the discrimination performance following the novel 2-s cue duration was on average significantly above chance, whereas when subjects were presented with the old, 6-s cue duration with a new response mapping, their discrimination performance was below chance. Accuracy on both durations was high after a few sessions for subjects in the relative group. We observed the reverse pattern in the absolute group and found that the proportion of correct trials was above chance following the old, familiar 6-s duration, while performance dropped below chance following the novel 2-s cue duration. As training progressed performance to the 6-s duration deteriorated despite the initial positive transfer. In the absolute group it again took many sessions for performance to approach baseline accuracy in both cues.

To evaluate these observations, we examined the proportion of correct responses during the first post-transfer session for the 6-s cue which was the common duration across all four groups. Our one-way ANOVA results indicated a significant overall difference in accuracy on 6-s trials, $F = (3, 28) = 95.31$, $p < .001$, $MSE = 1.10$, $n^2 = 0.91$. Importantly, the absolute group was able to benefit from preserving the response location associated with the 6-s cue, as the subjects' average discrimination accuracy level was at .77 ($SD_{absolute} = 0.13$), and not significantly different than that of the control group ($p = .45$; $M_{control} = 0.84$, $SD_{control} = 0.10$) in the first post-transfer session. Pairwise comparisons indicated that these two groups performed significantly better on 6-s trials than both the relative and reversal groups (all ps $< .001$) whose accuracy levels were well below chance and did not differ from one another ($p = .86$; $M_{relative} = 0.17$, $SD_{relative} = 0.12$; $M_{reversal} = 0.16$, $SD_{reversal} = 0.07$).

We then analyzed the absolute and relative groups' discrimination accuracy following the novel, 2-s cue in the first post-transfer session (Figure 2.3). We found that the relative group had highly accurate discriminations ($M = .75$, $SD = 0.15$) to the novel cue, whereas the absolute

group's accuracy was well below chance following the 2-s cue ($M_{absolute}$ = .22, $SD_{absolute}$ = 0.10; independent samples *t*-test; $t(14) = -8.64$, $p < .001$, $d = -4.32$). These findings again show that preserving the relative identity of the short and long levers before and after the shift resulted in immediate and appropriate performance following the novel cue duration.

We then examined how fast the new task was learned in Phase 2 (also see Figures 2.S3 and 2.S4 in the supplementary text for individual acquisition curves). Similar to Experiment 1, the relative group's discrimination accuracy increased and reached asymptote in only a few sessions after transfer, whereas the reversal and absolute groups required more extensive training to approach baseline accuracy. As expected, our one-way ANOVA analysis on the number of sessions the animals took to achieve at least 75% overall discrimination accuracy for two consecutive sessions after completing at least 10 sessions indicated that relearning speeds differed across groups, $F(3, 28) = 12.34$, $p < .001$, $MSE = 203.95$, $n^2 = 0.57$. Specifically, the absolute and reversal groups ($M_{absolute}$ = 22.75, $SD_{absolute}$ = 6.56; $M_{reversal}$ = 21.63, $SD_{reversal}$ = 3.29; p = .58) were slower than the relative and control groups ($M_{relative}$ = 14.75, $SD_{relative}$ = 3.41; $M_{control}$ = 12.50, $SD_{control}$ = 0.76; p = 0.56) to achieve high duration discrimination accuracy (all other ps < 0.01).

When we calculated the number of training days that the animals needed to achieve at least 75% accuracy in their short and long duration categorizations separately for two consecutive sessions (Figure 2.4), we again found that in the relative group, the positive transfer for the novel, 2-s cue was almost immediate. The 6-s cue took several days to reach high accuracy levels. In the absolute group, there was enough initial positive transfer to the 6-s cue that the criterion was met almost immediately for that duration. In contrast, it took extensive training of the 2-s cue to reach criterion in that group. In the reversal group, both durations had to

be retrained extensively to achieve high accuracy, though it took longer to train the 6-s cue than the 18-s one. Further, as in the Upshift experiment, most animals in the reversal and absolute groups learned to correctly categorize long durations faster than short durations. Our mixed ANOVA on the number of sessions needed to reach the ≥ 75% accuracy (for the first time) provided support for these conclusions and revealed significant main effects of Duration, $F(1, 28) = 18.57$, $p < .001$, $MSE = 315.06$, $n_p^2 = 0.40$, and Group, $F(3, 28) = 25.51$, $p < .001$, $MSE = 517.60$, $n_p^2 = 0.73$, as well as a significant interaction, $F(3, 28) = 17.48$, $p < .001$, $MSE = 296.60$, $n_p^2 = 0.65$. Follow-up simple main effects analyses showed that the speed with which the short versus long durations was learned varied within each group (all ps < .02) except the control group ($p = .44$), and that there was an overall difference in how fast the cue durations were learned across groups (short cue: $p < .001$; long cue: $p = .045$).

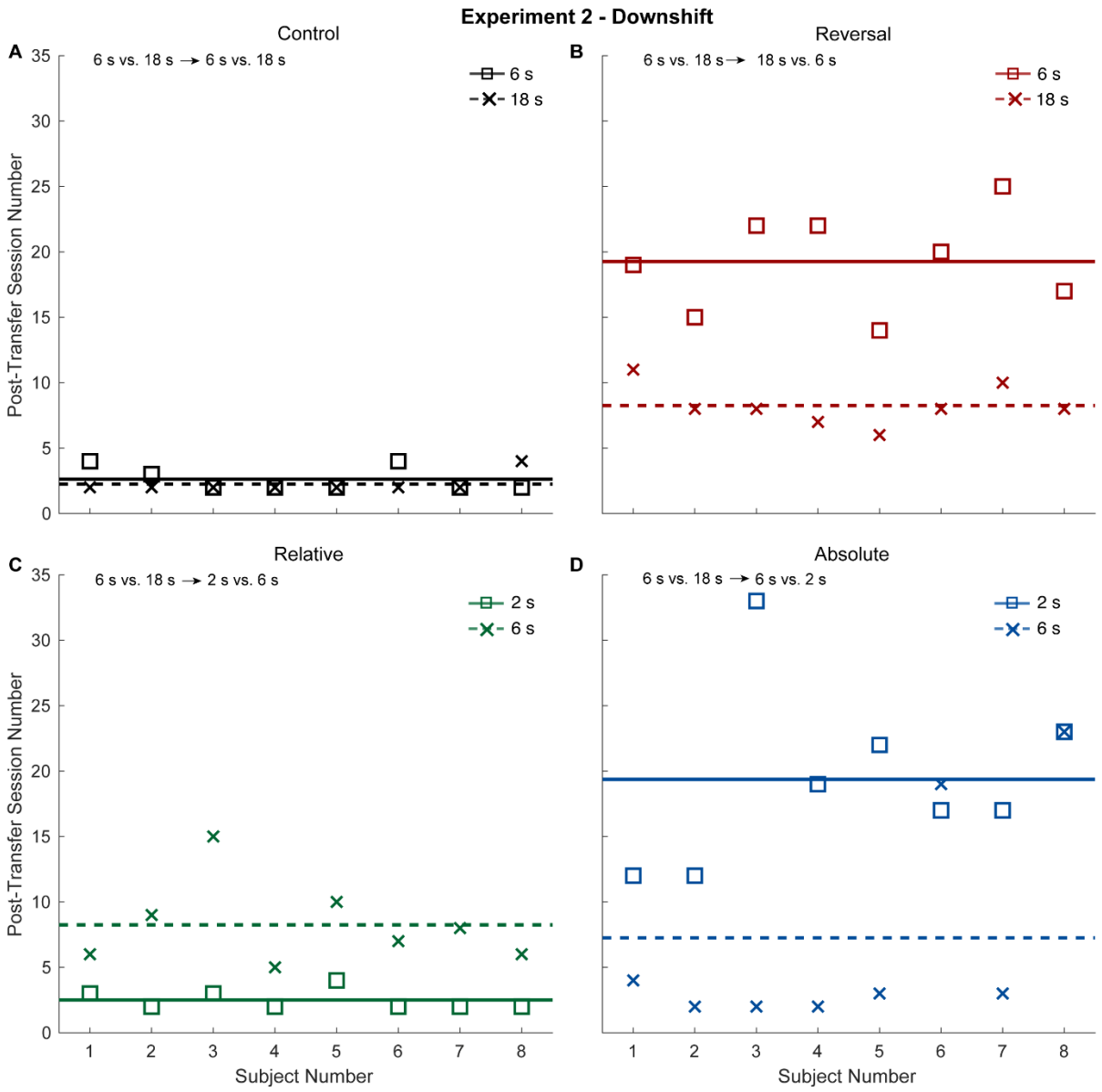

Figure 2.4. Relearning speed in Experiment 2. Number of sessions required to perform at ≥ 75% accuracy shown separately for short and long cue durations in Phase 2 transfer test. Lines (solid: short duration, dotted: long duration) show the mean session numbers and symbols (square: short duration, cross: long duration) illustrate individual subjects' data.

Finally, after completing the transfer test with either the 6 vs. 18s (control and reversal) or the 2 vs. 6s (relative and reversal) duration pairs, we tested the animals in one final transfer

condition with a new set of durations (4 vs. 12 s) in Phase 3. In all groups, one of these time intervals was the arithmetic mean of a previously learned duration pair in Phase 2. If absolute times control responding, we expected one element of this new duration pair to create ambiguity and drive the discrimination accuracy down to chance level (Molet & Zentall, 2008; Zentall et al., 2004). This should occur for the 12-s cue duration in Group$_{MeanLong}$ that was previously tested with 6 and 18 s (control and reversal groups of Phase 2), and for the 4-s cue duration in Group$_{MeanShort}$ that was previously tested with 2 and 6 s in Phase 2 (relative and absolute groups of Phase 2). Despite these changes in absolute time intervals, however, we hypothesized that preserving the relative mapping would result in rapid acquisition. The 6-s short time was shifted to the even shorter 4-s time in Group$_{MeanLong}$, and the 6-s long time was shifted to the even longer 12-s time in Group$_{MeanShort}$. As can be visualized in Figure 2.5, even though there was an initial drop in discrimination accuracy for the ambiguous cue durations, the performance was still above chance level, and further improved and stabilized after only one or two days of training with the new duration pair.

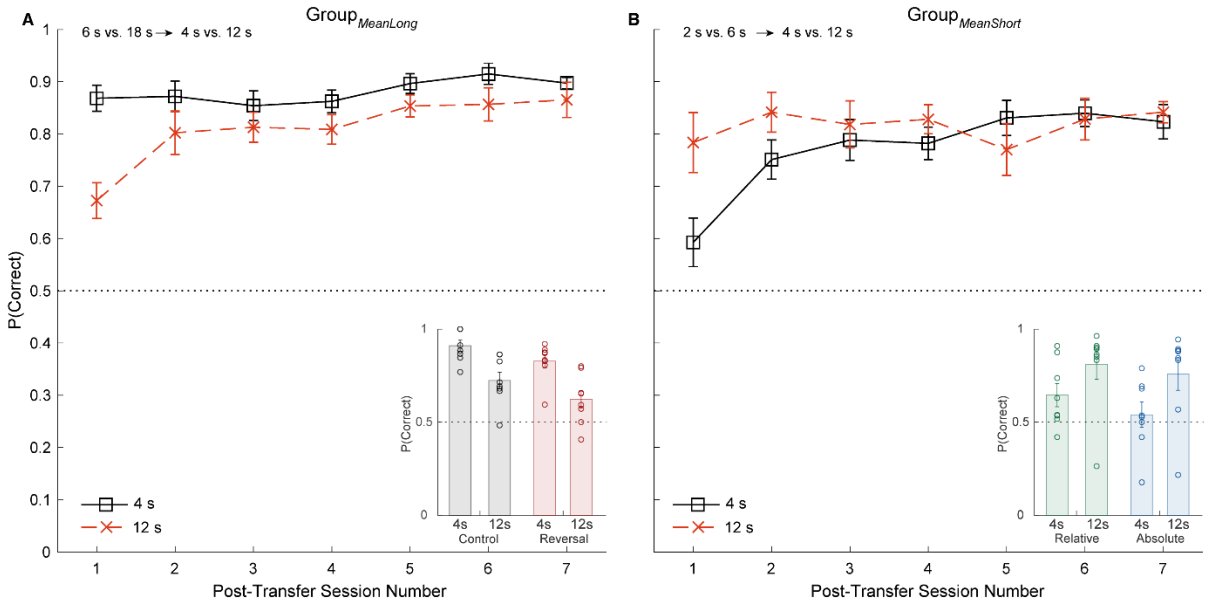

Figure 2.5. Discrimination accuracy in Phase 3 of Experiment 2. Proportion of correct responses on short and long trials after transfer are shown separately for both experimental groups in Phase 3 of Experiment 2. Group$_{MeanLong}$ (A) consisted of the control and reversal groups of Phase 2, Group$_{MeanShort}$ (B) consisted of the relative and absolute groups of Phase 2 (refer to the inset plots for each sub-groups' discrimination accuracy in the first post-shift session). Note that we only included the first 7 post-transfer sessions in this graph, as some animals completed Phase 3 in 7 sessions, whereas others required more prolonged training. Error bars show *SEM*.

A mixed ANOVA analyzing the proportion of correct responses on short (4 s) and long (12 s) trials in the first transfer session of Phase 3 revealed a significant main effect of Group, $F(1, 30) = 4.53$, $p = .04$, $MSE = 0.11$, $n_p^2 = 0.13$, and a Duration x Group interaction, $F(1, 30) = 17.55$, $p < .001$, $MSE = 0.60$, $n_p^2 = 0.37$. Simple main effects analyses showed that the proportion of correct responses for the short cue (4 s; $p < .001$), but not for the long cue (12 s; $p = .11$), differed between two groups. We also found significant differences in the accuracy of short and long cue categorizations both in Group$_{MeanLong}$ ($p < .001$) and Group$_{MeanShort}$ ($p = .04$). Group$_{MeanLong}$, which was formerly tested with 6 and 18 s, responded accurately in 87% of 4-s trials ($SD = .10$), whereas the group-average accuracy dropped to 67% on the now ambiguous

12-s trials (*SD* = .14). On the other hand, Group$_{MeanShort}$, which was tested with 2 and 6 s in Phase 2, showed the opposite pattern during the final transfer test and performed accurately in 78% of 12-s trials (*SD* = .23), whereas the average accuracy dropped to 59% on the ambiguous 4-s trials (*SD* = .19). Finally, an independent-samples *t*-test on the number of sessions needed to reach criterion of at least 75% correct in two consecutive sessions in Phase 3 revealed that training length did not differ between the two groups, $t(30) = -0.98$, $p = 0.33$ (Group$_{MeanLong}$: $M = 7.75$, $SD = 1.53$; Group$_{MeanShort}$: $M = 9.31$; $SD = 4.25$ sessions).

Discussion

As in the Upshift Experiment, we again found evidence for the use of both absolute and relative representations in a temporal discrimination task. Our results from the first transfer test (Phase 2) indicated that preserving the absolute and/or relative relations of durations and the associated response locations helped with initial discrimination performance. Furthermore, preserving relative mapping also led to faster adaptation to new temporal and spatial mappings. On the other hand, preventing animals from relying on such relative relations, as in the case of the absolute group, or on both relative and absolute relations, as in the case of the reversal group, resulted in a significant increase in the difficulty of achieving sustained accurate performance in the transfer phase. Furthermore, when we tested the subjects with two novel durations and preserved the relative mapping in Phase 3, we found that the discrimination accuracy showed only slight deterioration, despite the use of an ambiguous duration that was at the arithmetic mean of a previously learned duration pair. Although there were several subjects whose discrimination performance deteriorated for the ambiguous cue duration, group accuracy levels were still above chance. Taken together, these findings indicate that animals have knowledge

about absolute durations and use them to guide behavior, but they also have knowledge of the relative relationship between durations and this knowledge is not easily modified.

2.4 General Discussion

In the present study, we investigated the nature of temporal encoding in a duration discrimination task. We were particularly interested in understanding whether animals use absolute or relative representations of time to guide their temporal choices. To this end, we trained mice to correctly discriminate different time intervals and then either increased or decreased the cue durations and/or switched the associated response locations, which resulted in changes in relative and/or absolute mappings of duration-lever assignments. Specifically, in the absolute groups, the response that earned reward for one of the durations was preserved from the initial training to the transfer phase. In this group, the subjects had to learn new responses based on the new relative durations. Thus, we would expect positive transfer in this group based on absolute durations and negative transfer based on relative duration. We expected the opposite to be true in the relative groups. These subjects had to learn to make a new response to the duration that was preserved across phases, but the correct action based on relative durations was preserved. Here, the use of absolute times would lead to negative transfer, but the use of relative times would produce positive transfer. In the reversal groups, subjects had to learn to make new responses based on both absolute and relative representations, and negative transfer should predominate. Overall, our findings indicate that the transfer occurred most readily when relative relationships of durations and response locations were maintained. In contrast, when the animals had to re-map these relative relations, their temporal discrimination ability was impaired, and they required extensive training to re-establish temporal control. However, preserving the response location of one of the absolute cue durations in such conditions enabled animals to

initially maintain accurate post-shift duration categorization performance. These results demonstrate that animals encode both absolute and relative representations of duration.

At first glance, our findings might seem at odds with previous studies providing evidence for mainly one type of time encoding in humans and nonhuman animals (e.g., Carvalho & Machado, 2012; Thomaschke et al., 2015; Zentall et al., 2004). However, we think that our evidence for the use of both types of temporal representations is not surprising, as it is well-established that organisms can process, learn, and adapt to varying temporal and stimulus contexts (e.g., Akdoğan & Balcı, 2016; Avlar et al., 2015; De Corte et al., 2018; Jazayeri & Shadlen, 2010). Even though our findings indicate that categorizations heavily rely on relational control of timing behavior, they also provide clear evidence that animals also utilize absolute temporal information.

Before going into a more detailed discussion on temporal representations, we would like to note that in the absolute and reversal groups, there was a trend for accurate performance to emerge faster to the longer cue than the shorter one. This is, of course, not surprising for the Downshift absolute group that had to press the same lever after the long (6 s) tone presentations in both phases. However, this finding for the Upshift absolute group is particularly puzzling, as the response location of the short cue (6 s; former long cue) was maintained, which initially produced positive transfer, leading us to expect that the short cue might be learned faster throughout the transfer phase. It is unclear to us whether training history plays a role in learning speeds. More interestingly, why this asymmetry would also be true in the reversal groups is not obvious. One possibility is that larger magnitudes may be more salient than smaller ones in general. In fact, a small but diverse body of evidence has shown that discriminations based on odor, sound, physical length, and temporal durations are acquired faster when the outcome is

signaled with the larger of the two magnitudes (for a review see Inman & Pearce, 2018). Although most timing research focuses on steady-state timing behavior and overlooks how temporal control over behavior is acquired (but see Balci et al., 2009; Machado, 1997), however, Church and Deluty (1977) did not report any differences in the acquisition of short vs. long duration discriminations in their seminal temporal bisection study. Therefore, we think that the question of whether the asymmetry appears during the initial training phase or is specific to reversal learning is an interesting one but not settled.

Absolute durations control initial transfer

In all transfer conditions in all of our experiments the initial transfer seems to be guided by the absolute time durations. In Experiment 1, subjects were first trained on a 2-s vs. 6-s discrimination. When switched to a 6-s vs. 18-s discrimination, the absolute group showed initial positive transfer to the 6-s cue, while the relative and reversal groups showed initial negative transfer. This pattern of results is consistent with the hypothesis that subjects learned to press a particular lever in the presence of the 6-s duration, and initial transfer depended on whether this mapping was preserved. Similarly, in Experiment 2, subjects were first trained on a 6-s vs. 18-s discrimination. When switched to a 2-s vs. 6-s discrimination, the absolute group showed initial positive transfer to the 6-s cue, while the relative and reversal groups showed initial negative transfer. This pattern of is also consistent with the hypothesis that subjects learned to press a particular lever in the presence of the 6-s duration, and initial transfer depended on whether this mapping was preserved. In the last phase of Experiment 2 (Phase 3), subjects were transferred to a 4-s vs. 12-s discrimination. These values were the arithmetic means of the duration pairs used in Phase 2. Specifically, the 12-s cue was the arithmetic mean of the 6- and 18-s cue durations learned by the control and reversal mice, and the 4-s cue was the arithmetic mean of the 2- and 6-

s cues that were used for the relative and absolute mice. The relative mapping was the same as in the previous phase for all groups. Based on generalization from the absolute durations of Phase 2, we expected that the duration that was at the mean of the previous durations to neither help nor hinder the transfer, and that the novel cue to be facilitated. For example, in the groups transferred from the 6-s vs. 18-s discrimination we expected that the tendency to press the former 6-s lever would generalize strongly to a 4-s cue, and there would be an equal tendency to press both levers after the 12-s cue. This is indeed what we found in the first session. Similarly, in the groups transferred from the 2-s vs. 6-s discrimination, we expected that the tendency to press former 6-s lever would generalize strongly to a 12-s cue and there would be an equal tendency to press both levers after the 4-s cue. This pattern too was confirmed in the first session data for these groups. Overall, our data provide strong evidence that the dominant representation that governs performance at the start of a transfer test is an absolute one. Clearly, the relation between absolute durations and specific responses is encoded during temporal discriminations.

Relative representations exert long lasting control over responding

Several features of the data suggest that relative representations of durations are also encoded and used in temporal discrimination learning. In both experiments, there was initially positive transfer to the preserved duration in the absolute transfer groups. In these groups, subjects had to learn to make new responses based on relative durations. Surprisingly, despite the positive initial transfer, performance to the preserved duration deteriorated over the first few sessions of training and most subjects never recovered baseline accuracy.

One explanation for this decline in discrimination performance to the preserved duration is that the transfer test temporarily reduced stimulus control by cue durations and strengthened stimulus control by lever locations (Carvalho & Machado, 2012; Carvalho et al., 2016). Take the

case of the Downshift absolute group: After learning to press the left lever following the 6-s cue and the right lever following the 18-s cue, the animals learned to press the right lever following the 2-s cue and continued to press the left lever following the 6-s cue. Due to temporal generalization, most of these mice started to choose the left lever after the 2-s cue and experienced extinction for pressing the left lever. The occasional reinforcements following presses on the right lever as well as the extinction episodes could explain the generalized bias towards the right lever, and thus the marked decline in discrimination accuracy on 6-s trials. This competition between stimulus control by cue durations and lever locations could explain the acquisition trade-off observed in most of the transfer sessions in the absolute groups. If this were the explanation for the deterioration in performance, then we should see identical changes in the Downshift relative group. After learning to press the left lever following the 6-s cue and the right lever following the 18-s cue, the animals learned to press the left lever following the 2-s cue and the right lever following the 6-s cue. Due to temporal generalization, most of these mice started to choose the left lever after both 2- and 6-s cues. As training continues for the 6-s cue, the reinforcements following presses on the right lever as well as the extinction on the left lever should strengthen the tendency to make right lever responses. Generalization of this tendency to the 2-s cue should produce a deterioration of performance on the left lever as it did in the absolute group. Instead, despite initially showing negative transfer to the preserved duration, subjects in the relative groups of both experiments recovered high accuracy very rapidly without any deterioration in performance. Based on generalization alone we would not expect a difference between absolute and relative groups. We, therefore, think that relative representations also play a particularly crucial role when animals are learning a new duration pair.

Taken together, these patterns of results suggest that animals might use single or multiple types of temporal representations to learn task contingencies, which might change during the course of training or when tested under different experimental conditions. One possibility is that subjects had learned to respond on the basis of absolute durations in Phase 1, but the relative relation between durations guided the remapping of responses. It is also possible that, subjects learn temporal discriminations with relative representations and with training they switch to using absolute representations. When the absolute representations fail to work in the transfer phase, perhaps they then revert to using relative representations. Alternatively, perhaps they initially learn with absolute representations and only switch to relative ones when the first strategy fails. It is not clear why one type of representation would be favored over the other one in this task, as both could result in successful performance.

The third phase of Experiment 2 is consistent with these speculations. In that phase, one of the durations was the mean of the durations experienced in the previous phase. This meant that based on control by absolute time the two choices ought to be of approximately equal strength. Furthermore, in this test the relative mapping of durations to responses was preserved. Here, where there was no basis for negative or positive transfer from an absolute encoding perspective, we observed positive transfer in many subjects and rapid acquisition of the new discrimination, as would be expected from having preserved the relative mapping. Although we think that our results pose a challenge for an absolute coding model, data simulations might be required to more fully assess both absolute and relative coding approaches.

Metric representations of time

Even though our study shows that animals form both absolute and relative temporal representations, one particularly interesting finding was the amount of individual differences in

how fast the animals adapted to the uncertainty owing to the changes in temporal and spatial contingencies. Our statistical test results as well as the visual inspection of individual subjects' data consistently revealed that some animals showed immediate transfer, whereas others required extensive training to learn new task parameters, even when tested under the same experimental conditions. These findings collectively raise the questions of at what point mice learn relative and absolute time representations and to what extent they utilize either type of temporal information to guide their time judgments in stable and dynamic task structures. It is possible that animals mainly rely on one type of representation in a stable temporal context and then flexibly adjust the type of temporal code after facing more uncertain environments in which there is variability in the number and the length of time intervals, as well as in the associated responses. However, it is a methodological challenge to examine such "subjectivity" of temporal representations and response strategies, as it is unclear when individual animals detect or perceive changes in temporal contexts and update their beliefs accordingly. Nonetheless, these outstanding questions about how time is represented under stable and uncertain conditions should be addressed at both the behavioral and the neural levels to provide a more thorough understanding of the nature of temporal codes.

As a way of understanding the different ways in which time can guide behavior, we suggest that the underlying representation of time has the properties of a ratio scale. Consequently, when asked to discriminate between two durations, one can use this metric knowledge in multiple ways. Subjects could learn to respond in the presence of a specific duration. This is what we have referred to as the absolute representation. Alternatively, via subtraction or addition from a standard in memory, subjects can respond to the larger of two magnitudes. Alternatively, via division the larger or smaller alternative can be determined. The latter two computations are

what we have referred to as the relative representations. While we can conclude that absolute and relative representations were used in the current experiments, we cannot say which of the possible computations underlie the formation of relative representations.

Relevantly, there is mounting evidence that there are reliable neural correlates of a metric representation of time that suggest that both absolute and relative relationships are computed. For instance, Mello et al. (2015) recorded from individual striatal neurons in rats during a *serial fixed-interval* task in which they varied the length of fixed time intervals in different test blocks, after which food rewards became available. Their results showed that at the population level, two-thirds of striatal neurons exhibited a similar temporal trajectory in firing patterns and maintained their ordinal peak activity position across different target intervals ranging from 12-60 s. The rescaling of the firing patterns with changes in duration, provides support mainly for relative encoding of time in the striatum. A particularly relevant study by Shimbo et al. (2021) recently investigated the hippocampal CA1 neuronal activity in rats during a duration discrimination task, in which duration pairs were scaled up or down in different blocks of trials (e.g., block 1: "10s/Left, 5s/Right"; block 2: "20s/Left, 10s/Right"; block 3: "10s/Left, 5s/Right". Their findings revealed that activity patterns of an assembly of CA1 pyramidal cells compressed or stretched in time as a function of the increases or decreases in the durations of timed intervals, suggesting scalable temporal representations in the rat hippocampus.

Such compression or dilation observed in neural activity in response to changes in temporal contingencies, referred to as *temporal scaling*, has been reported also in other brain regions and behavioral tasks (e.g., Emmons et al., 2017; Gouvêa et al., 2015; Komura et al., 2001; Leon & Shadlen, 2003; Wang et al., 2018; Xu et al., 2014). However, despite the clear evidence for such relative neural signals in the brain, which are potentially useful for adaptive

behavior and efficient neural processing, Mello et al. (2015) also reported that one-third of neurons in the striatum did not preserve their ordinal position in their firing activity across different target durations. Perhaps these neurons were encoding absolute temporal information which could be multiplexed in striatal neuron populations (Motanis & Buonomano, 2015). Previous research on the neural basis of temporal encoding also provided evidence consistent with the existence of temporally specific neurons in the striatum by demonstrating that neurons show distinct firing patterns for individual durations (Matell et al., 2003). Furthermore, other studies show that only a subset of neurons involved in timing rescaled (e.g., Meirhaeghe et al., 2021; Mello et al., 2015; Shimbo et al., 2021), which indicates that a proportion of neurons possibly also encodes absolute temporal information (Motanis & Buonomano, 2015).

Here it is important to note that, most previous studies on the neural basis of temporal processing assume that an absolute temporal representation takes the form of neural activity responding to specific durations, whereas relative temporal representations refer to the adaptive rescaling of neural activity to different time intervals. Based on our study as well as other behavioral findings mainly from the Machado lab, we would like to caution the reader against this dichotomy and suggest that time is represented in a way that allows for multiple representations to be computed to guide performance.

In our experiments we pitted the different types of representations against one another in the transfer tests. It appears that while only one type of temporal representation dominates at different points of training, multiple representations may exist. In the absolute groups, there was an initial positive transfer based on retraining the same relationship between an absolute time and a specific response. However, as training progressed it appeared the subjects became aware that the preserved duration, though of the same absolute duration, was no longer in the same relative

position (longer or shorter). This conflict produced a very pronounced deterioration of performance that only slowly recovered with extensive training. Whether this strong control by the relative representation is a general principle or specific to the current task and training procedure is worthy of future study, as task structures might contribute to the variability observed in how the brain encodes time (Zhou et al., 2022). Another important aspect of understanding the neural basis of timing and our findings will be to understand how time-varying patterns of neural activity (e.g., population clocks, ramping activity, etc.) are mapped onto motor outputs during learning.

In sum, the current set of experiments show that mice can represent experienced durations both as having a certain magnitude (absolute representation) and as being shorter or longer of the two durations (an ordinal relation to other cue durations), with relational control having an enduring influence in governing temporal discriminations. We think that it is actually this ability of describing temporal relations and event durations in various ways that enables organisms to perform arithmetic operations of time intervals in an effective manner. Consequently, individuals are able to make common relativized descriptions such as identifying the shortest and or the longest durations, or computing the average of different time intervals in various contexts to adapt behavior and guide decisions. It also enables brains to compute the sum or difference of a number of durations (Gibbon & Church, 1981; Takahashi & Watanabe, 2015; Wearden, 2002), which helps individuals to adaptively execute behaviors at the correct time and in the correct amount of time.

2.5 Supplemental Material

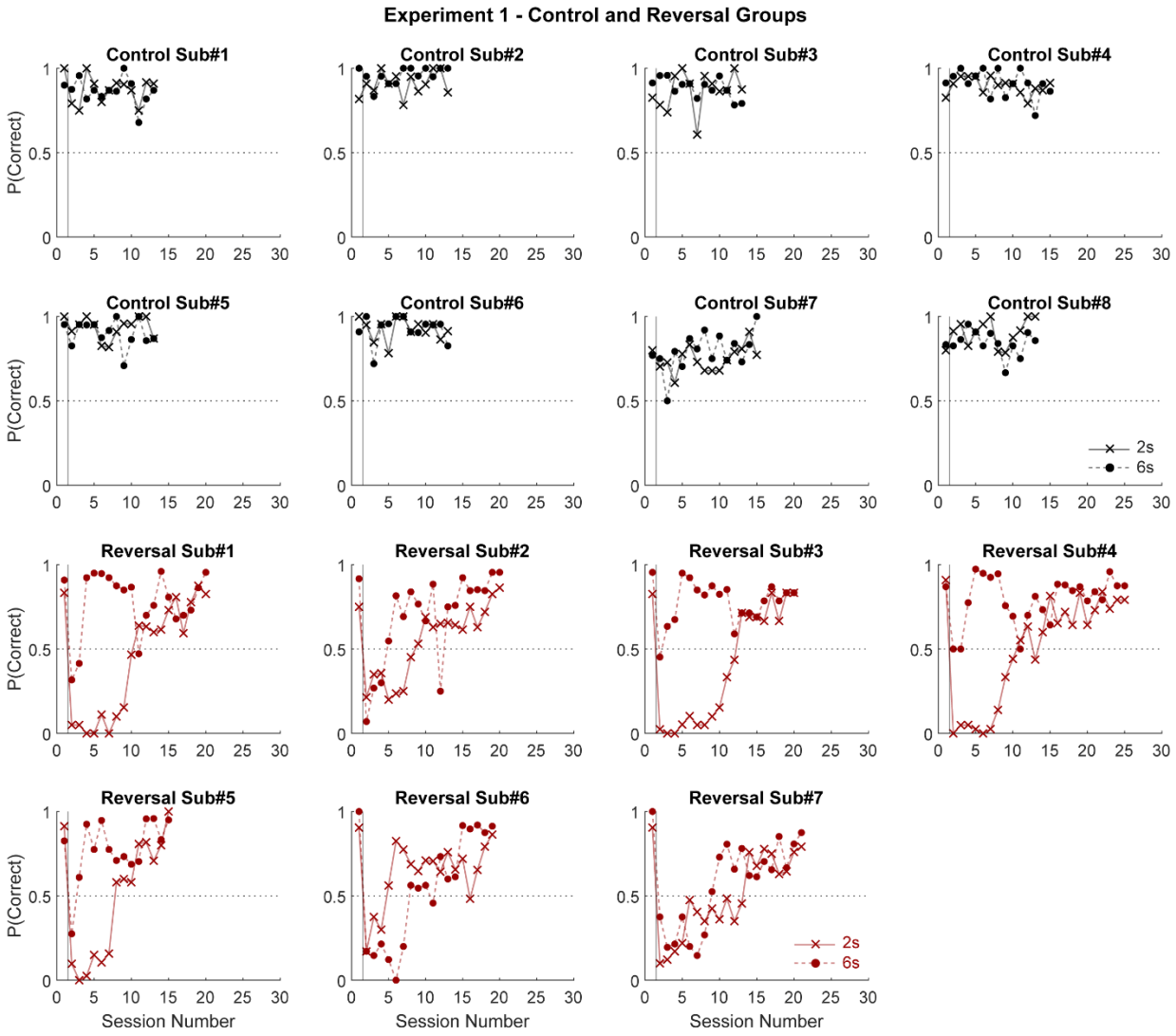

Figure 2.S1. Individual acquisition curves of control and reversal subjects in Experiment 1. Vertical lines indicate the start of the transfer test. The proportion correct of responses on short and long trials in both the last pre-transfer session and all post-transfer sessions are shown for each individual animal.

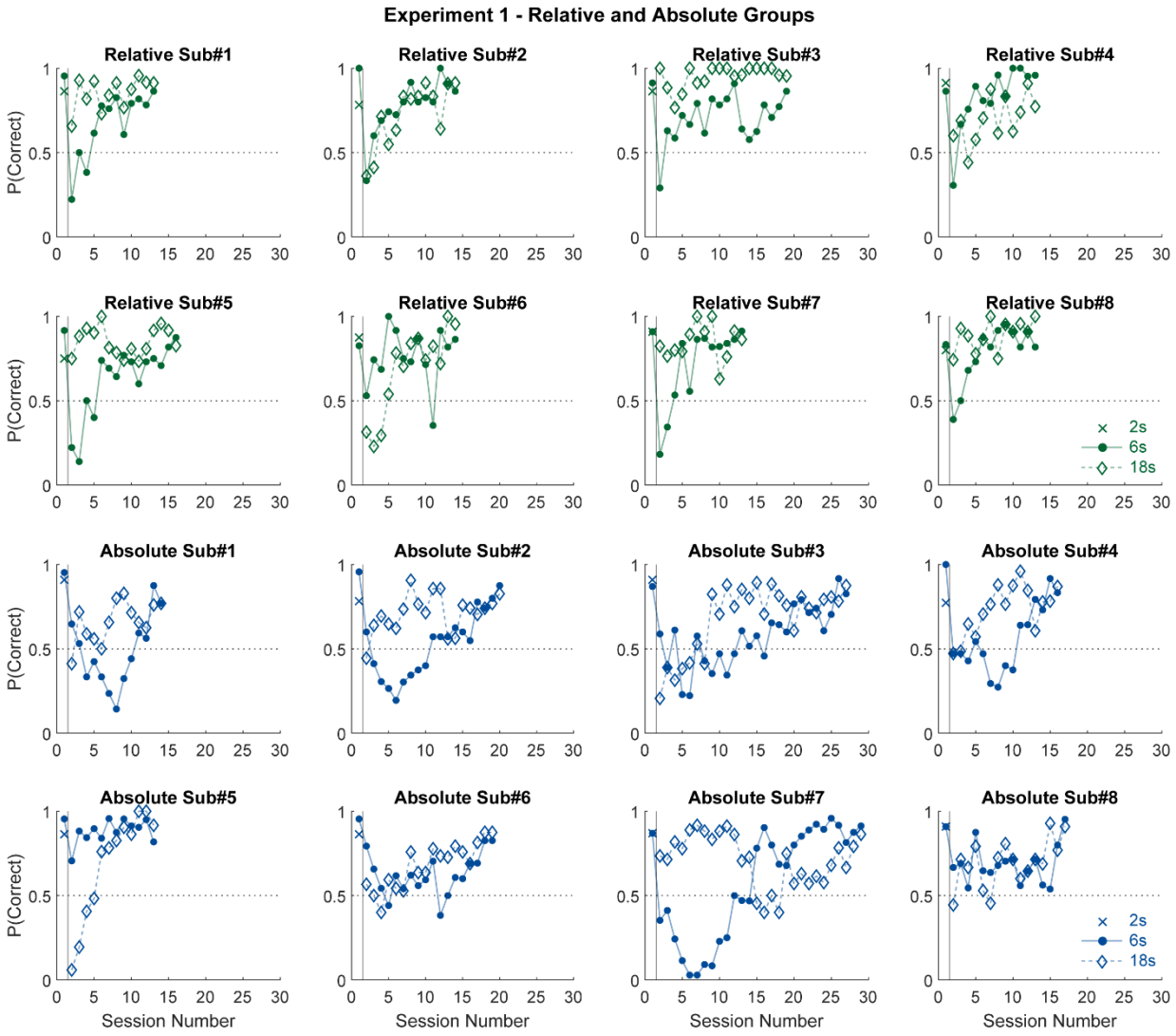

Figure 2.S2. Individual acquisition curves of relative and absolute subjects in Experiment 1. Vertical lines indicate the start of the transfer test. The proportion correct of responses on short and long trials in both the last pre-transfer session and all post-transfer sessions are shown for each individual animal.

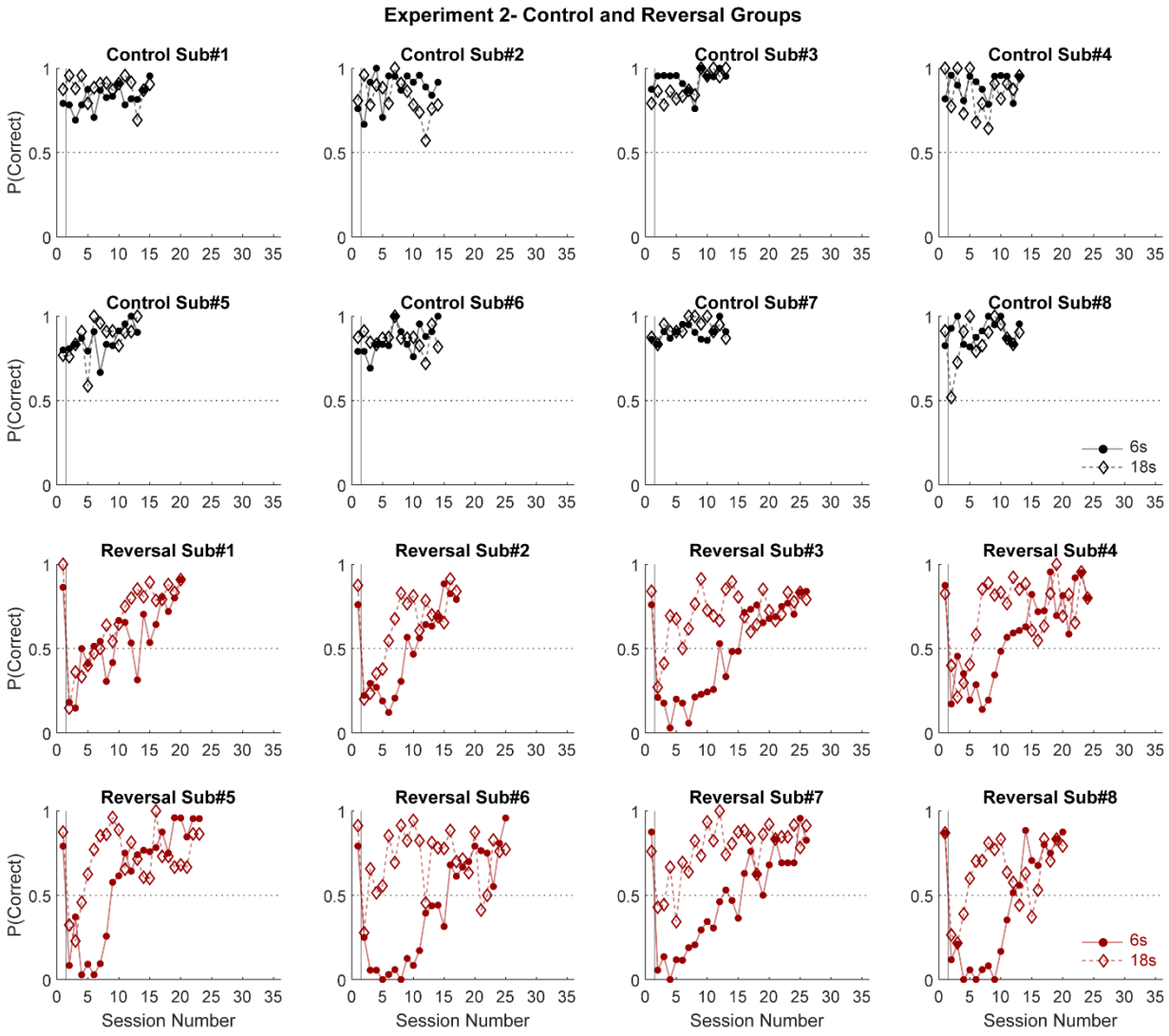

Figure 2.S3. Individual acquisition curves of control and reversal subjects in Experiment 2. Vertical lines indicate the start of the transfer test. The proportion correct of responses on short and long trials in both the last pre-transfer session and all post-transfer sessions of Phase 2 are shown for each individual animal.

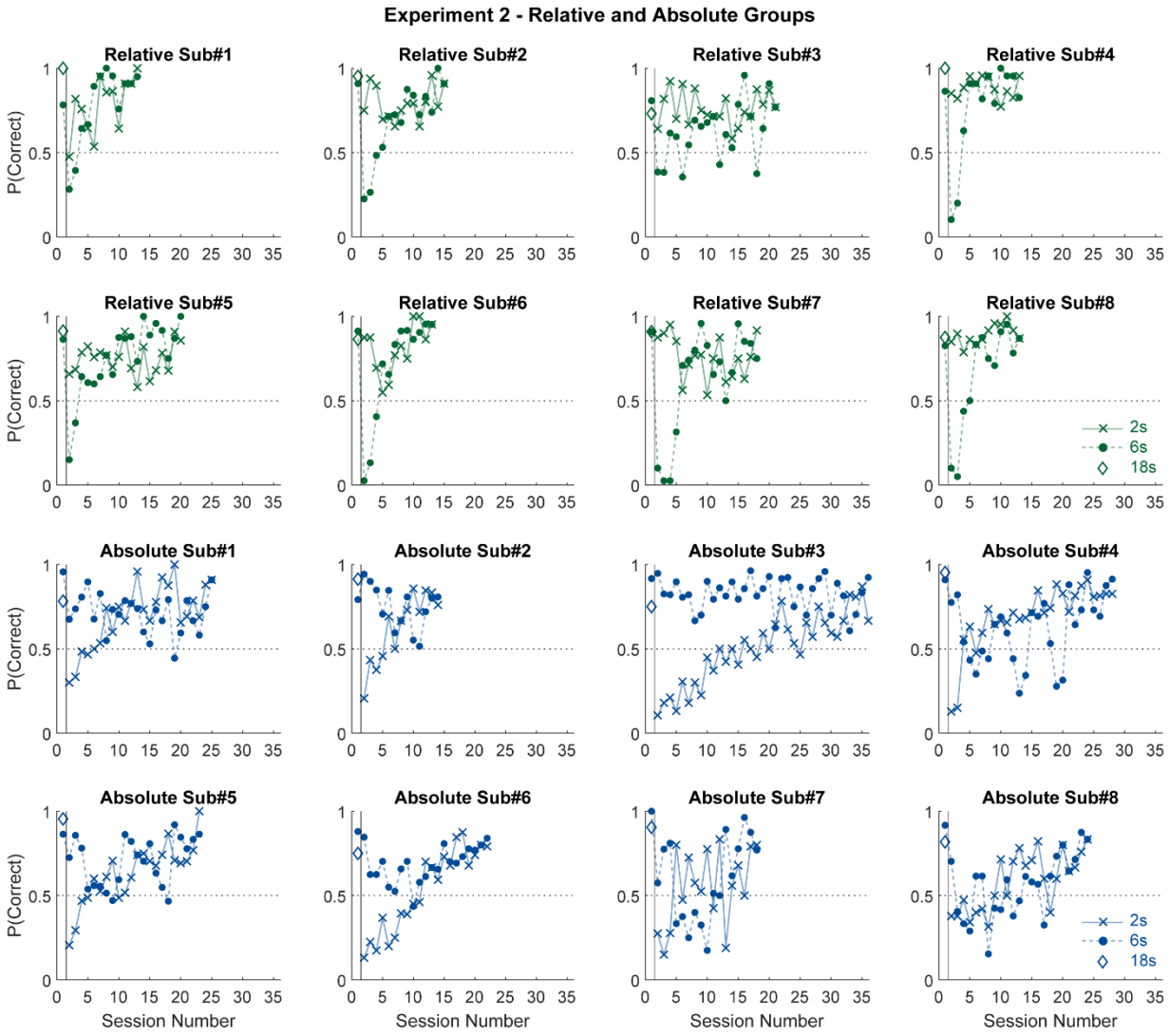

Figure 2.S4. Individual acquisition curves of relative and absolute subjects in Experiment 2. Vertical lines indicate the start of the transfer test. The proportion correct of responses on short and long trials in both the last pre-transfer session and all post-transfer sessions of Phase 2 are shown for each individual animal.

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Chapter 3: Temporal Arithmetic: Mental Addition of Time Intervals

3.1 Introduction

Accurate and flexible use of time is a pervasive element of life. One must be constantly able to adaptively execute behaviors both at the correct time and in the correct amount of time. Indeed, the need for this capability is present from the very moment of waking up. Knowing the length of a morning routine requires being able to add the durations of multiple activities such as brushing teeth, getting dressed, and eating breakfast. This information is then used to determine when to get out of bed to leave enough time to get ready for and then travel to school or work. In the cases of waking up early or oversleeping, adjustments are made to spend greater or lesser amounts of time on certain activities of a morning routine. This example illustrates that a working use of time underlies even the seemingly simple act of reaching the first destination of a day and that its absence could lead to problematic premature or delayed arrivals. Perhaps it is then not surprising that distinct impairments related to temporal processing are characteristic of various neuropsychiatric disorders that can lead to difficulties in maintaining both personal and professional commitments (e.g., Bauermeister et al., 2005; Bonnot et al., 2011; Bschor et al., 2004; Meck, 2006; Pastor et al., 1992). It is thus crucial to understand the behavioral properties of temporal processing as well as the mental operation of time intervals to advance how they contribute to functional and impaired behavior.

Accordingly, a substantial amount of research has been conducted on timing and time perception in a variety of species. Important insight regarding the use of single durations has been gained using various timing tasks, such as the peak-interval (PI) procedure (Catania, 1970; Roberts, 1981). During a PI procedure, fixed-interval trials in which responses after a fixed

duration are always reinforced become intermixed with peak trials in which responses at a to be timed duration are required but not reinforced. Subject responses during peak trials typically become centered around the to be timed duration and exhibit variability scaled to this criterion (Balsam et al., 2009; Gibbon, 1991; Meck & Buhusi, 2010). This supports the scalar timing theory (Gibbon, 1977; Gibbon, Church, & Meck, 1984) which posits that timing of single durations exhibits scalar variability, meaning estimates of durations are roughly linear with actual durations but precision is relative to duration length. An increased criterion duration should therefore coincide with an increased standard deviation of estimates (Gibbon, 1991). While there is considerable evidence to support these two main statistical properties in the timing of single durations, they remain largely unexplored in relation to the timing of multiple durations as well as to the mental operations of time intervals.

In fact, timing of multiple intervals has overall been little studied despite its behavioral relevance. It should be noted that executing appropriate behavior most often requires mentally manipulating two or more durations of time, as well as the integration of event memories across timescales (e.g., Clewett et al., 2019; Sols et al., 2017). Of the limited research examining these types of computations, most studies have indirectly focused on factors that may create differences between timing of single intervals and timing of multiple intervals (e.g., Block, Hancock, & Zakay, 2010; Jazayeri & Shadlen, 2010; Seifreid & Ulrich, 2011). First, it is known that encoding multiple durations requires more attention and memory than encoding a single duration does. This is of importance because it has been found that increased cognitive load, referring to attentional and working memory demands, leads to longer retrospective duration judgments (Enns et al., 1999; Block et al., 2010). Second, it is known that the presence of multiple intervals inherently means the existence of a temporal context, referring to the presence

of other durations before or after a duration to be timed. This is of relevance because it has been found that temporal contexts cause duration judgments to tend towards the mean of all durations presented (Jazayeri & Shadlen, 2010). Such an outcome is likely tied to the fact that memory prototypes are based on both specific information, linked to the current to be timed duration, and average representations, gathered from all presented to be timed durations (McClelland & Rumelhart, 1985). Third, it is known that use of multiple durations requires simultaneous mental manipulations. This is of interest because it has been found that duration judgments are less precise when attention must be divided between timing and a concurrent task (Seifreid & Ulrich, 2011). Cognitive load and temporal context may uniquely affect representation of multiple intervals while their use may be subject to the effects of simultaneous manipulations.

In relation to manipulation of multiple intervals, one recent area of research has offered further understanding by studying mental addition of time intervals. Notably, Fortin et al. (2000) has examined how temporal judgments are affected by breaks in time. Using a gap procedure, they presented a tone signaling a target interval broken into two parts and then required participants to produce the target interval without including the break in its presentation. Thus, there was a potential requirement to sum pre-break and post-break durations. It was found that lengths of durations were overestimated when there was a break in time in comparison to when there was no break in time, perhaps suggesting an overestimation bias for summation (Fortin et al., 2000). Interestingly, Takahashi & Watanabe (2015) conducted a study using a temporal reproduction task in which participants were explicitly instructed to produce both two durations (0.6-1.1s) successively and the sum of two durations and similarly found relative overestimation for summation in both visual and auditory modalities.

It is possible that these results occurred because a requirement to sum multiple intervals produces a high cognitive load and, as aforementioned, therefore leads to longer perceived durations (Takahashi & Watanabe, 2015). Further, it should also be considered that overestimation during summation of time may result from similar processes involved in overestimation found in summation of spatial durations and numbers (Walsh, 2003). A representational momentum effect has been identified in which overestimation occurs for the addition of spatial durations (Walsh, 2003). In addition, several studies have shown an operational momentum effect in which answers for addition problems are overestimated whereas answers for subtraction problems are underestimated (Knops et al., 2014; Knops et al., 2009; McCrink et al., 2007). It has been proposed that spatial-numerical associations present in a "mental number line" may be responsible for the tendency to process addition as exaggerated rightward movement and subtraction as exaggerated leftward movement (Knops et al., 2009; McCrink et al., 2007). It is not unreasonable then that studies requiring summation of temporal durations demonstrated an overestimation bias.

However, it should not be dismissed that the overestimation observed may have also resulted from a strategy other than summation in these studies. In the study by Fortin et al. (2000), it is possible that participants stopped timing at the gap and resumed timing after the gap, therefore using a stopwatch like strategy to time rather than mental addition (Narkiewicz et al., 2015). In the study by Takahashi & Watanabe (2015), participants knew in advance that they would be summing the next durations presented. Therefore, they similarly could have used a stopwatch like strategy by learning to stop timing after the end of the first duration and resume timing after the start of the second duration (Narkiewicz et al., 2015). In both cases, using a "stopwatch" strategy would certainly require less exertion than repeated mental summation.

To address this limitation in previous research and ensure that results observed are in fact due to mental addition of time, this study limited the ability to use a "stopwatch" strategy by requiring participants to sum time intervals without advance knowledge of an addition requirement. To further understand arithmetic operations are how time intervals are manipulated, we tested participants in a temporal reproduction task. In this procedure, they were serially presented with two different durations, and then were randomly instructed to produce the first duration, second duration, or sum of the durations. They were thus required to first encode durations and then perform mental computations. Temporal judgments were analyzed with respect to their accuracy and precision characteristics by using hierarchical models to fully capture the nature of mental addition and to account for individual differences that are largely neglected in the timing field.

3.2 Method

Subjects

Thirty-four adults (M_{age} = 19.94, SD_{age} = 2.94, $range_{age}$ = 18-35) participated in the experiment. They were recruited through subject pools for Introductory to Psychology students at Barnard College and Columbia University in return for partial course credit. Participants were screened for a history of psychiatric or neurological conditions and medication use, which was considered in the statistical analyses presented below. The experiment was approved by the Institutional Review Board at Barnard College, and all participants provided written informed consent prior to the experiment.

Stimuli and Apparatus

Participants were seated at a viewing distance of approximately 50 cm. The visual stimuli used were either a blue square or an image of two blue squares with a centered black plus sign

presented in the center of a gray background. Stimulus presentation was controlled via PsychoPy2, and responses were collected with a standard computer keyboard.

Procedure

Participants were tested on a computer-based *temporal reproduction* task, in which they were first presented with different durations and were then instructed to estimate either a single duration or the sum of two durations as accurately as possible. There were two types of trials: *addition* and *serial-interval* trials (see Figure 3.1). Both in serial interval and addition conditions, participants initiated the successive presentations of two different time intervals signaled by separate blue squares. After the duration presentations in serial trials, participants were randomly instructed to produce either the first or second duration, whereas in addition trials, they were asked to produce the sum of the two durations.

In both conditions, durations were a fixed 1.5 s plus a random value drawn from a mixture of two exponential distributions, one with a mean of 3 s (in 75% of trials) and the other with a mean of 5 s (in 25% of trials). Initiation of all target duration presentations was made by pressing the spacebar, and time productions were made by pressing the spacebar for a start time and then once again for a stop time when the participants thought that the produced duration has matched the target duration. During productions of single durations (in serial trials), an image of a single blue square appeared, and during productions of sums of durations (in addition trials), an image of two blue squares with a centered plus sign appeared on the computer screen. Each trial was followed by an intertrial interval (ITI) which was a random value sampled from a uniform distribution ranging between 1.5 s and 2 s. No performance feedback was provided to the participants. The experiment consisted of a randomized set of 40 serial trials and 40 addition

trials, and lasted approximately 35 min. Participants were allowed to take a break every 20 trials and were instructed not to count throughout the experiment.

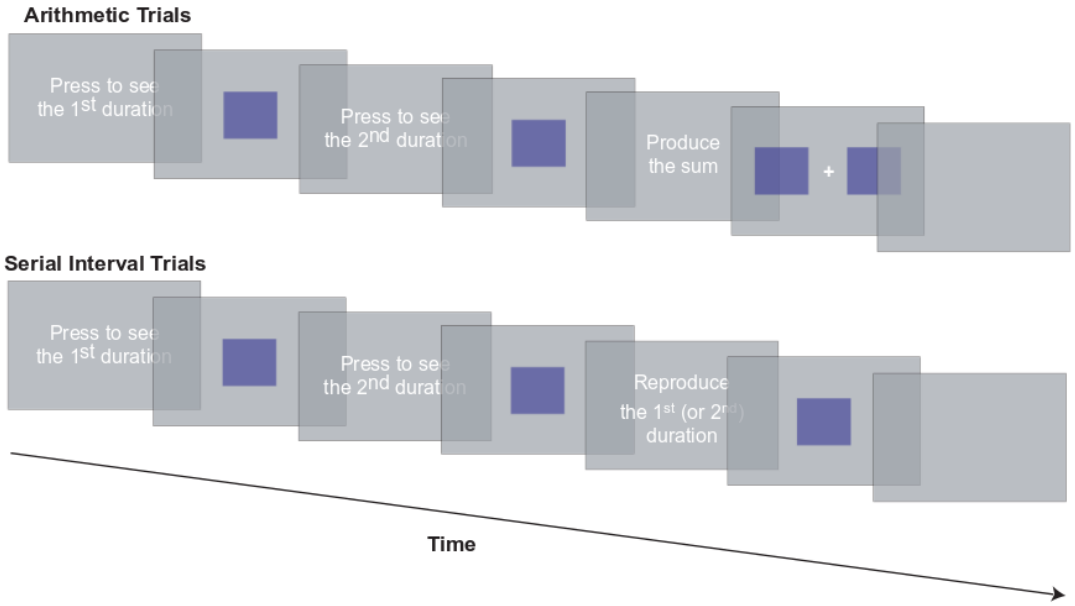

Figure 3.1. Schematic illustration of addition and serial-interval trials. After the sequential presentations of two variable durations, participants were prompted to estimate either the first or the second duration (serial trials) or the sum of the two durations (addition trials). See text for further details.

Data Analysis

To analyze the accuracy of temporal productions, we first normalized each time estimate with the target interval on each trial. Consequently, a value of 1 indicated a perfectly accurate reproduction, whereas values above and below 1 indicated over- and under-production of the target duration, respectively. For instance, if a participant was presented with 2 and then 3 s on a given addition trial, and produced 4.5 s, the normalized produced time was $4.5 \,/\, (2 + 3) = 0.9$,

revealing a slight underproduction of the target. To eliminate the effect of extraordinarily short and long production times in our analysis, we then conducted outlier detection based on normalized produced times. Outliers were 2.5 × *IQR* or more above or below the median normalized productions calculated separately for each participant (total data loss: 3.75%). We also computed to coefficients of variation to quantify participants' trial-to-trial variability (i.e., internal timing uncertainty). For each subject and trial type, we calculated the standard deviation of normalized productions and divide it by the mean of normalized productions. In addition to accuracy and precision of normalized time estimates, we also analyzed how long the participants took to start their temporal judgments after the screen display identifying the target interval [the 1st or 2nd duration or the sum], hereafter referred to as *start times*.

To analyze the effects of addition on the accuracy and precision of temporal estimates and start times, we used the linear mixed-effects model analysis via the *lmer* function from package *lme4* in R (Pinheiro & Bates, 2000). In each analysis, we generally started with the model containing all main effects and interactions, and then compared the more complex and reduced models with a likelihood ratio test using the *anova* function from the package lme4 to select the best-fitting model. The *lmerTest* package was used to assess significance. All models were run both by including the participants with neurological and psychiatric condition history (n = 8) and excluding them from the analyses (n = 26). Since we obtained qualitatively similar results, we opted to include all subjects in the analyses and integrated a fixed factor (coded as 0 = without the neuropsychiatric history, 1 = with history) into models.

3.3 Results

We first analyzed whether how long participants took to initiate their temporal judgments (or "timers") differed between serial and addition trials. The start times was submitted as the

dependent variable to a hierarchical model by including target interval (in s), trial type (serial-interval or addition), the interaction between target interval and trial type, and trial number (normalized by the total number of trials) as fixed effects. Visual inspection of Figure 3.2 as well as the hierarchical model estimates (Table 3.1) suggest that subjects waited an average of 1.29 s before starting to report their estimates of the sums of two durations, which was only 1 ms shorter than the start times on serial trials (Addition: b = -0.09, SE = 0.07, t = -1.28, p = .20; Serial: b = 1.30, SE = 0.09, t = 15.07, p < .001). These findings reveal that wait times of participants did not differ between serial and addition trials, indicating that the necessary mental computations before starting temporal judgments (e.g., retrieving the length of two time intervals) was completed in the same amount of time in both trial types. The length of target intervals or whether participants had a history of neuropsychiatric condition also did not affect start times (both ps > .12). We only observed a main effect of trial number, indicating that when compared to the start of the experiment, the participants were 34 ms faster to initiate their productions at the end of the experiment (b = -0.34, SE = 0.06, t = -5.46, p < .001). This overall decreasing trend of trial effects on start times can be visualized in Figure 3.2B. Please refer to Table 3.1 for the full model specifications and relevant statistical results.

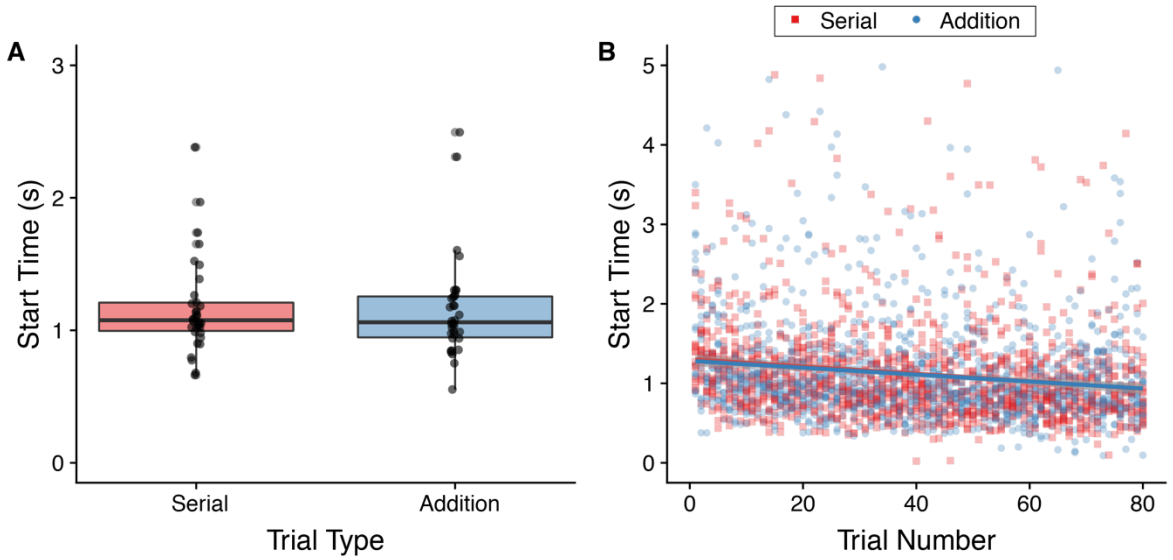

Figure 3.2. Start times depicted separately for serial and addition trials. Left panel shows the average start times for each subject, and right panel depicts individual start times from all trials and linear fits to these data. Note that both the individual data points and linear fits in the right panel are only used for visualization purposes.

Table 3.1

Summary of the Linear Mixed-effects Analysis for Start Times.

Factor	*b*	*SE*	*t*	*p*
Intercept	1.30	0.09	15.07	<0.001***
Target interval	0.01	0.01	1.52	0.13
Trial type_add	-0.09	0.07	-1.28	0.20
Trial number	-0.34	0.06	-5.46	<0.001***
NeuroPsych	-0.07	0.15	-0.50	0.62
Target interval:Trial type_add	0.002	0.01	0.28	0.78

***$p < 0.001$

After confirming that the average times to start productions in serial and addition conditions suggested that the mental arithmetic of time intervals did not differentially alter start times, we analyzed the accuracy of temporal productions. As can be seen in Figure 3.3A, time

estimates had a monotonic relation to the timed duration in both serial and addition trials. To quantify and further probe this relationship, we analyzed normalized productions (Figure 3.3B) with a hierarchical model. The following changes were made to the model specified above for start times: Serial trial type (production of the 1st or 2nd duration) and start times were also entered as fixed effects, and varying intercepts and slopes of trial type and target interval as well as of their interaction were estimated for each subject in the model.

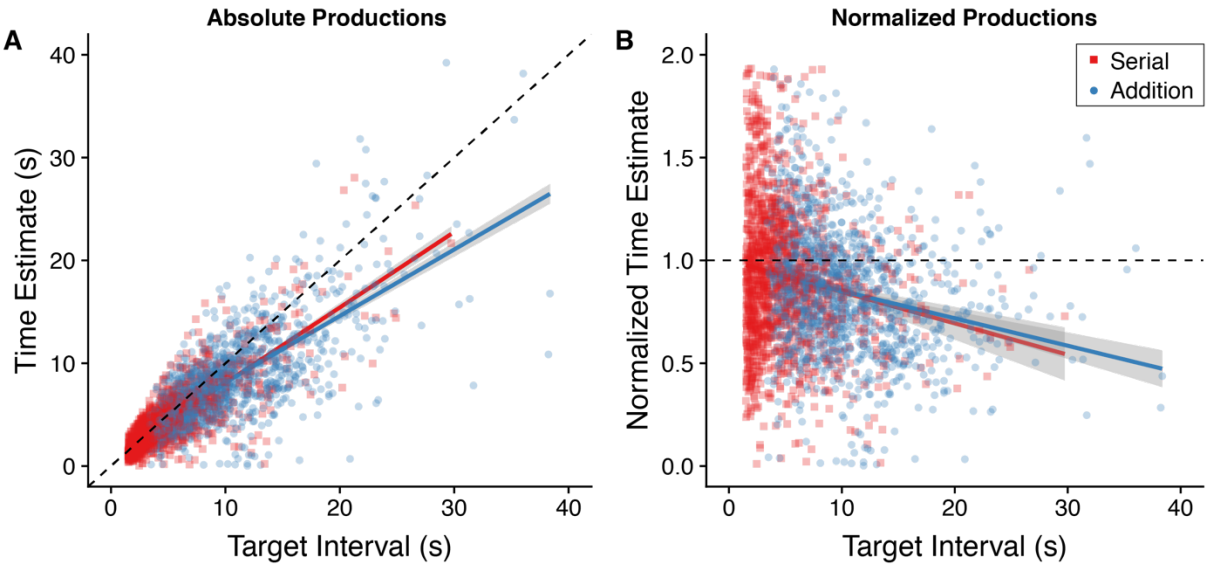

Figure 3.3. Time estimates in serial and addition trials. Left panel (A) shows absolute time estimates (s) and right panel (B) shows normalized time estimates as a function of target intervals in all serial and addition trials. Note that both the individual data points and linear fits are only used for visualization purposes.

As expected, participants were overall highly accurate in their temporal judgments (Table 3.2). We found that when asked to produce the first duration in serial trials, participants' estimates were approximately 10% shorter than the target interval ($b = 0.91$, $SE = 0.04$, $t = 22.44$,

$p < .001$). On the other half of serial trials in which the target was the second duration, participants' estimates were 0.07 closer to target intervals, almost reaching an average value of 1 signaling perfect accuracy ($b = 0.07$, $SE = 0.02$, $t = 4.60$, $p < .001$). Participants performed similarly in addition trials, with the estimated average normalized production of .93 indicating only a slight underproduction of objective sums ($b = 0.02$, $SE = 0.03$, $t = 0.74$, $p = .47$). Furthermore, we found a main effect of the length of target intervals, such that every increase in targets of 1 s led to a .02 decrease in normalized productions ($b = -0.02$, $SE = 0.003$, $t = -4.74$, $p < .001$; Figure 3.3B). However, this inverse relationship between targets and estimates were not differentially affected in serial and summation trials ($b = 0.002$, $SE = 0.003$, $t = 0.79$, $p = .44$), further showing that participants performed similarly in conditions where they had to estimate single durations or their sums. Please refer to Table 3.2 for the full model specifications and the rest of statistical results, and to Figure 3.4 to visualize the variability in individual estimates in three different trial types (serial 1st, serial 2nd, and addition).

Table 3.2

Summary of the Linear Mixed-effects Analysis for Normalized Time Estimates.

Factor	*b*	*SE*	*t*	*p*
Intercept	0.91	0.04	22.44	<0.001***
Target interval	-0.02	0.003	-4.74	<0.001***
Trial type_add	0.02	0.03	0.74	0.47
Serial trial type_second	0.07	0.02	4.60	<0.001***
Trial number	0.09	0.02	4.77	<0.001***
Start time	0.03	0.01	4.88	<0.001***
NeuroPsych	-0.06	0.06	-0.96	0.34
Target interval:Trial type_add	0.002	0.003	0.79	0.44

***$p < 0.001$

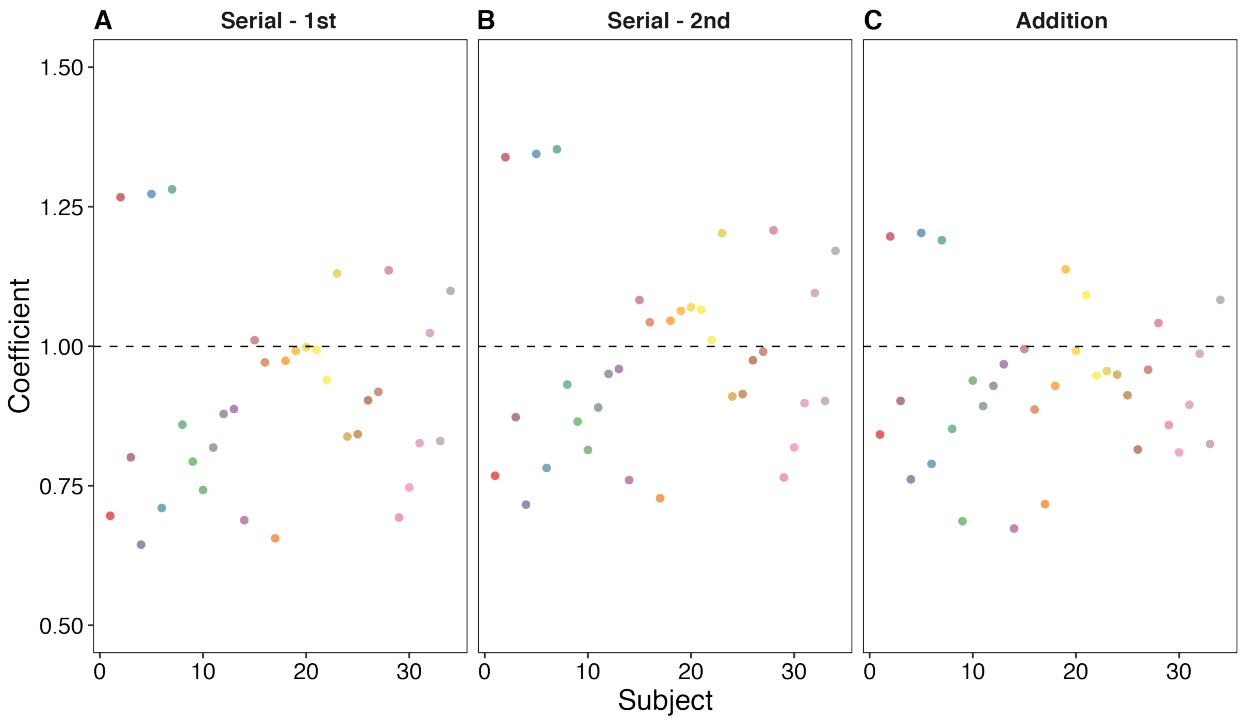

Figure 3.4. Normalized produced duration estimates in serial and addition trials. The coefficients were calculated by using the *ranef* and *fixef* functions of the lme4 package, and are shown separately for each subject (color coded). Note that these estimates do not take into account the effects of target interval, trial number, or start times.

Our analysis of the accuracy of time estimates revealed that, even though participants had a slight tendency to underestimate target intervals, there were not any meaningful differences between serial interval and arithmetic trials. To further analyze the nature of temporal estimates, we also examined the variability in duration productions by computing the coefficients of variation (CVs). We then submitted them as the dependent variable to a hierarchical model, by including trial type (serial-interval or addition) and serial trial type (1^{st} or 2^{nd}) as fixed effects, and subject as random effects. It should be noted that since this analysis requires summarizing the data by calculating the standard deviation and mean of normalized productions, we ended up with only three values per subject, one for each trial type (serial 1^{st}, serial 2^{nd}, and addition). Visual inspection of Figure 3.5 suggests that participants had similar levels of precision (trial-to-

trial variability) in their time estimates across addition and serial-interval trials. Even though this observation was supported to a great extent with our hierarchical model results, there were still slight differences across trial types. The findings revealed when participants were asked to produce the first duration in serial trials, they had the lowest levels of temporal precision with an average of 0.36 (b = 0.36, SE = 0.02, t = 18.84, p < 001). On the other hand, when they had to produce the second duration in serial trials or the sums of both durations in addition trials, their judgments of target durations varied less from one another (Serial 2nd: b = -0.03, SE = 0.01, t = -2.08, p = 0.04; Addition: b = -0.44, SE = 0.01, t = -2.99, p = 004).

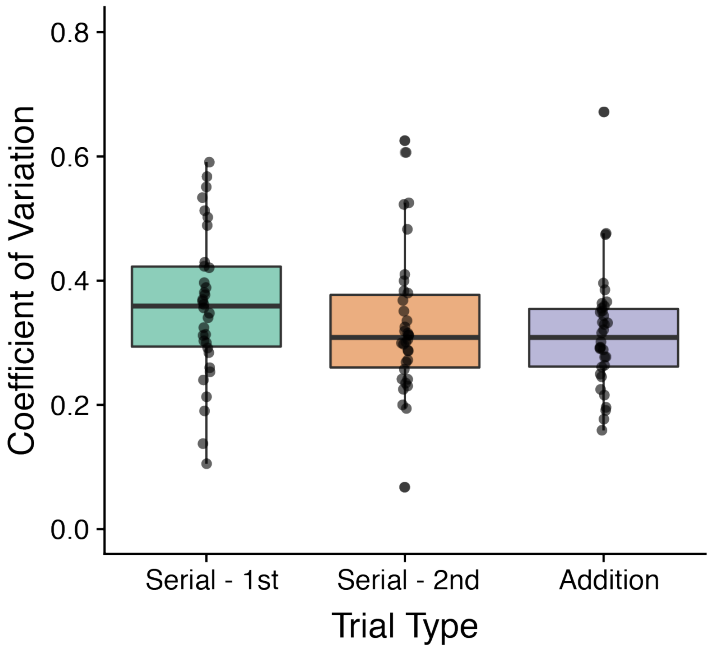

Figure 3.5. Precision of time estimates in serial and addition trials. Trial-to-trial variability is quantified by coefficients of variation of normalized time estimates in serial and addition trials.

3.4 Discussion

In this experiment, we tested the effects of mental addition on time estimates. Briefly, participants were presented with two durations and then were asked to produce either a first or

second duration or the sum of two durations. Crucially, they did not have prior knowledge on the type of the required temporal judgment on a given trial, which we expected to affect not just the temporal strategies but also the times to initiate reproductions. Our analysis of start times did not reveal any significant differences between the average times to start production in the serial and addition conditions. Importantly, these results suggest that the additional mental computations in the serial and addition trials were performed when instructed and thus that the experimental manipulation worked as expected. They also suggest that the serial and addition conditions required a similar level of cognitive effort, perhaps because they both required encoding of two durations and performing subsequent computations according to instructions not known in advance.

We then analyzed the accuracy of time estimates and found that there were actually no significant differences in the average normalized productions between serial and addition trials. Even though there were several factors that led the participants to over- or under-estimate the target intervals such as the length of targets, start times, and trial number, the requirement of mental addition was not one of them, which is at odds with previous findings reporting overestimation biases for summation in spatial and numerical domains as well on a few studies suggesting overestimation bias for summation in the temporal domain (Fortin et al., 2000; Knops et al., 2014; Knops et al., 2009; McCrink et al., 2007; Takahashi & Watanabe, 2015; Walsh, 2003). This discrepancy in the results might suggest that summation operates differently in a temporal domain than it does in spatial and numerical domains. While it is relatively well-established that overestimation biases are present for addition of space and number, there are a limited number of studies that have investigated summing time and it is unclear that their findings were truly due to mental addition.

First, in previous studies on summation of time, participants had known when addition would be required and thus had been able to utilize other strategies. For example, in the study conducted by Fortin et al. (2000) which required addition of pre-break and post-break durations, participants may have simply stopped timing at the break and resumed timing after the break. Similarly, in the study conducted by Takahashi & Watanabe (2015), participants knew in advance when they would be required to sum the next two durations and may have stopped timing after the presentation of the first duration and resumed timing at the beginning of the presentation of the second duration. Use of such stopwatch like strategies is likely because they require less cognitive effort than repeated mental summation. Notably, the results of the current study have an increased likelihood of being due to mental summation alone because participants were prevented from utilizing other strategies. As instruction for production was always given only after participants had encoded presented durations, mental computations were consistently required.

Second, previous experiments explicitly examining temporal addition utilized target intervals ranging only from 0.6 seconds to 1.1 seconds (Takahashi & Watanabe, 2015). As such, it is possible that overestimation was observed simply because duration lengths were too short. Moreover, motor response times may have been confounded with production times. In addition to eliminating the possibility for use of timing strategies unrelated to mental addition, the present experiment also increased validity of results by utilizing a wide range of durations with a fixed minimum of 1.5 seconds. The potential for observations being due to certain duration lengths or affected by motor response times was greatly reduced.

However, it should be noted that in our study we observed slight underproduction patterns in all conditions, particularly with longer target intervals. A close inspection of the absolute and

normalized productions presented in Figure 3 further reveals that shorter durations were somewhat overproduced. These findings are not surprising given that duration estimates tend to shift towards the mean of all durations previously presented (Gu & Meck, 2011; Jazayeri & Shadlen, 2010). These respective over- and under-production patterns of shorter and longer durations may have been the result of the specific temporal context created within the experiment. Furthermore, since we used exponential distributions for the selection of durations, trials with longer durations were less frequent than those with shorter durations. This asymmetry might have caused the estimates for longer durations to be further pulled towards the lower end of the duration range and thus led the participants to underestimate most durations.

Finally, we also analyzed the trial-to-trial variability in time estimates in serial and addition conditions. Even though when participants were less precise in their judgments when they were asked to produce the first duration in serial trials, on average, the coefficient of variation values did not differ greatly across serial and addition trials. Even though one might expect that producing combinations of durations would be a more difficult task than producing single durations, as producing sums of durations demands more attention and memory than simply producing a single duration does (Enns et al., 1999; Block et al., 2010), our findings again showed that mental addition of time does not result in more error and imprecision in time estimates.

It should be noted, however, that since the variability analysis relies on the calculation of coefficient of variation values, we had to summarize the data and obtain at most 3 values per subject. Given that the length of time intervals altered the accuracy of time estimates, with longer targets being underproduced, the reader should be cautious in interpreting these results which were obtained without considering the durations themselves. This is particularly of importance,

as one might mistakenly suggest that our findings regarding the variability of time estimates provide support for various theoretical accounts such as the scalar timing theory (Gibbon, 1991) which assumes a constant coefficient value for each organism. Even though this assumption might well be supported by future analyses, the current findings are insufficient to suggest the possibility that scalar variability is a part of timing both single durations as well as sums of durations.

Future studies should also investigate other arithmetic operations to have a better understanding of mental computations of time. We recently conducted studies where we were able to generalize our findings to other duration ranges, as well as to the subtraction operation. As there has already been research done on these processes in spatial and numerical domains, it would be of interest to determine whether results in the temporal domain are consistent (Katz & Knops, 2014; Knops et al., 2009; Knops et al., 2014; McCrink et al., 2007, Shaki & Fischer, 2017). Further, to better understand the cognitive processes such as attention and working memory as well as the neural mechanisms underlying all forms of temporal arithmetic, methodologies such as pupillometry and electroencephalography could be pursued in future studies.

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Chapter 4: Serotonergic Modulation of Temporal Discriminations

4.1 Introduction

The perception of time is central and fundamental to virtually all our daily activities. Temporal processing that occurs over the seconds to minutes timescale, denoted as *interval timing*, is particularly important for everyday activities and is linked to various cognitive and motor functions (Buhusi & Meck, 2005). Therefore, distortions in time perception experienced in many psychiatric disorders, including schizophrenia (Allman & Meck, 2012; Davalos & Opper, 2015; Thoenes & Oberfeld, 2017; Ward et al., 2012) are expected to have functional consequences. Schizophrenia is characterized by a variety of cognitive impairments that include disturbances in memory, attention, and executive processing (e.g., Gold et al., 2017). These cognitive processes are all highly crucial for intact temporal processing, as individuals need to attend to perceive, attend to and remember time intervals to be able to decide whether or when to execute a behavioral response (Gibbon et al., 1984). Therefore, it is not surprising that individuals with schizophrenia experience substantial timing deficits (e.g., Carroll et al., 2008, 2009; Ciullo et al., 2016; Elvevåg et al., 2003; Davalos et al., 2003; Johnson & Petzel, 1971; Lošák et al., 2016; Papageorgiou et al., 2013; Stevenson et al., 2017; Tysk, 1983; Wahl & Sieg, 1980).

The serotonergic system is implicated in both timing behavior and the pathogenesis of schizophrenia, particularly in relation to its positive symptoms such as hallucinations. Recently, the therapeutic effects of psychedelic drugs that act on serotonergic receptors and induce hallucinations on anxiety, depression, and mood have received extensive interest (Dos Santos & Hallak, 2020; Fuentes et al., 2020). However, the effects of serotonergic hallucinogens on cognition, and more specifically, on timing behavior have been understudied. We, therefore,

believe that to better understand their therapeutic potential in treatment-resistant psychiatric conditions, it is crucial to study the effects of serotonergic hallucinogens on temporal cognition. Further, since it has even been hypothesized that impairments in timing performance might be one of the core cognitive deficits in schizophrenia (Bonnot et al., 2011; Gómez et al., 2014; Ward et al., 2012), the characterization of the effects of psychedelic drugs on temporal processing is particularly important.

Indeed, there is some evidence that psychedelic drugs such as lysergic acid diethylamide (LSD) and psilocybin lead to altered temporal processing (Wackermann et al., 2008; Wittmann et al., 2007; Yanakieva et al., 2019). For instance, Wittmann et al. (2007) tested humans on a temporal reproduction task where subjects were trained to estimate and reproduce durations ranging between 1.5 and 5 s and tested their performance after psilocybin, a serotonin (5-$HT)_{2A/1A}$ receptor agonist, intake. Their results revealed that subjects tended to under-reproduce time intervals between 4 and 5 s but not those between 1.5-3 s. By contrast, Yakanieva et al. (2019) reported that microdose LSD leads to selective over-reproductions of time intervals between 2 and 4 s, but not those between 800 ms and 1.6 s. Although it is not clear why psilocybin and microdose LSD changed temporal estimates in the converse direction, both psychedelic drugs affected the processing of time intervals mainly in the suprasecond range.

One hallucinogen that is of particular interest is the 5-$HT_{2A/2C}$ receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) that has been shown to elicit head twitch responses in mice (González-Maeso et al., 2007; Halberstadt & Geyer, 2013) and adopted as a behavioral proxy for hallucinogenic effects. Similar to the effects of other psychedelic drugs, DOI has also been found to alter the accuracy and precision of temporal estimates in different timing tasks, although not always in the same direction (e.g., Asgari et al., 2006a,b; Body et al., 2003,

2006a,b; Cheung et al., 2007; Halberstadt et al., 2016; Hampson et al., 2010). Most of such DOI effects have been studied in timing tasks that require animals to categorize experienced time intervals as shorter or longer than a cutoff duration (e.g., 10s), or to expect food rewards after fixed time intervals. In one such timing task, levers or response keys are available during timed intervals, which allows animals to dynamically regulate their behavior based on the passage of time. DOI administration in those free-response paradigms revealed that rats start responding early in a peak-interval timing task (Asgari et al., 2006a) and categorize durations as long more frequently in free-operant psychophysical procedures (Body et al., 2003, 2006a,b; Cheung et al., 2007), resulting in leftward shifts in response curves. These latter changes are consistent with the idea that the drug makes the internal clock speed up. Conversely, more recently Halberstadt et al. (2016) tested mice in another timing task where the levers extended only after a cue of variable duration (2.5-10.5s) had elapsed. In this "retrospective" version of the temporal discrimination task, they found that DOI led to an increased tendency to report longer durations (>6.5s) as short, but the reports about shorter durations (<6.5s) did not change (see also Asgari et al., 2006b; Hampson et al., 2010 for similar findings in rats). This raises the possibility that the drug only distorts timing of particular intervals.

Taken together, these studies consistently revealed that serotonergic hallucinogens, particularly DOI markedly alter temporal processing, albeit not in a consistent manner. Despite the lack of consensus on the direction of shifts seen in response functions, however, these drugs seem to affect time estimates for either the shorter or longer, but not all durations in tasks where the subjects experienced multiple time intervals. Therefore, the current study is aimed at better characterizing whether DOI alters the processing of time intervals that are of only specific duration length. Since DOI effects have been more widely studied in retrospective temporal

discrimination tasks, we also tested performance in two temporal bisection tasks while targeting the 5-$HT_{2A/2C}$ receptor activation by systemic DOI administration prior to testing. Crucially, we tested animals with different, partially overlapping, sets of stimulus durations ranging from 2 to 24 s, such that longer durations in one set constituted the shorter durations in another set. This allowed us to explore if DOI affects how the animals perceive durations either in an absolute manner or in relation to one another in each temporal context.

4.2 Experiment 1

Method

Subjects

Nine C57BL/6j male mice (The Jackson Laboratory, Bar Harbor, ME, USA) that were previously used in a temporal discrimination study were tested in this experiment. They were housed in groups of four or five and kept with 12:12h light–dark cycle. Experimental sessions were conducted during the light phase. Water was available ad libitum in home cages, and the mice were maintained at 85-90% of their free-feeding weight. All animal procedures described in this study were in accordance with Columbia University and New York State Psychiatric Institute Institutional Animal Care and Use Committees and Animal Welfare regulations.

Apparatus

A total of thirty matching operant chambers (ENV-307W; Med-Associates, St. Albans, VT) placed inside sound and light attenuating boxes were used in this and following experiments. A feeder trough was centered on one wall of the chamber and used to deliver liquid reward of evaporated milk (0.01 ml). Two retractable levers (ENV-312-2W: 2.2 cm above the grid floor and 11 cm apart) were mounted on either side of the feeder trough. An audio speaker (ENV-324W) was used to deliver tone (90 dB, 2500 Hz) serving as auditory stimulus. A house

light (ENV-315W) was located on the opposite wall, at the top of the chamber. An exhaust fan was activated in each chamber at the start of an experimental session. The experimental protocol was controlled via Med-PC computer interface and Med-PC IV software, and behavioral events were recorded with a temporal resolution of 10 ms.

Procedure

In the temporal bisection procedure, mice categorize the length of time intervals as *short* or *long* based on their subjective temporal similarity to two reference durations (Figure 4.1). The mice used in Experiment 1 had previous experience with the task in which the short reference duration was 6 s and the long reference duration was 18 s. Therefore, in the current experiment, we skipped the initial training steps which are described below for Experiment 2 and re-trained the animals on the temporal bisection task with the following set of durations: 6.00, 7.21, 8.65, 10.39, 12.48, 14.99, and 18.00 s. Briefly, the animals were presented first with a tone lasting for one of the randomly selected stimulus durations on each trial, after which both levers remained extended until a response was made. Correct categorizations of the reference durations (6 or 18 s) resulted in reward delivery for 5 s, whereas responses following intermediate durations were not reinforced. Incorrect categorizations or failure to respond within 10 s of lever extension terminated the trial immediately. Each trial was then followed by a random inter-trial interval (ITI) that was selected from an exponential distribution with a mean of 45 s. Once mice reached a stable baseline of responding, we tested temporal discrimination performance after injecting the animals with DOI prior to test sessions. Following Halberstadt et al. (2016), DOI was dissolved in saline (0.9% NaCl) and administered at a volume of 5 mL/kg body weight. All animals were tested in the bisection task approximately 10 min after intraperitoneal injection with DOI or saline. They all received three different doses (0.3, 1.0, and 3.0 mg/kg) in

counterbalanced order. Each drug day was separated by one vehicle day of saline injections, resulting in 18 testing days in the drug phase. Sessions ended after 70 mins or until 40 rewards were earned, whichever occurred first.

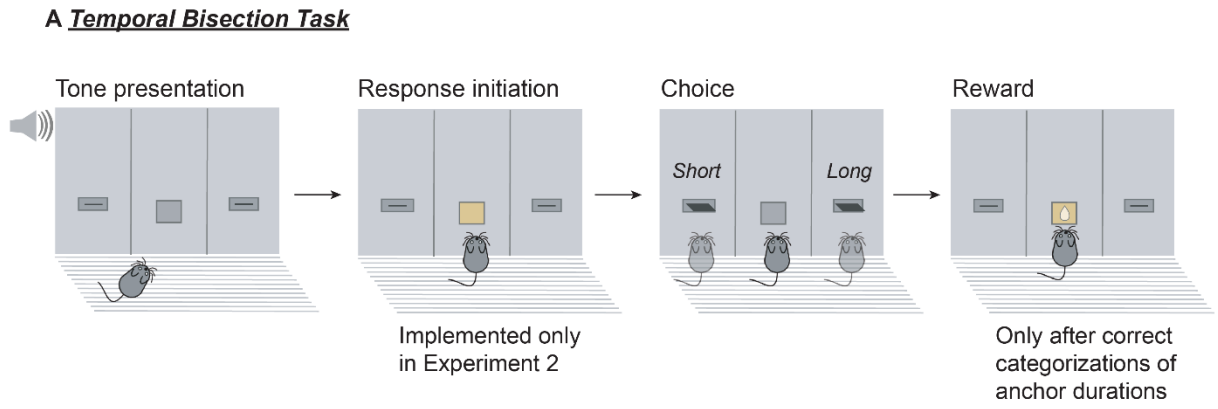

Figure 4.1. Schematic illustration of a trial sequence in the temporal bisection task. See text for further details.

Data analysis

To investigate the changes in temporal discrimination performance under different DOI conditions, we analyzed the proportion of long responses and response times (RTs) associated with short and long choices, and calculated the proportion of trials where the animals failed to make a response within 10 s of lever extension. These units of analysis were then submitted to repeated-measures ANOVAs. In all statistical analyses presented in this paper, an alpha level of .05 (two-tailed) was used. When necessary, Holm-Bonferroni corrections were used to adjust for all multiple comparisons. Initial data analyses were done using custom scripts written in MATLAB, and follow-up statistical analyses were run in JASP (Version 0.16.1).

Results

We first analyzed the changes in the proportion of long responses as a function of stimulus durations under different DOI conditions. As can be visualized in Figure 4.2A, the proportion of long responses increased as a function of test durations and psychometric curves were generally sigmoidal in form in all drug conditions. Further, DOI administration led to a dose-dependent change in psychometric curves, such that higher doses of DOI resulted in larger effects on temporal choices. Interestingly, the DOI-dependent shifts in the psychometric functions were asymmetrical: Subjects tended to judge the experienced durations as short more often following longer durations, whereas choices following shorter durations were not affected. A repeated-measures ANOVA (Duration x Drug Condition) run on choice data supported these findings and revealed main effects of both Duration, $F(6, 48) = 121.66$, $p < .001$, $n_p^2 = 0.94$, and Drug Condition, $F(3, 24) = 7.88$, $p < .001$, $n_p^2 = 0.50$, and a Duration x Drug Condition interaction, $F(18, 144) = 1.86$, $p = 0.02$, $n_p^2 = 0.19$. Follow-up simple main effects analyses showed that in all four drug conditions, there was an increase in the proportion of long responses as a function of stimulus durations (all ps $< .001$), and that different doses of DOI altered temporal judgments on 10.39-s (dur #4) and 18.00-s (dur #7) trials (both ps $\leq .01$; all other ps $\geq .08$).

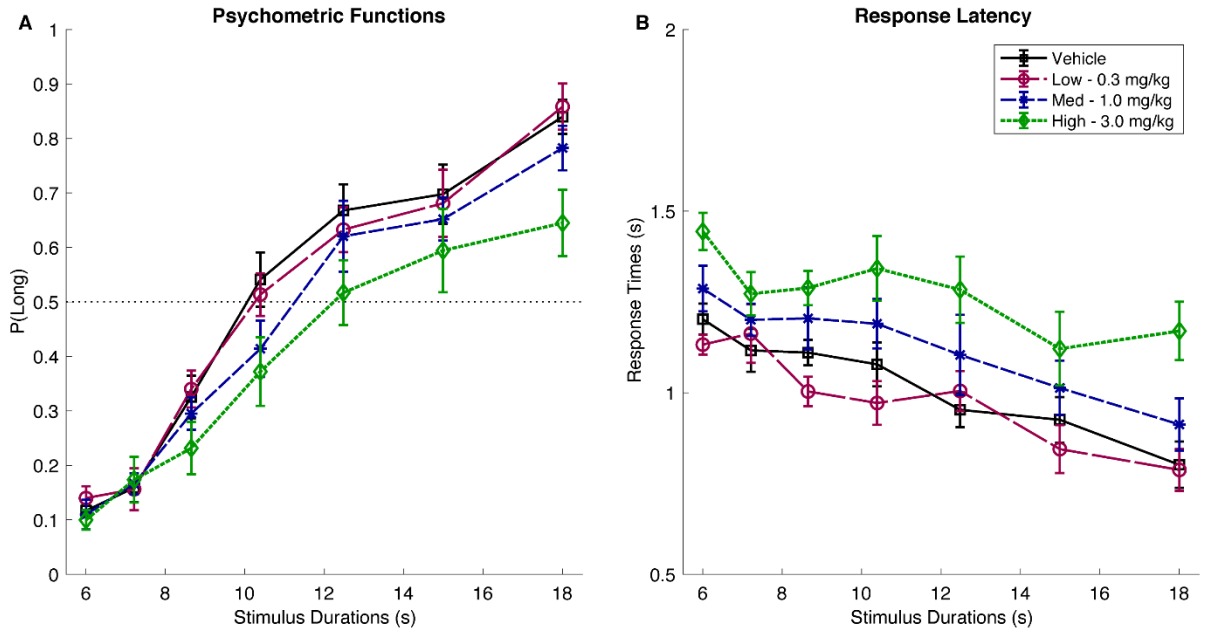

Figure 4.2. Effects of DOI on temporal bisection performance. The proportion of long responses (A) and response times (B) are shown as a function of stimulus durations, separately for each drug dose. Error bars depict *SEM*.

We then analyzed the response times (RTs) associated with choices under different DOI conditions. Our results revealed that the mice were faster to press the levers following longer stimulus durations in all conditions and that higher doses of DOI led to slower response latencies (Figure 4.2B). A repeated-measures ANOVA (Duration x Drug Condition) run for overall RTs revealed a main effect of Drug Condition, $F(3, 24) = 21.86$, $p < 0.001$, $n_p^2 = 0.73$, and a main effect of Duration, $F(6, 48) = 8.94$, $p < 0.001$, $n_p^2 = 0.53$, without a significant interaction, $F(18, 144) = 1.10$, $p = 0.36$. Holm-corrected post hoc comparisons showed that average RTs in both medium and high dose conditions were higher than those in vehicle and low dose conditions (vehicle vs. low: $p = 0.31$; all other ps ≤ 0.03). Further, pairwise comparisons conducted for the main effect of Duration revealed that the animals were faster to report their choices following longer durations (18.00s vs. durations $\leq$ 10.39s: $p \leq 0.004$; 14.99s vs. durations $\leq$7.21s: $p \leq$

0.009; 12.48s vs. 6.00s: $p = 0.045$; all other ps > 0.06). Critically, even though the subjects were slower to make categorizations, DOI administration did not affect their ability to complete trials, as there were no changes in the proportion of missed trials on drug days, $F(3, 24) = 2.13$, $p = 0.12$.

Discussion

In Experiment 1, we replicated the effects of systemic delivery of DOI on temporal discrimination performance reported in previous studies (e.g., Asgari et al., 2006; Halbertstadt et al., 2016; Hampson et al., 2010) and found dose-dependent shifts in temporal choices. Specifically, higher doses of DOI increased the animals' tendency to categorize longer durations as short and lead to an overall increase in response times. Since DOI affected categorizations mainly following longer cue durations, instead of resulting in symmetrical shifts in psychometric functions, in the next study we tested two naïve groups of animals with different short and long durations. These duration sets had an overlap, such that the longer durations in one group constituted the short durations in the other group. This manipulation allowed us to investigate if DOI injections would again affect only the higher end of the duration set regardless of the absolute duration values, or those following only specific time intervals that are shorter or longer than a particular duration (e.g., < 10 s).

One key finding in Experiment 1 was that irrespective of the DOI dose the animals were faster to respond following longer durations. A close inspection of RTs reveals that this change in RTs as a function of duration mostly occurs on long choice trials (Figure 4.S1). It has been argued that with elapsing time subjects would become more confident that it is a long duration trial and might move to the long response location prior to the cue offset (Balcı et al., 2011; Balcı & Simen, 2014; Gouvêa et al., 2014; Wiener et al., 2019). When the levers (or response keys) are

made available, animals can then immediately classify the duration as long, resulting in long choice RTs to be faster than short choice RTs (e.g., Akdoğan & Balcı, 2016; Balcı & Simen, 2014; Klapproth & Wearden, 2011; Tipples, 2015). Even though we have not explicitly recorded the subjects' movement patterns during delay periods (but see Cambraia et al., 2019; Gouvêa et al., 2014; Wiener et al., 2019), our analysis of short and long choice RTs (refer to the supplemental material) also revealed that RTs associated with long classifications were faster than short classification RTs. Therefore, it is possible that our subjects adopted a particular movement pattern during cue presentation. Given the overall increase in motor latency under drug conditions, in the next experiment we tried to minimize such time-based behavioral sequences that might have been developed during cue presentations. Consequently, instead of extending the levers automatically following cue presentation, we trained the animals to make a head poke into the central (food) hopper following each cue to extend the levers and then report their choices. We expected this additional step should ensure that subjects had to use the information about the duration of the cues to make choices.

4.3 Experiment 2

To further investigate the effects of DOI on temporal discrimination, we tested 28 naïve mice with two different sets of durations. The first group (*short-interval* group, $n = 13$) was tested with shorter set of stimulus durations ranging between 2 and 8 s, whereas the second group (*long-interval group*, $n = 15$) was tested with longer stimulus durations ranging between 6 and 24 s. The ratio between the reference durations was kept constant at 1:4 between the two groups. One key difference between Experiments 1 and 2 was that in the latter the animals were required to make a head poke into the illuminated central port to insert the levers after tone presentation to minimize the occurrence of a time-based motor response (e.g., switching from the

short lever to long lever) during cue presentation. Even though the animals could still choose to manifest a dynamic motor response during the presentation of a timing stimulus, we expected them to occur less frequently and be idiosyncratic.

Methods

Subjects and apparatus

Twenty-eight naive C57BL/6j male mice (The Jackson Laboratory, Bar Harbor, ME, USA) were used in Experiment 2. Mice were approximately 9 weeks old upon arrival. The apparatus was the same as in Experiment 1.

Procedure

Lever press training. Lever press training consisted of two phases. The first phase involved a two-day magazine training in which the animals were trained to consume the liquid reward from the raised dipper. On the first day, reward delivery ended 9.5 s after the first head entry, and a random intertrial interval (ITI) sampled from an exponential distribution with a mean of 30 s initiated. The session ended after either 30 min or 20 dipper presentations. On the second day, the animals were trained with the same protocol except this time the dipper was up for 8 s. All mice collected 20 rewards in both sessions. In the second phase of lever press training, animals were trained to press a lever to earn the liquid reward. Each trial began with the insertion of either the left or the right lever, randomly selected on each trial. A lever press within the first 30 s of lever insertion resulted in immediate reward for 5 s (*Fixed-ratio* 1 [FR1]). The dipper was presented also when the lever has been extended for 30 s without a press (*Fixed-time* 30 s [FT30s]). In both cases, the lever was retracted the moment the dipper was raised, and then a random ITI (with a mean of 45 s) initiated after reward delivery. Sessions ended after 60 min elapsed or when the mice earned 40 reinforcements, whichever came first. Mice continued

receiving sessions like this until they earned 30 rewards in two consecutive sessions. If the mice could not reach the performance criterion in two days with the FR1&FT30s schedule, they were then trained on FR1 trials in which reward delivery was contingent upon a lever press. The ITI and performance criterion were the same as in FR1&FT30s trials.

After the animals pressed the levers at least 30 times in two consecutive sessions, they proceeded to the *response-initiated lever press* training which required them to make a head poke into the illuminated hopper to extend one of the randomly chosen levers. The mice were run on this program for a total of 7 days. On Days 1-3, no limited hold period was applied for the head entry requirement, whereas starting Day 4, animals had to make a head poke into the illuminated hopper to insert the levers within 20 s of trial initiation. If they failed to make a head entry within 20 s, the trial ended and an ITI started. On the last 2 days, a brief tone was presented for 500 ms before turning on the feeder light cuing the head entry requirement. This step was implemented to familiarize mice with tone presentation at trial onset. On average, all experimental steps before the duration discrimination training lasted 12.27 sessions (*SD* = 0.73, *range* = 11-14).

Duration discrimination training. In this phase, we varied the duration of tone presentation at trial onset and trained the mice to categorize different tone durations as short or long (Figure 4.1). Cue duration pairs were 2 vs. 8 s or 6 vs. 24 s in the short- and long-interval groups, respectively. We mainly followed the training protocol described in Ward et al. (2009; Experiment 2) and integrated the response step into the protocol. On Day 1 of the *single-lever, single-duration* program, after the presentation of the short duration (2 s for short interval and 6 s for long interval groups) and a head entry into the central port following tone offset, only the lever associated with a correct short categorization was extended. A press on this lever within 10

s was rewarded with 5 s of reinforcement delivery. The lever was retracted after 10 s and the trial was terminated. On Day 2, only the long duration (8 s for short interval and 24 s for long interval groups) and the corresponding lever were presented. Duration-lever mapping was counterbalanced across subjects. On Days 3 and 4, the mice completed the *interspersed single-lever, single-duration* program in which each of the two durations were randomly presented with their respective corresponding levers. The mice then completed 3 sessions of *50% choice response trials* (50% single-lever and single-duration trials), 3 sessions of *75% choice response trials* (25% single-lever and single-duration trials), and 3 sessions of *100% choice response trials* programs which had an increasing presentation probability of trials that involved the extension of both levers after tone presentation and required animals to choose the lever that was associated with the just-presented cue duration to earn a reward. During choice trials, an incorrect categorization was followed by a correction trial in which the house light was illuminated for 1 min and following an ITI (with a mean of 45s), another choice response trial with the same cue duration initiated. The correction procedure was then removed, and 5 more sessions of 100% choice response trials were completed. Mice continued the duration discrimination training until their discrimination accuracy for each reference duration was at least 75% for two consecutive sessions. The short-interval group completed the last phase of the duration discrimination training (100% choice response trials) in an average of 14.93 days (*SD* = 8.43, *range*: 7-35), whereas the long-interval group took an average of 22.27 days (*SD* = 6.67, *range* = 11-34).

Temporal bisection testing. After training mice with the two reference durations, we introduced five intermediate durations that were spaced equally on a logarithmic scale and randomly presented on 50% of trials. The complete set of durations consisted of 2.00, 2.52, 3.17, 4.00, 5.04, 6.35, and 8.00 s in the short-interval group and of 6.00, 7.56, 9.52, 12.00, 15.12,

19.05, and 24.00 s in the long-interval group. We tested the mice with these durations for a minimum of 27 days (the last 7 sessions with vehicle injections) until the discrimination accuracy in the last 2 days as well as in the average of last 5 days was at least 75% separately for each of the two reference durations. Mice advanced to the drug phase approximately after 27 days of baseline temporal bisection testing (short-interval group: M = 27.67, SD = 2.49, *range* = 27-37; long-interval group: M = 27.87, SD = 1.15, *range* = 27-30). As in Experiment 1, DOI was administered at one of the three doses (0.3, 1.0, 3.0 mg/kg; i.p.) 10 min prior to testing. All animals received each DOI dose three times in counterbalanced order, and all drug days were followed by a vehicle day, resulting in 18 testing days.

Results

The analysis of choice proportions under different drug conditions in the current experiment revealed similar changes to those observed in Experiment 1. We again found that the administration of higher doses of DOI resulted in the increased frequency of short responses following longer stimulus durations (Figure 4.3). Two separate two-way repeated-measures ANOVAs (Duration x Drug Condition) run for the choice data in both groups corroborated these findings and revealed a main effect of Duration both in the short-interval, $F(6, 72) = 344.44$, $p < 0.001$, $n_p^2 = 0.97$, and the long-interval groups, $F(6, 84) = 261.01$, $p < 0.001$, $n_p^2 = 0.95$. Importantly, higher doses of DOI resulted in an increase in the frequency of short responses, as supported by a main effect of Drug Condition in both the short-interval, $F(3, 36) = 6.53$, $p = 0.001$, $n_p^2 = 0.35$, and long-interval groups, $F(6, 84) = 6.28$, $p = 0.001$, $n_p^2 = 0.31$. Our findings also revealed a Duration x Drug Condition interaction in both groups (short-interval group: $F(18, 216) = 1.72$, $p = 0.038$, $n_p^2 = 0.13$; long-interval group: $F(18, 252) = 3.79$, $p < 0.001$, $n_p^2 = 0.21$). Follow-up simple main effects analyses showed that the proportion of long responses as a

function of stimulus durations in all drug conditions in both groups of animals (all *p*s < 0.001), and that different doses of DOI altered temporal judgments on 4.00, 6.35, and 8.00 s trials in the short-interval group (*p*s ≤ 0.03; all other *p*s > 0.06) and on 6.00, 12.00, 19.05, 24.00 s trials in the long-interval group (*p*s ≤ 0.01; all other *p*s > 0.05).

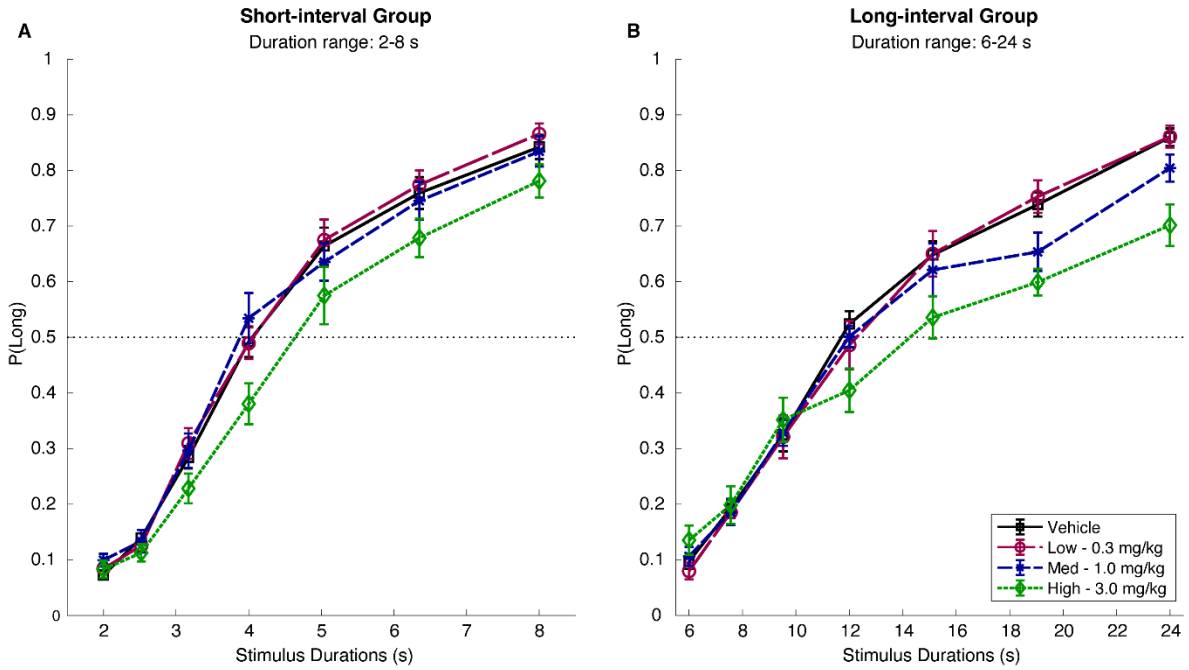

Figure 4.3. Effects of DOI on temporal choices in short- and long-interval groups. The proportion of long responses in short-interval (A) and long-interval (B) groups are shown separately for each drug dose. Error bars depict *SEM*.

To assess the effects of DOI on motor responses, we analyzed the change in head-poke latency as a function of stimulus durations in all drug conditions (Figure 4.4A-B). Our results indicated that higher doses of DOI resulted in slower head poke times to initiate the presentation of the choice levers after cue presentation in both duration range groups. Separate repeated-measures of ANOVAs run for the two duration range groups revealed a main effect of Drug

Condition both in the short-interval, $F(3, 36) = 14.45$, $p < .001$, $n_p^2 = 0.55$, and the long-interval groups, $F(3, 42) = 32.41$, $p < .001$, $n_p^2 = 0.69$. Holm-corrected post hoc comparisons showed that, when compared to other drug levels, both groups of animals took the longest time to make a head poke when tested at the highest DOI dose (short interval group: all *p*s $\leq .005$; long interval group: all *p*s $< .001$). Even though they still had relatively slow poke latencies at the medium dose in both groups, this change between dose pairs was slightly significant only when compared to the latencies at the low dose in the short-interval group ($p = .05$). Differences in poke latencies between all other dose pairs were non-significant (short-interval group: both *p*s > .17; long interval group: all *p*s > .09).

We further found the long-interval group animals were slightly faster to make head pokes on long trials, as revealed by a main effect of Duration on poke latencies, $F(6, 84) = 3.65$, $p = .003$, $n_p^2 = 0.21$. This slight decrease in poke times following longer durations was significant only between 6.00- and 19.05-s trials ($p = .04$) and 6.00- and 24.00-s trials ($p = .001$; all other *p*s > .08). Although the visual inspection of Figure 4.4A also suggests a similar reduction in poke latencies with increasing durations in the high dose condition, the main effect of Duration was not significant in the short-interval group, $F(6, 72) = 1.61$, $p = .16$. Finally, Drug x Duration interactions were not statistically significant in either the short-interval, $F(18, 216) = 1.59$, $p = .06$, or the long-interval group, $F(18, 252) = 1.38$, $p = .14$.

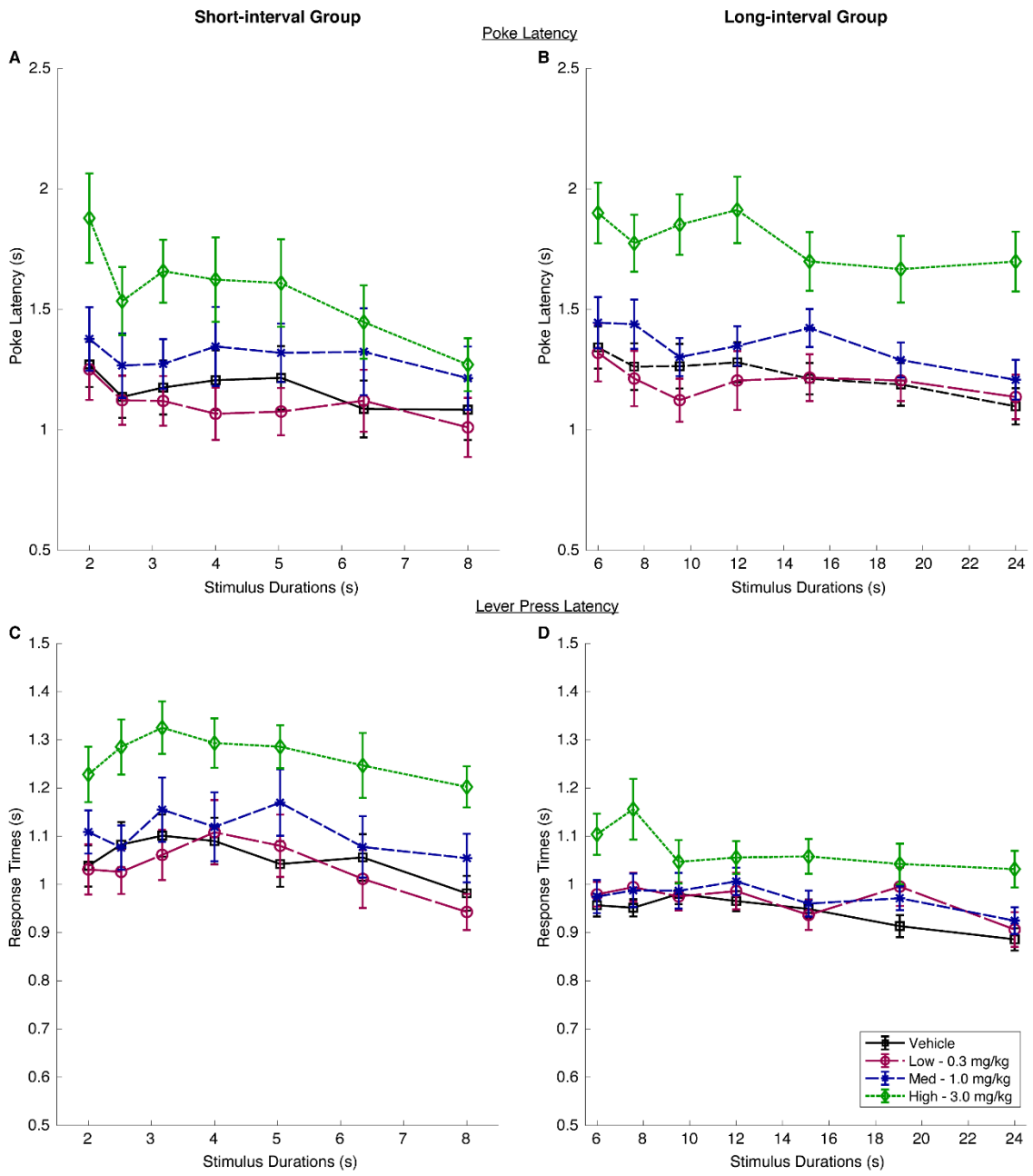

Figure 4.4. Effects of DOI on response speed. Response initiation (head poke) latency and choice (lever press) latency in short-interval (A, C) and long-interval (B, D) groups are shown separately for each drug dose. Error bars depict *SEM*.

Next, we analyzed lever press response times following each duration presentation and expected the animals to be slower after DOI administration. Furthermore, since we trained the

animals to make a head poke to insert the levers, instead of automatically extending them after the timed stimulus presentation, we expected that the decreasing trend in RTs with increasing durations observed in Experiment 1 would be less pronounced, if not completely absent, in Experiment 2. As can be seen in Figure 4.4C&D, DOI administration slowed down the response times in both duration range groups, as supported by a main effect of Drug Condition in both the short-interval, $F(3, 36) = 28.28$, $p < .001$, $n_p^2 = 0.70$, and the long-interval groups, $F(3, 42) = 12.90$, $p < .001$, $n_p^2 = 0.48$. Holm-corrected post hoc comparisons revealed a significant increase in RTs only under the highest DOI condition in both groups (short-interval group: all ps $\leq .001$; long-interval group: all ps $\leq .01$). No RT differences were found between the remaining drug condition pairs in either duration ranges (short-interval group: all ps $> .17$; long-interval group: all ps $> .13$). Although our results also indicated a main effect of Duration in both the short-interval, $F(6, 72) = 4.20$, $p = .001$, $n_p^2 = 0.26$, and the long-interval groups, $F(6, 84) = 3.77$, $p = .002$, $n_p^2 = 0.421$, Holm-corrected post hoc comparisons revealed significant differences in RTs only between 3.17- and 8.00-s trials ($p = .01$) and between 5.04- and 8.00-s trials ($p = .02$) in the short-interval group (all other ps $\geq .08$). No significant RT changes were found in any of the duration pairs in the long-interval group (all ps $> .10$). Finally, Drug Condition x Duration interactions were not statistically significant in either the short-interval, $F(18, 216) = 1.15$, $p = .31$, or the long-interval groups, $F(18, 252) = 1.42$, $p = .09$.

As in Experiment 1, even though the animals were slower to respond under drug conditions the animals on average missed 1-4% of trials, and DOI administration did not alter the proportion of missed trials in either duration range group (repeated-measures ANOVAs; short-interval group: $F(3, 36) = 2.48$, $p = 0.08$; long-interval group: $F(3, 42) = 2.24$, $p = 0.10$).

Discussion

In this experiment, we again found that particularly the highest dose of DOI increased the animals' tendency to categorize longer durations as short and led to an overall increase in head poke latencies and response times in a temporal bisection task. Critically, these biases towards emitting short responses did not depend on the absolute durations of cues, they instead occurred only on longer trials in a given temporal context. That is, we did not observe a bias for short choices only when the cue durations were longer than an absolute cutoff duration, e.g., 12 s. For instance, if we examine the choices following the 8-s cue in the short-interval group, we can see that the highest dose of DOI increased the frequency of short judgments on these long trials. On the other hand, despite being one of the shorter cue durations in the long-interval group and Experiment 1, we did not observe such short biases in temporal choices following the 8-s cue in Experiment 1 or other cues that were similar in duration length, but on the shorter end of the stimulus duration range, in the long-interval group (i.e., 7.56 and 9.52 s). These findings perhaps indicate that DOI administration affects how the animals perceive durations in relation to one another in each temporal context, but not in an absolute manner per se.

4.4 General Discussion

In this study, we investigated the effects of serotonin 2A receptor activation on temporal discriminations by systemic administration of a 5-HT_{2A} agonist, DOI, prior to testing. Crucially, we tested three groups of animals with different, partially overlapping, sets of cue durations ranging from 2 to 24 s, such that longer durations in one set constituted some of the shorter durations in another set. This allowed us to explore if DOI alters the processing of time intervals that are of only specific duration length (i.e., absolute durations), or if it affects how the animals perceive cue durations in relation to one another (i.e., relative durations).

Our findings revealed that DOI injections, particularly at the highest dose (3.0 mg/kg), consistently increased the animals' tendency to make more frequent *short* choices and their response speed. Interestingly, these biases towards short categorizations mainly occurred following longer durations in a given temporal context and did not depend on absolute cue durations (e.g., for those longer than 10 s). For instance, a close look at choice proportions on 6.35-s trials in the short-interval group of Experiment 2 (Figure 4.3A), where the durations ranged from 2-8 s, revealed a response bias towards short choices. However, when another cue that was similar in duration length (i.e., 6 s) was used as a shorter stimulus duration in Experiment 1 (Figure 4.2A), or in the long-interval group in Experiment 2 (Figure 4.3B), DOI did not lead to any shifts in temporal choices on those 6-s trials. Taken together, these findings indicate that DOI leads to a relative shortening of perceived durations and does not change how the animals categorize durations in an absolute manner per se.

The flattening of the higher end of psychometric curves in all duration sets indicates that it is unlikely that higher memory and attentional demands required for timing longer durations are the sole reason for the changes in temporal choices under DOI. One might argue that these findings point to a more central timing deficit that is related to the speed of the internal clock and is independent from the length of timed durations. However, this seems rather implausible, as faster or slower internal clocks that were mostly manipulated in various pharmacological studies been shown to affect duration judgments following, most, if not all, stimulus durations, thus leading to symmetrical horizontal shifts in psychometric curves (e.g., Matell et al., 2004; Meck, 1983; 1996). Furthermore, any theoretical model that assumes an internal clock and changes to its speed via certain experimental manipulations would seem to imply that such clock speed changes should produce similar disruption in different timing tasks (Zeiler, 1998). However, the

leftward shifts in response curves at higher doses of DOI in free-response paradigms where the animals are free to respond during the timing of target intervals (e.g., peak-interval: Asgari et al., 2006a; or free-operant psychophysical procedure: Body et al., 2003, 2006a,b; Cheung et al., 2007), suggest that DOI does not simply alter clock speed.

The asymmetrical changes in the psychometric functions indicate that DOI selectively decreased accuracy on long-duration trials in each temporal context. One prominent theory of this *choose-short* effect, the *subjective-shortening* hypothesis (Spetch & Wilkie, 1983), suggests that the remembered duration of cues shortens with longer retention intervals. Even though there were no programmed delays between cue offsets and lever extensions, the retrospective nature of the task required the animals to wait until the end of the cue presentation to report their choices. Since choice-response latencies systematically increased in most DOI conditions, it is indeed possible that the decrease in response speed might have introduced longer delays after tone offsets, which in turn might have shortened the subjects' working memory for stimulus durations. Furthermore, since working memory may actively contribute to the encoding of durations (Gu et al., 2015), any possible disruptive DOI effects on memory (Meneses, 2007) would be expected to negatively impact the temporal discrimination performance.

Another possible explanation for DOI effects is through a disruption of attention to cue durations. Relevantly, high doses of DOI were found to disturb attentional and vigilance processes in rats that were tested on a two-lever choice reaction task (Nakamura & Kurasawa, 2000). Therefore, it is possible that the performance decrements seen at the higher endpoint of the psychometric functions might also result from inattention to stimulus duration presentations. It has been indeed argued that when attention is diverted during duration encoding, the transfer of total amount of pulses counted by the internal pacemaker to working memory might be

disrupted, which might then result in smaller pulse counts in the working memory (i.e., accumulator) and underestimations of durations (Block & Zakay, 1997).

Disruption of attention and memory processes under DOI, and how such disturbances during the presentation of timed intervals might result in longer sample durations' being consistently categorized as shorter is not surprising, given the involvement of those cognitive processes in temporal cognition (e.g., Gibbon et al., 1984; Polti et al., 2018; Pouthas & Perbal, 2004; Wittmann, 2013; Zakay & Block, 1996). Here it is important to note that, the temporal bisection task involves both retrospective and prospective timing processes in that the subjects both need to attend to the presentation of the timing stimulus in real-time (i.e., prospective) and classify the experienced duration as short or long after cue offset (i.e., retrospective). Therefore, it is important to test if the observed choose-short effects at higher DOI doses would be replicated in other timing tasks where the animals can report elapsed time in prospective timing protocols. However, as we have shown in this study, it is crucial for those studies to test multiple target intervals to better investigate the nature of DOI's effects on timing performance. Such future experiments would provide a more comprehensive picture of how 5-HT_{2A} receptor activation alters time-keeping and other timing-related cognitive process such as attention and memory.

Although we consistently found that DOI leads to subjective shortening of longer durations in different temporal discrimination contexts (see also Halberstadt et al., 2016), the exact nature of these effects is unclear. This is perhaps not surprising as 5-HT_{2A} receptors are widely distributed in the nervous system (Barnes & Sharp, 1999) and can be found in many brain regions including the prefrontal cortex, hippocampus, and basal ganglia (e.g., Jakab & Goldman-Rakic, 1998; Pazos et al., 1985; Raote et al., 2007), with varying degrees of expression. These

brain areas are extensively studied in learning and memory (e.g., Zhang & Stackman, 2015), as well as in the interval timing field (De Corte et al., 2022; Paton & Buonomano, 2018), but their exact contributions to those cognitive functions are not always clearly defined. Given the increasing awareness of serotonin's role in cognition, as well as the development of drugs acting on the serotonergic system that are used for the treatment of a variety of psychiatric conditions, we, therefore, believe that it is important for future studies to examine how the activation or blockade of different serotonin receptor subtypes affect temporal cognition.

4.5 Supplemental Material

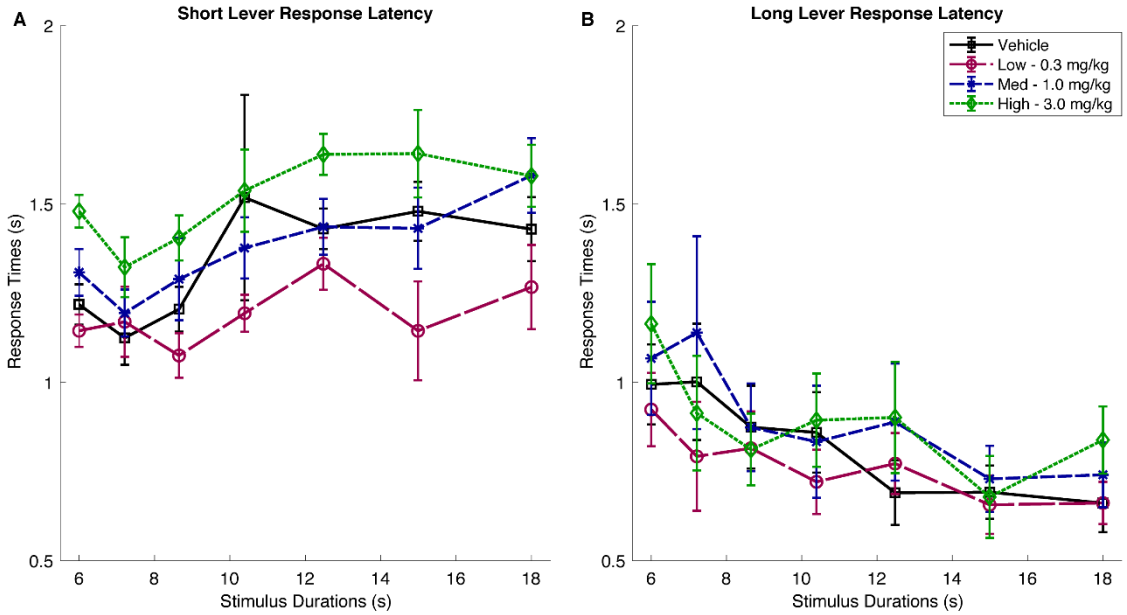

Figure 4.S1. Effects of DOI on short and long choice latency in Experiment 1. Response times are shown separately for short (A) and long (B) choice trials. Error bars depict *SEM*.

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Chapter 5: Examination of the Role of Striatal Direct and Indirect Pathway Medium Spiny Neurons in Action Timing

5.1 Introduction

The ability to anticipate when behaviorally and biologically relevant events will occur is among the most fundamental functions of the animal brain. It allows organisms to plan their actions, adapt to changes in temporal structures in the environment, and understand the relationships between actions and consequences. This fundamental ability is dependent on the animal brain's capacity to tell time across different time scales in the short-interval (e.g., seconds and minutes) and circadian ranges (approximately 24 hours). In particular, most of our daily tasks such as deciding when to hit the brakes as traffic light turns yellow requires the brain to accurately estimate the passage of time on a scale of hundreds of milliseconds to a few minutes (Buhusi & Meck, 2005). Despite the abundance of research on the behavioral properties of this timing ability, the neural mechanisms underlying time representations and temporal processing remain unknown.

A growing body of literature has reported that the timing mechanism is distributed across the brain. Specifically, the neural processing of time on the scale of seconds to minutes has been argued to be distributed across multiple brain structures (Buhusi & Meck, 2005; for reviews see: Paton & Buonomano, 2018; Tallot & Doyère, 2020), including the parietal cortex (Jazayeri & Shadlen, 2015; Leon & Shadlen, 2003), prefrontal cortex (Emmons et al., 2017; Kim et al., 2013; Narayanan & Laubach, 2009), hippocampus and entorhinal cortex (Eichenbaum, 2014; Heys & Dombeck, 2018; MacDonald & Tonegawa, 2021; Mau et al., 2018; Meck et al., 2013; Shimbo et al., 2021), cerebellum (Gooch et al., 2010; Ivry & Keele, 1989; Teki et al., 2012), and basal ganglia (Cruz et al., 2020; De Corte et al., 2019; Emmons et al., 2020; Soares et al., 2016). This

is perhaps not surprising given the links between temporal cognition and sensory processing and other psychological functions such as memory and attention (Matthews & Meck, 2016).

While numerous brain regions have been implicated in temporal information-processing, multiple lines of research suggest the basal ganglia (BG) as a locus of temporal representations. For instance, Fan et al. (2012) showed that GABAergic neurons in the substantia nigra of mice trained to press a lever and hold it down for a certain minimum amount of time changed their activity for duration of the action. A recent study by Hamilos et al. (2020) trained mice in a different motor timing task in which the subjects learned to initiate licking after withholding response following cue onset and recorded dopaminergic (DAergic) signals in the substantia nigra pars compacta (SNc). Their results revealed a ramping activity before movement initiation, and the steepness of this pre-movement ramp predicted movement timing, that is, how long the animals waiting before lick initiation on individual trials. A more direct assessment of the role of midbrain DA neurons in time estimation in a duration categorization task showed that trial-to-trial variations in DAergic responses correlated with temporal judgments, and that decreases or increases in DA activity altered temporal categorizations, illustrating a link between time estimations and the activity of DA neurons in SNc (Soares et al., 2016).

A major input area of the BG, the striatum (Alexander & Crutcher, 1990; Gerfen et al., 1990) is also involved in temporal processing. Several studies that trained rodents to anticipate food rewards delivered on a fixed interval reinforcement schedule and to respond for reward after a fixed delay revealed changes in neuronal firing rates during timed intervals, suggesting the involvement of striatal neurons in temporal information-processing (Bakhurin et al., 2017; Emmons et al., 2020; Matell et al., 2003; Mello et al., 2015). Importantly, in the Mello et al. (2015) study, response profiles of most striatal neurons rescaled with timed intervals that ranged

from 12-60 s, indicating that the dorsal striatum adapts to changes in temporal contingencies. Furthermore, a recent study by Gouvêa et al. (2015) recorded and manipulated the activity of striatal ensembles in rats performing a duration categorization task. Their findings revealed that the dynamics of striatal neurons predicted duration judgments, such that different neuronal subpopulations fired at different times within the timed intervals and their response trajectories guided temporal choices (short vs. long) on individual trials. Finally, muscimol infusions in the dorsal striatum impaired the animals' duration sensitivity, demonstrating a role of striatal populations in guiding time-based choices.

Consistent with these observations, patients with neurological and psychiatric conditions that are characterized by a loss of dopamine neurons in the SNc or affect the basal ganglia circuits such as Parkinson's disease (e.g., Malapani et al., 1998; Parker et al., 2013), Huntington's disease (Freeman et al., 1996), attention deficit hyperactivity disorder (Slater & Tate, 2018), and schizophrenia (Ward et al., 2012) have reported altered experience of time and timing deficits. Similarly, lesions, temperature manipulations, and pharmacological interventions targeting the substantia nigra and the striatum also led to changes in time estimations and disturbances in timing behavior (e.g., Gouvêa et al., 2015; Meck, 2006; Mello et al., 2015; Monteiro et al., 2020; Tunes et al., 2021). Furthermore, D2 type dopamine receptor over-expression in the striatum was also found to disrupt timing behavior (Drew et al., 2007).

Taken together, these findings suggest that the BG circuits, particularly the SNc and dorsal striatum, have access to information about elapsed time and are particularly important for timekeeping (Paton & Buonomano, 2018). However, it is still unclear how the two major BG pathways, direct and indirect pathways contribute to time encoding. The direct pathway medium spiny neurons (MSNs) in the striatum directly target the midbrain, whereas indirect pathway

MSNs in the striatum influence basal ganglia output through the external segment of the globus pallidus and subthalamic nucleus (Alexander & Crutcher, 1990; Parent et al., 1984). In addition, direct pathway MSNs and indirect pathway MSNs express dopamine D1 receptor and D2 receptor, respectively (Gerfen et al., 1990). These subpopulations of neurons are thought to play important roles in movement and action performance (Graybiel, 1998; Jin et al., 2014), although there is still no consensus on whether these subpopulations of neurons work in an antagonistic (Kravitz et al., 2010; Tai et al., 2012) or a coordinated (Tecuapetla et al., 2014; Tecuapetla et al., 2016) manner. It is, therefore, particularly important to further examine striatal direct and indirect pathway MSNs in timing tasks which involve movement initiation, action suppression, and movement termination to better probe the functional roles of these neuronal subpopulations in how the animals decide when to respond in various behavioral procedures (Cruz et al., 2022; De Corte et al., 2019).

Consequently, this project is aimed at investigating the functional significance of direct and indirect pathway MSNs in the dorsal striatum in action timing. To probe the role of D1 and D2 MSNs in action timing we first developed a behavioral task in which mice are trained to withhold responding for a fixed amount of time to gain access to food rewards. Crucially, this task required animals to emit only a single motor response in anticipation of the end of a target time interval to minimize the contribution of lever press-related activity to changes in the neural activity the striatum during delay periods (e.g., Emmons et al., 2017). After validating the task, we then inhibited the activity of striatal D1 and D2 MSNs during the waiting task, using a viral Cre-dependent approach to express a Designer Receptor Exclusively Activated by Designer Drugs (DREADD) in the DMS. Taken together, we think that these experiments will have important implications for understanding how time is processed in the brain and used to guide

behavior, as well as the nature of timing deficits observed in neurological and psychiatric conditions with striatal dysfunction, such as Parkinson's disease (e.g., Malapani et al., 1998; Pastor et al., 1992), Huntington's disease (Beste et al., 2007; Freeman et al., 1996), and schizophrenia (Ward et al., 2012).

5.2 Methods

Experimental Subjects

Male and female D1-Cre and A2a-Cre mice (> 3 months old) were maintained on a 12-h light-dark cycle with ad libitum access to water. 13 A2a-Cre and 10 D1-Cre animals were used. Mice were housed 2-5 per cage. Experimental sessions were conducted during the light phase. During behavioral testing, the mice were maintained at 85-90% of their free-feeding weight. All animal procedures were in accordance with the Columbia University and NYSPI Institutional Animal Care and Use Committees and Animal Welfare regulations.

Stereotaxic Surgery

All surgeries were performed under sterile conditions and isoflurane (1%–5%, plus oxygen at 1-1.5 L/min) anesthesia on a stereotactic frame (David Kopf Instruments). Throughout each surgery, mouse body temperature was maintained at 37°C using an animal temperature controller (ATC1000, World Precision Instruments) and afterward, each mouse was allowed to recover from the anesthesia in its home cage on a heating pad. The mouse head was shaved and cleaned with 70% alcohol and betadine. Prior to virus injections, the skull was exposed, and the periosteum was removed using 3% hydrogen peroxide. The animals were bilaterally injected with a total of 420 nl of either AAV5.hSyn.DIO.hM4D(Gi).mCherry (Addgene; $9.7x10^{12}$ GC/ml titer) or AAV5.hSyn.DIO.mCherry (Addgene; $1.1x10^{13}$ GC/ml titer) into each site using a Nanoject III Injector (Drummond Scientific, USA) at a rate of 30 nl per pulse every 45 s. Two

sites of injection were used for bilaterally targeting the dorsomedial striatum (Site A: AP: 0.75 mm, ML: ±1.3 mm, DV: -3.0 mm from bregma; Site B: AP: 1.15 mm, ML: ±1.3 mm, DV: -3.0 mm from bregma). The injection pipette was left in place for ~10 min post-injection before it was slowly removed. After the injection, the skull was cleaned, and the skin was sealed with tissue adhesive (Vetbond, 3M). Post-operative pain was prevented with Rimadyl after surgery. After the virus was allowed to express for at least 4 weeks, mice were intraperitoneally injected with either a DREADD ligand, JHU37160 (J60; 0.2 mg/kg; Hello Bio, UK, HB6261) or saline 30 min prior to behavioral testing. Six mice in the A2a group and 5 mice in the D1 group were injected with the hM4Di virus. Viral expression was validated histologically in all mice after the completion of data collection.

Behavioral Task

Behavioral training started at least seven days after the stereotaxic surgeries. For mice that received only viral injections, training started approximately one week after the surgery, whereas for mice that underwent fiber optic or GRIN lens implantation surgeries, training started at least 3 weeks after the surgery to ensure sufficient virus expression. In all experiments, we ran animals for a maximum of 60 minutes and 6-7 days per week.

Apparatus

In all experiments, standard operant chambers (ENV-307W; Med-Associates, St. Albans, VT) placed inside sound and light attenuating boxes were used. A feeder trough was centered on one wall of the chamber and used to deliver liquid reward of evaporated milk (0.01 ml). Two retractable levers (ENV-312-2W; 2.2 cm above the grid floor) were mounted to either side of the feeder trough, and a triple LED stimulus lights were placed directly above the levers (ENV-322W). Only the middle, yellow stimulus light (0.4 cm diameter) was used in the experiments. A

house light was turned on throughout the daily sessions. The experimental protocol was controlled via Med-PC computer interface, and behavioral events were recorded with a temporal resolution of 10 ms using the Med-PC IV software. Animals' movements and locations were also recorded using a USB camera (30 fps) that was controlled by Bonsai (https://open-ephys.org/bonsai).

Procedure

Magazine Training. Animals were trained to consume milk rewards (0.01 ml) from a raised dipper. Ten seconds after the first head entry, the dipper was lowered. A variable inter-trial interval (ITI) randomly selected from a list of durations with a mean of 30 s was presented after reward delivery. Sessions ended either after 30 min or 20 dipper presentations, whichever came first. On the subsequent day, animals were trained with the same protocol except this time the dipper was presented for 8 s. All mice were able to collect 20 rewards in both sessions.

Lever press training. Animals were trained to press a lever to earn the liquid reward. Each trial began with the insertion of either the left or the right lever, randomly selected on each trial and the illumination of the middle LED light above the extended lever. A lever press within the first 30 s of lever insertion resulted in immediate reward for 5 s (*Fixed-ratio* 1 [FR1]). The dipper was presented also when the lever has been extended for 30 s without a press (*Fixed-time* 30 s [FT30s]). In both cases, the lever was retracted and the LED light turned off the moment the dipper was raised. After reward delivery, a random ITI (with a mean of 45 s) initiated. Sessions ended after 60 min elapsed or when the mice earned 40 reinforcements, whichever came first. Mice continued receiving sessions like this until they earned 30 rewards in two consecutive sessions. If the mice could not reach the performance criterion in two days with the FR1&FT30s schedule, they were then trained on FR1 trials in which reward delivery was contingent upon a

lever press. The ITI and performance criterion were the same as in FR1&FT30s trials. After the animals completed the lever press training, they did one more FR1 session where the ITI duration was reduced to an average of 10 s.

Waiting task, Phase 1. After mice learned to press the levers, they were trained to wait a certain minimum amount of time before making a lever press (Figure 5.1). In Phase 1, the target duration was 4 s, and the animals could wait at most 10 s to press the lever. Each trial started with a brief tone presentation for 500 ms. Half of the animals were presented with a high frequency tone (10kHz, 80dB), and whereas the other half was presented with a low frequency tone (3kHz, 80dB). After a 1-2 s delay, either the right or the left lever extended and the LED stimulus light turned on. A lever press after 4 s since lever extension turned off the LED light and retracted the lever. After 1-2 s delay, the dipper was presented for 5 s, and an ITI initiated. If an animal failed to wait for at least 4 s before making a lever press, or waited more than 10 s, the lever was retracted and a random ITI was presented. Sessions ended after either 60 mins or 80 rewards, whichever occurred first. After the animals were trained on one lever (e.g., left), they were then trained on the opposite lever (e.g., right). After the animals learned to withhold lever pressing for at least 4 s on the new lever, they were randomly presented with either the left or the right lever in each session. Upon reaching stable performance, mice were injected with saline 30 min prior to testing. At least 5 days of vehicle injections all animals received intraperitoneal injections of a DREADD ligand, JHU37160 (J60; Hello Bio, UK, HB6261) at 0.2mg/kg 30 min prior to behavioral testing for two days. Each J60 day was separated by two vehicle days. After the second 0.2 mg/kg J60 day, animals were injected with vehicle for 1-2 weeks, and then the same J60 injection protocol was repeated at a higher dose (0.8 mg/kg).

Waiting task, Phase 2. After the animals were trained and tested on 4-s waits, they all upshifted to 8-s waits. In this phase, the mice had to wait at least 8 s, but no more than 20 s to make a press. If the animals started with the left lever and a low frequency tone in Phase 1, they started the 8-s waiting training with the right lever and a high frequency tone in Phase 2. All remaining experimental procedures were the same as in Phase 1.

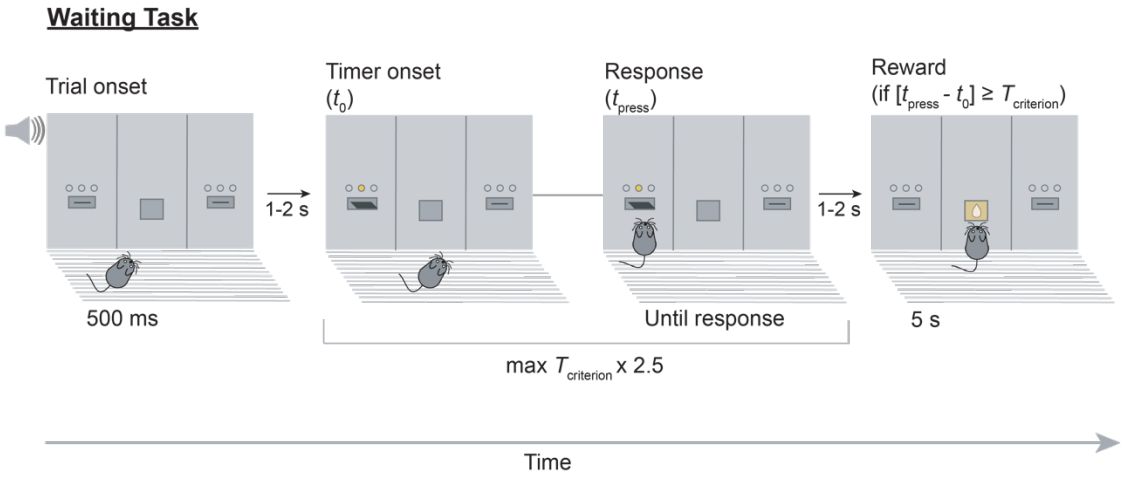

Figure 5.1. Illustration of a reinforced trial sequence in the waiting task. Mice were trained to wait for a fixed interval of either 4 or 8 s before pressing a randomly selected lever to earn a milk reward. Early presses ($[t_{press} - t_0] < T_{criterion}$) terminated the trial and initiated a random ITI (~10s). Trial terminated also when the animals failed to respond within 10 or 20 s of trial onset in 4-s and 8-s target conditions, respectively.

Histology and anatomical verifications

For tissue analysis, the mice were anesthetized with a mixture of ketamine (150 mg/kg) and xylazine (20 mg/kg) solution and transcardially perfused with 1x phosphate-buffered saline (PBS) followed by 4% paraformaldehyde (PFA) solution. Whole brains were then harvested and

post-fixed overnight in 4% PFA at 4°C. Brains were transferred to PBS the next day and stored at 4°C for up to one week. They were then sliced into 40μm coronal sections on a vibratome (Leica) and stored in 1xPBS at 4°C. mCherry expression from DIO-hM4Di and DIO-mCherry in sections containing the striatum was imaged without the addition of fluorescent antibodies.

5.3 Results

Chemogenetic inactivation of striatal indirect pathway medium spiny neurons on waiting performance

We first examined A2a-Cre animals' wait time accuracy on vehicle days and calculated median press latencies in both 4-s and 8-s conditions (Figure 5.2 A&B). Our results indicated that both groups of animals were highly accurate in their waiting performance. On average, the hM4Di group waited 4.35 s (*SD* = 0.85) on 4-s trials, and 8.55 s (*SD* = 1.00) on 8-s trials. Similarly, mCherry controls waited an average of 4.31 s (*SD* = 0.81) and 9.07 s (*SD* = 1.16) on 4-s and 8-s trials, respectively. We then normalized median press latencies for each subject with target wait times (Figure 5.2 C&D). Here, a value of 1 indicates perfect accuracy, whereas values below 1 indicates early responses and values above 1 indicates late responses. The visual inspection of Figure 5.2 C&D panels reveal that J60 administration led hM4Di animals to respond early on both 4-s and 8-s trials, but did not impact mCherry-control animals' press latencies. When we ran a 2x3 repeated-measures ANOVA with Duration (4 and 8 s) and Drug Condition (Vehicle, Low J60 [0.2 mg/kg], High J60 [0.8 mg/kg]) factors separately for hM4Di and mCherry groups, we only found a main effect of Drug Condition, $F(2, 8) = 20.38$, $p < .001$, $n_p^2 = 0.45$ in the hM4Di group. Follow-up Holm-corrected pairwise comparisons revealed significant differences in normalized wait times between vehicle and low J60 dose conditions ($p = 0.003$), and between vehicle and high J60 dose conditions ($p < 0.001$). There was not a

significant mean difference between the two J60 conditions ($p = 0.20$). Same analysis for mCherry animals' waiting performance did not reveal any significant effects of J60 or duration changes (all Fs < 2.38, ps < 0.62).

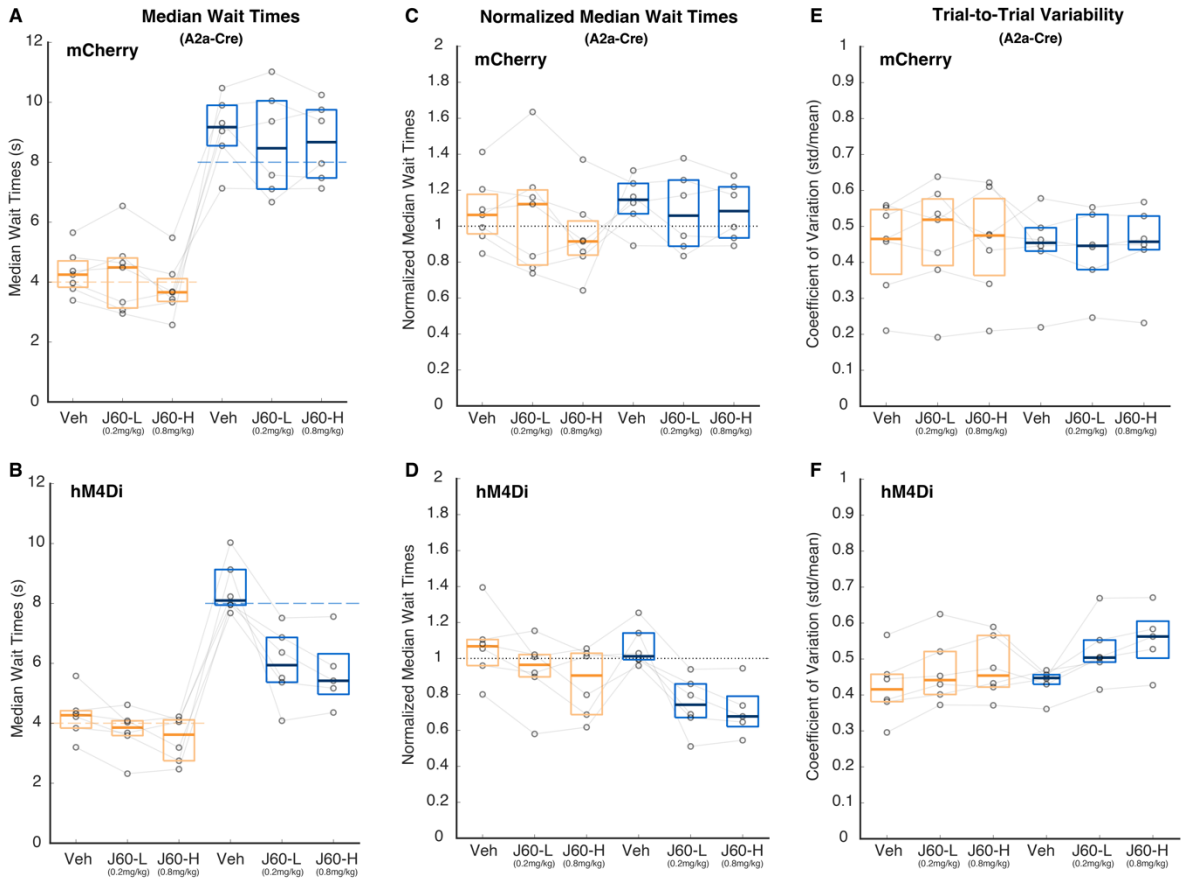

Figure 5.2. Effects of J60 on waiting behavior in the A2a-Cre group. Changes in wait times (A, B), normalized wait times (median wait times/target wait time; C, D), and trial-to-trial variability (E, F) due to the chemogenetic inactivation of indirect pathway medium spiny neurons are shown for mCherry control and hM4Di animals.

To better understand the effects of J60 on waiting behavior, we also examined the trial-to-trial variability in wait times and calculated the coefficient of variation (CV; std/mean of wait times) values for each subject. As can be visualized in Figure 5.2 E&F), J60 administration reduced hM4Di animals' precision in their waiting behavior. Supporting these findings, a 2x3 repeated-measures ANOVA (Duration x Drug Condition) run on CV values revealed only a main effect of Drug Condition, $F(2, 8) = 20.06$, $p < .001$, $n_p^2 = 0.84$, in the hM4Di group. Follow-up pairwise comparisons again showed significant increases in trial-to-trial variability of wait times between vehicle and both drug conditions (veh vs. low J60 dose: $p = 0.002$; veh vs. high J60 dose: $p = 0.001$). Similar to our accuracy findings, the amount of increase in CVs did not differ between the two J60 doses ($p = 0.36$). Finally, we found no effects of J60 or target wait duration on mCherry animals' timing precision, (all Fs < 0.64, ps < 0.80).

Chemogenetic inactivation of striatal direct pathway medium spiny neurons on waiting performance

We found that D1-Cre animals were also highly accurate in their waiting behavior on vehicle days in both 4-s and 8-s conditions (Figure 5.3 A&B). On average, the hM4Di group waited 4.05 s ($SD = 0.67$) on 4-s trials, and 8.06 s ($SD = 0.77$) on 8-s trials. Similarly, mCherry controls waited an average of 4.48 s ($SD = 0.46$) and 7.70 s ($SD = 1.08$) on 4-s and 8-s trials, respectively. We then normalized median press latencies for each subject with target wait times (Figure 5.3 C&D), and found that J60 administration had no effect on normalized wait times in both hM4Di and mCherry-control animals. A 2x3 repeated-measures ANOVA (Duration x Drug Condition) run for the hM4Di group supported these observations and did not reveal any significant effects of J60 or duration changes on normalized press latencies (all Fs < 1.89, ps < 0.79). The same analysis for the control animals only revealed a slight main effect of Duration on normalized

wait times, $F(1, 4) = 9.59$, $p = .04$, $n_p^2 = 0.71$. Follow-up pairwise comparisons revealed slightly longer wait times on 4-s trials as compared to 8-s trials ($p = 0.04$). We did not find a significant main effect of Drug Condition, or a Duration x Drug condition interaction (both Fs < 1.26, ps < 0.73).

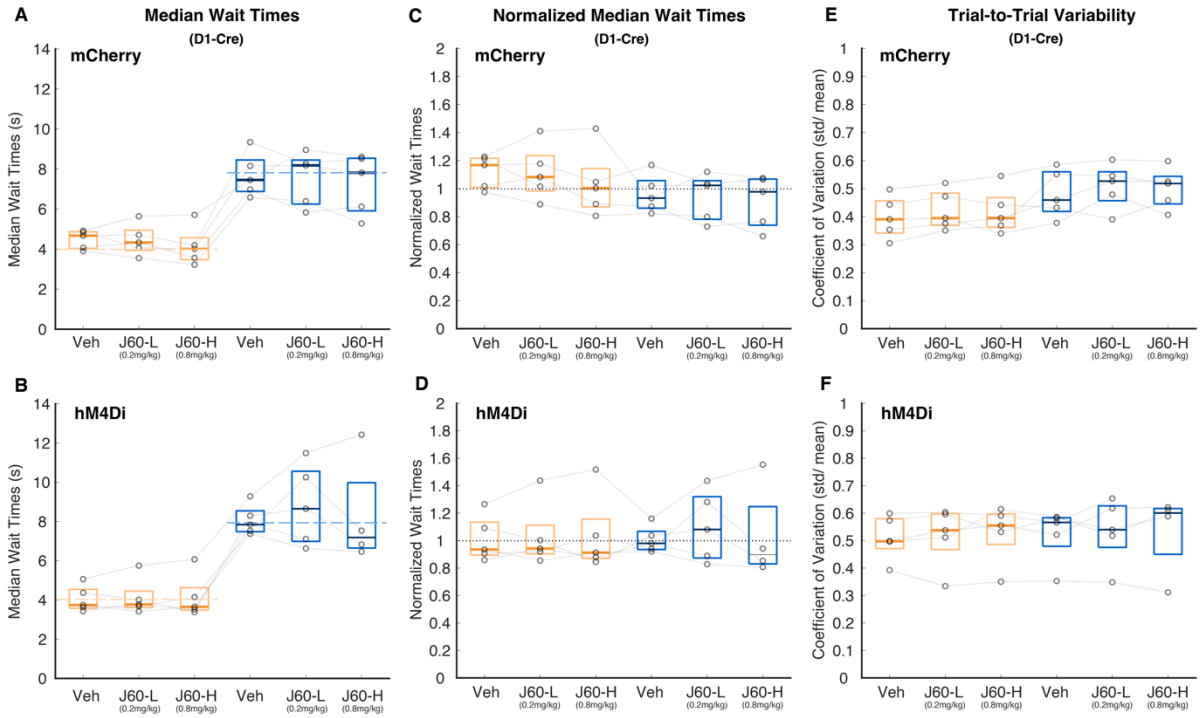

Figure 5.3. Effects of J60 on waiting behavior in the D1-Cre group. Changes in wait times (A, B), normalized wait times (median wait times/target wait time; C, D), and trial-to-trial variability (E, F) due to the chemogenetic inactivation of direct pathway medium spiny neurons are shown for mCherry control and hM4Di animals.

We then analyzed the trial-to-trial variability of wait times, quantified as coefficient of variation (CV), and did not find an effect of J60 or the change in target wait times (Figure 5.3 E&F). Our analyses of CV values in both groups by separate 2x3 repeated measures ANOVAs

did not reveal any changes in subjects' precision in their waiting behavior (hM4Di: all Fs < 1.06, ps < 0.38; mCherry: all Fs < 5.02, ps < 0.09).

Although J60 did not affect the accuracy or the precision of waiting behavior, it led to an increase in the proportion of missed trials in the hM4Di group, indicating that the animals waited too long to press the lever and missed the response window on some trials. A 2x3 repeated-measures ANOVA (Duration x Drug Condition) indeed revealed a main effect of drug administration, $F(2, 6) = 6.00$, $p = .04$, $n_p^2 = 0.67$, and follow-up pairwise comparisons showed a difference in the proportion of missed trials between vehicle and low J60 dose conditions (p = 0.04; other ps > 0.32). We did not find a significant main effect of Duration, or a Duration x Drug Condition interaction (both Fs < 9.14, ps < 0.76). Same analysis for the control animals only revealed a main effect of Duration, $F(1, 4) = 11.88$, $p = .03$, $n_p^2 = 0.75$, with higher number of missed trials on 4-s trials (p = 0.03). There was not a significant change under different drug conditions, or due to a Duration x Drug Condition interaction (both Fs < 2.20, ps < 0.75).

5.4 Discussion

In the current set of experiments, we examined the effects of chemogenetic inactivation of direct and indirect pathway medium spiny neurons in the dorsomedial striatum during a waiting task. Since timing effects are expected to produce proportional shifts in behavioral measures of interest (i.e., response latencies in our experiments), we used two different target wait times. Consequently, we expected that any effect of chemogenetic inactivation of direct and indirect pathway MSNs on timing would lead wait times to shift in proportion to the interval being timed, i.e., 4 or 8 s. In contrast, any pure motoric effects would be expected to be revealed through absolute shifts in behavioral measures (e.g., 2 s increase or decrease in response speed in both duration conditions).

Our results indicated that the DREADD-induced inhibition of indirect pathway MSNs resulted in shorter and more variable wait times. Critically, the magnitude of changes in how long the animals could withhold lever pressing in the A2a-Cre animals was proportional to target durations (10-20%), rather than the same absolute amount (e.g., 3 s) in both 4-s and 8-s conditions. Conversely, the inhibition of direct pathway MSNs did not lead to any changes in the accuracy and precision of waiting behavior of D1-Cre animals. However, it increased the proportion of missed trials, as animals waited too long and failed to press the levers during the response window on some trials.

These findings are in line with previous studies demonstrating a more prominent role of the striatopallidal pathway in temporal processing than the striatonigral pathway of the basal ganglia. In a recent study, De Corte et al. (2019) trained rats on a peak-interval (PI) task and infused a D1 or D2 antagonist into the dorsomedial or dorsolateral striatum. The results indicated that D2 receptor blockade in both regions caused later start and stop times in the PI task, whereas blocking D1 receptors delayed the decision to stop responding only in the dorsomedial striatum and did not impact start times. Similarly, Drew et al. (2003) evaluated the effects of dopamine D1 and D2 antagonist drugs on rat's timing performance in the PI procedure with two time intervals. They found that the systemic administration of haloperidol, a D2 antagonist, led to increases in start and stop times for both intervals, whereas SCH23390, a D1 antagonist, decreased response rates, but did not have any pronounced effects on the timing of responses. Finally, Kamada and Hata (2021) investigated the effect of D1 dopamine receptor (D1DR) blockade within the dorsal striatum on rats' timing performance also in the PI procedure. Their results showed that SCH23390, a D1DR antagonist, infusions into the dorsocentral striatum mainly reduced maximum response rates and increased the time to start responding, without

affecting the times to terminate responding on the PI task. Although these manipulations of D1 or D2 receptors do not always lead to consistent shifts in timing behavior, it is clear that the indirect pathway of the basal ganglia plays a more significant role than the direct pathway in temporal cognition.

However, the null effect of striatal direct pathway MSN activity inactivation on waiting behavior may not definitively imply a lack of effect. It is well-established that the dorsomedial striatum receives and integrates information from multiple brain regions including the cortex and thalamus, and plays essential roles in movement, goal-directed behavior, instrumental learning, and temporal processing (e.g., Emmons et al., 2020; Lex & Jauber, 2010; Lu et al., 2021; Yin et al., 2005). Since individual neurons in a given neural subpopulation might respond differentially to a variety of behavioral variables (e.g., movement, reward encoding, decision accuracy, etc.), as in the case of dopamine neurons in the midbrain (Engelhard et al., 2019), it is then possible that there are different striatal neuronal subpopulations of various sizes that encode specific behavioral processes in the waiting task. Although pharmacological and chemogenetic studies advance our understanding of the neural circuitry of temporal processing, it is necessary to selectively target different neuronal populations in the striatum to better probe the functional activity of individual D1 and D2 MSNs. This would allow us to better understand what percentage of cells in a given neuronal subpopulations encode specific behavioral variables (e.g., response initiation, reward retrieval, etc.), or whether individual striatal MSNs multiplex various behavioral variables and temporal information.

Furthermore, our task design and the animals' behavioral strategies during the waiting period might have also affected the extent of D1 and D2 MSNs' differential contributions to waiting performance. Although we specifically trained the animals to withhold responding

during the waiting period and emit a single lever press after a fixed amount of time has elapsed since trial onset, animals were still free to generate diverse movement profiles. Since D1 and D2 MSNs have been shown to have differential roles in regulating movements, action sequences, and action suppression (Barbera et al., 2016; Cruz et al., 2022; Klaus et al., 2017; Tecuapetla et al., 2016; Vicente et al., 2016), the subjects' selection of distinct behavioral strategies during waiting periods might have modulated the impact of D1 and D2 MSN inactivation on waiting behavior. This also necessitates the investigation of spatially and genetically defined neurons, coupled with behavioral monitoring, to allow for selective targeting of neuronal subpopulations to better account for animals' motor behavior before and during responding in timing tasks.

In conclusion, in this study we investigated the roles of direct and indirect pathway medium spiny neurons in the dorsomedial striatum in timing behavior by using a virally-mediated chemogenetic approach to remotely and temporarily inhibit neural activity in D1 and D2 MSNs during a waiting task. Our results indicated that the DREADD-induced inhibition of D2 MSNs led to earlier and more variable wait times. Critically, the shifts in wait times were mostly proportional to target durations (10-20%), rather than a fixed absolute amount (e.g., 3 s) in both 4-s and 8-s conditions. On the other hand, the inhibition of D1 MSNs did not result in any significant changes in press latencies. These findings shed light on the distinct contributions of direct and indirect pathways to timing, and provide evidence for a significant role of the striatopallidal pathway in modulating time-dependent behaviors.

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Chapter 6: Conclusion

The work presented in this dissertation provides evidence for adaptive timing behavior and strengthens the evidence for the existence of a distributed neural network underlying temporal information-processing. After providing a brief introduction to theoretical and methodological approaches to interval timing in Chapter 1, we first examined the nature of temporal representations in Chapter 2, with the aim of understanding how the temporal distance between behaviorally relevant events is encoded to guide behavior. To this end, we tested mice in a duration discrimination procedure in which they learned to correctly categorize tones of different durations as short or long. After being trained on a pair of target intervals the mice transferred to conditions in which cue durations and corresponding response locations were systematically manipulated so that either the relative or absolute mapping remained constant. Our results demonstrated that mice can represent experienced durations both as having a certain magnitude (absolute representation) and as being shorter or longer of the two durations (an ordinal relation to other cue durations), with relational control having a more enduring influence in temporal discriminations.

We think that it is this fundamental ability of describing temporal relations and event durations in various ways that enables organisms to perform mental operations of time in an effective manner. This is highly crucial, as executing appropriate behavior at the correct time and in the correct amount of time most often requires mentally manipulating two or more durations of time. Therefore, in the next experiment (Chapter 3) we investigated the mental operation of time. Specifically, we tested the effects of mental addition on time estimates in a temporal reproduction task. Briefly, participants were presented with two durations and then were asked to produce either a first or second duration (*serial* condition) or the sum of two durations (*addition*

condition). Our results indicated that humans are able to successfully perform mental operations on time intervals, as evidenced by the lack of significant differences in time judgments between serial and addition trials. Although we did not present those data here, we also conducted studies where we were able to generalize our findings to other duration ranges and to the subtraction operation.

These two behavioral studies presented in Chapters 2 and 3 demonstrate that time is represented in various ways, either in parallel or by converting from one to another. This is quite important, as previous studies investigating the nature of temporal representations typically argue for one type of temporal code over the other (e.g., absolute vs. relative). Instead, we would like to caution the reader against a dichotomy and suggest that time is encoded on a ratio scale which enables the computation of nominal, ordinal and even metric (interval and ratio) representations of time to guide behavior.

This discussion on the nature of temporal representations has contributed to the differences in the assumptions of timing theories. Several theories posit that the brain encodes durations as distinct temporal objects and treat them as unordered stimulus categories (e.g., Gershman & Uchida, 2019; Hardy & Buonomano, 2018; Karmarkar & Buonomano, 2007; Matell et al., 2003), whereas others suggest that time is encoded in units and compared linearly (e.g., Church, 1984; Gibbon et al., 1984; Treisman, 1963). If one assumes that remembered cue durations are categorical and subjectively unordered, it is hard to understand how our subjects were able to (1) discriminate novel cue durations with high accuracy when the relative mapping of duration and lever assignments were maintained between pre- and post-transfer sessions in Chapter 2, or (2) perform mental operations on time intervals such as addition in Chapter 3. It should be noted, however, that even though our findings provide support mostly for theoretical frameworks

assuming a linear metric of time, those different accounts of timing might not be mutually exclusive for different timescales and more complex environmental contexts. Needless to say, further research, particularly those carefully integrating many levels of organization from behavioral to neuronal are needed to help guide theoretical interpretations.

After presenting the two behavioral studies that provided evidence for the flexible timing ability in humans and mice, we then sought to understand the neural bases of adaptive temporal processing. The neural processing of time on the scale of seconds to minutes has been argued to be distributed across multiple brain structures, and the serotonergic and dopaminergic pathways play crucial roles in the timing circuit (Buhusi & Meck, 2005; Hinton & Meck, 1997; Ho et al., 2002). To further probe the neural bases of flexible temporal processing, we first investigated the effects of serotonin (5-HT) 2A receptor activation on temporal discriminations by systemic administration of a 5-HT_{2A} agonist, DOI, prior to testing in a temporal bisection task in Chapter 4. Our findings consistently revealed that a higher dose of DOI leads to the relative shortening of longer durations and does not affect how the animals categorize durations in an absolute manner per se. We think that such relative timing changes in temporal discriminations are possibly mediated by DOI's potential effects on attention and memory processes which are closely related to temporal cognition (e.g., Polti et al., 2018; Pouthas & Perbal, 2004; Wittmann, 2013; Zakay & Block, 1996).

In addition to the serotonergic system, dopaminergic pathways have been heavily implicated in temporal processing. Previous studies suggest that the basal ganglia (BG) circuits, particularly the SNc and dorsal striatum, have access to information about elapsed time and are particularly important for timekeeping (Buhusi & Meck, 2005; Paton & Buonomano, 2018). However, it is still unclear how the two major BG pathways, direct and indirect pathways, contribute to time

encoding (Cruz et al., 2022). Therefore, in Chapter 5 we investigated the roles of direct and indirect pathway medium spiny neurons (MSNs) in the dorsomedial striatum in timing behavior. We used a virally-mediated chemogenetic approach to remotely and temporarily inhibit neural activity in D1 and D2 MSNs during a waiting task. Our results revealed that the DREADD-induced inhibition of D2 MSNs led to earlier and more variable wait times, whereas the inhibition of D1 MSNs did not lead to any major changes in press latencies. Importantly, the changes in wait times observed due to inactivation of the indirect pathway MSNs were proportional to the target durations (10-20%), rather than a fixed absolute amount (e.g., 3 s). These findings provide crucial insight into the distinct contributions of direct and indirect pathways to timing, and suggest a more prominent role of the indirect pathway in modulating time-dependent behaviors.

These data, along with those presented in Chapter 4, highlight the importance of conducting future studies with selective targeting of different neuronal populations in a variety of prospective and retrospective timing tasks. We think that the use of such sophisticated neural and behavioral methods is crucial for (1) advancing our understanding of the contribution of detailed neural circuitry to temporal cognition, as well as the interplay between temporal processing and other cognitive processes such as attention and memory, and (2) informing the theoretical models on why and how specific neural circuits are critical for temporal information-processing.

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