Tugging at time: the role of temporal processing in the organization of string-pulling

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ABSTRACT

TUGGING AT TIME: THE ROLE OF TEMPORAL PROCESSING IN THE ORGANIZATION OF STRING-PULLING

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Northern Illinois University, 2018
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Time perception is one of the most influential cognitive capacities shared across animal species. Interval timing, occurring in the range of seconds to minutes, dictates actions to events experienced in the environment. Operant conditioning techniques have traditionally been used to assess interval timing which relies heavily on extensive training. There may be alternative techniques to investigate processing of temporal information, including exploring the organization of an animal’s repertoire of spontaneously occurring behaviors. String-pulling behavior in rats may be an alternative way to provide a novel approach to investigate time perception in rodents. The current set of studies evaluates the effects of manipulating temporal and motivational factors on the organization of string-pulling behavior. The first study revealed that short and long strings elicited different behavioral responses during string-pulling behavior. The second study demonstrated that high and low reinforcement rates differentially influenced the organization of string-pulling behavior. The third study provided evidence that string odor/length pairing elicited an odor discrimination. The results of this work provide a basis for string-pulling behavior as a novel behavioral assessment of interval timing.
ACKNOWLEDGEMENTS

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CHAPTER 1
INTRODUCTION

Time perception is one of the most influential cognitive capacities shared across many animal species. Interval timing is a sensory dimension used to organize highly trained behaviors (i.e., instrumental conditioning) and spontaneously occurring behaviors (i.e., food protection, interval timing behavior). Motivation also influences behavior; however, the effects of motivational factors are distinct from temporal factors. Time and motivation are guiding factors of behavior, including hunger to seek and consume food. For example, it is advantageous for animals to compare the amount of time it takes to eat a food item, possibly in an open environment, to the time it takes to carry it back to a refuge for consumption (Farine & Lang, 2013; Wallace, Choudhry, & Martin, 2006; Whishaw, Haun, & Kolb, 1999). These natural tradeoffs provide optimal experimental situations to investigate different temporal and motivational processes. A wide range of spontaneous behaviors has been used across many tasks, including dodging, food hoarding, and exploration, to investigate the organization of animal behavior (Wallace, Choudhry, & Martin, 2006; Wallace, Hamilton, & Whishaw, 2006; Whishaw & Tomie, 1987). Although these behaviors may be observed both in the lab and in the wild, temporal characteristics cannot be manipulated easily. Therefore, researchers have relied on behavioral techniques in which temporal and motivational factors can be manipulated systematically to evaluate temporal processes.

Instrumental conditioning techniques (i.e., peak-interval procedure) have been used to investigate the role of temporal and motivational processes in interval timing behavior through
the use of reinforcement schedules across many animal species, including rats and birds (Henderson, Hurly, & Bateson, 2006; Roberts, 1981). The peak-interval (PI) procedure uses schedules of reinforcement, or food delivery, to evaluate temporal information processing. Schedules of reinforcement provide a tool to investigate multiple psychological phenomena, such as when and how a behavior is reinforced; this includes rules about response time and response rate. The fixed-interval (FI) schedule of reinforcement is the basis of the PI procedure and has been used to dissociate temporal and motivational processes (Roberts, 1981). For example, rats elicit responding consistent with estimating FI20 and FI40 s schedules and then were assessed in the production of these intervals using the PI procedure (Roberts, 1981). In addition, the FI schedule of reinforcement has been used to dissociate motivational processes from temporal processes by varying the food reward (Roberts, 1981). This dissociation has provided support for the PI procedure as a valid assessment of interval timing behavior. Interval timing behavior is highly conserved across animal species, and thus it is evaluated similarly in humans as in rats and birds (Gallistel, 1990; Gibbon, Church, & Meck, 1984; Hinton & Meck, 1997; Wearden & McShane, 1998). Similarities in behavior across species have allowed researchers to investigate disruptions in interval timing.

Disruptions in neural systems that support the organization of behavior in time are the basis for impairments observed across a range of pathologies. Specifically, disruptions in interval timing behavior are observed in patients with frontal lobe disorders, including ADHD, schizophrenia, Parkinson’s disease and Huntington’s disease (Barkley et al., 1997; Blekher et al., 2010; Davalos, Kisley, & Ross, 2003; Degardin et al., 2009; Toplak et al., 2006). For example, individuals with ADHD often underestimate temporal intervals and their estimations have
increased variability relative to controls (Pollak et al., 2009). Patients given chronic antipsychotic medication (i.e., dopamine antagonists) for schizophrenia have demonstrated an underestimation in interval production with auditory stimuli and an overestimation with visual stimuli (Meck & Church, 1982; Penney, Gibbon, & Meck, 2000). Patients with Parkinson’s disease underestimate intervals when off of levodopa medication. Accurate interval timing estimation returns to these patients following the administration of L-dopa (Malapani, Deweer, & Gibbon, 2002; Wearden & Lejeune, 2008). Huntington’s disease causes an increase in variability in interval timing behavior (Rao, Marder, & Rakitin, 2014). Novel assessments focused on more spontaneous behaviors may provide an alternative way to evaluate disruptions in interval timing behavior earlier in disease onset and, as a result, more adequate treatments may be developed for patients.

Investigating rats’ spontaneous behavior, such as in a string-pulling task, may provide a novel tool to dissociate the factors which may influence string-pulling behavior, such as temporal or motivation processes. Rats engage in string-pulling behavior rapidly, which may provide insight into neural systems that mediate temporal processing of a spontaneous behavior. In addition, by investigating a spontaneous behavior, compensation in movement organization may be detected following brain damage (i.e., stroke) or drug administration that may have not been detected while investigating a more extensively trained behavior (i.e., lever pressing). This is important as a spontaneous task may be able to detect disease onset sooner than traditional assessments and thus, treatment may begin early. The current set of studies used a spontaneous string-pulling behavior to dissociate temporal and motivational processes. This was done in several ways. The first experiment examined the role of temporal processing in string-pulling
behavior elicited by using strings of varying length. Next, the second experiment evaluated the role of motivational factors in string-pulling behavior elicited by different rates of food reinforcement. Finally, the third experiment investigated whether temporal characteristics of string-pulling behavior were sufficient to elicit odor discrimination.

**Behavioral Basis of Interval Timing**

The use of temporal information to organize behavior has been observed across many species, including ants, bees, rats, and humans (Bonabeau et al., 1997; Meck, 1996; Rakitin et al., 1998; Roberts, 1981; Spaethe, 2001). A traditional behavioral technique (i.e., PI procedure) used in interval timing is reviewed focusing on the production of time and the role of reinforcement rate in the organization of instrumental responding (Roberts, 1981). In addition, interval timing phenomena, which has guided most interval timing research for the past 30 years, is reviewed in light of the current research.

**Peak-Interval Procedure**

The PI procedure is a production task that has been used to investigate the influence of temporal and motivational factors on the organization of instrumental responding. The procedure involves the production of intervals according to a schedule of reinforcement, or food delivery, often used in instrumental responding. The temporal characteristics of food delivery have been demonstrated to alter the organization of animals’ behavior in time (see Figure 1). The FI reinforcement schedule is implemented in the PI procedure and involves reinforcing a single response after a specific amount of time has elapsed; this produces a unique pattern of responding (i.e., lever pressing) that has been attributed to encoding the time of food delivery, such that a gradual increase in response rate is observed that peaks at the time of food delivery.
The observation of the peak response rate at the time of food delivery has allowed researchers to use the PI procedure to investigate the organization of behavior in time in various ways.

Figure 1: Reinforcement schedules representing different response rates reconstructed. Reinforcement schedules are displayed based on interval schedules which may be fixed or variable. Variable schedules involve reinforcement after a certain number of responses, while fixed interval schedules consist of a constant number of responses.
Specifically, rats were first shaped to press a lever to receive a food reward on a FI20 s schedule (Roberts, 1981). For example, a stimulus (i.e., light or sound) is presented for an allotted amount of time (i.e., 20 s), and a rat is required to press a lever to receive a food reward. Only one response is required during the 20 s to receive a reward; thus, food was delivered at 20 s regardless of when the rat pressed the lever (i.e., 1 s or 19 s) or how many times the rat pressed the lever after onset of the stimulus. Probe trials are introduced once rats are trained on a FI conditioning schedule. During probe trials a food reward is not presented at the FI (i.e., 20 s), and the stimulus is at least twice as long as the FI (i.e., 80 s) regardless of lever presses (Roberts, 1981). Lever presses, or rate of response, made by rats were recorded during probe trials and used as measures of performance in the PI procedure. Rats exhibit a gradual increase in the rate of lever pressing from the onset of a stimulus until the FI was reached during the PI procedure. Several processes may be inferred from the rate of responding during the interval. First, peak time was calculated as the time of the maximum response rate measured from the beginning of the stimulus onset (i.e., light). Second, peak rate represented the value of the maximum response rate during the trial (Roberts, 1981). These measures have characterized behavior in the PI procedure across many species.

The PI procedure has been shown to dissociate processes related to interval timing and motivation. First, the time between stimulus onset and delivery of reinforcement has been observed to influence responding. For example, FI20 s vs. FI40 s results in the peak response times occurring at 20 s or 40 s after the stimulus is presented, respectively. However, the peak response rate is maintained across both intervals. Next, varying the amount of food reward has been shown to alter rats’ behavior (i.e., peak response). For example, rats will run down a
platform quicker to retrieve a larger food reward and slower to retrieve a smaller food reward (Wallace & Fountain, 2002). Further, the manipulation of a food reward has differential effects on peak time and peak rate; although manipulating the food reward has been observed to affect the peak rate in rats, it does not affect peak time (Roberts, 1981). Higher amounts of food reinforcement are associated with higher peak response rates, and lower amounts of food are associated with lower peak response rates; therefore, the strength of peak rate is altered in response to varying reward contingencies, while the time of peak response does not change. The matching law states this type of relative rate of responding is equal to the relative rate of reinforcement (Davison, 2016; Herrnstein, 1970). This law is highly conserved across various behaviors and species. These findings provide evidence for the dissociable processes related to interval timing and motivation. Thus, the PI procedure is important in investigating the processes and neural systems that contribute to interval timing behavior. This simple technique has been adapted to study many aspects of interval timing behavior.

Temporal Information Processing

The PI procedure is a task that has provided a technique to investigate the processing of temporal information in animals. The internal clock was first described by researchers to account for the timing abilities observed in animals in the PI procedure. An internal clock refers to biological processes controlling the ability to time events experienced in the environment (Church & Deluty, 1977; see Figure 2). The internal clock has three components that aid in the organization of temporal behavior, including a pacemaker, an accumulator, and a comparator. First, when a stimulus is recognized, a continuously running pacemaker begins to generate pulses.
Figure 2: The three stages of the internal clock model are displayed with each of their components. Pulses are generated by a pacemaker and fed into a gate which integrates the pulses into reference memory. The incoming pulses are compared to pulses stored in reference memory until a ratio of pulses is matched which allows an organism to respond or not to respond to a stimulus in the environment.
Second, pulses build up in an accumulator where the numbers of pulses stored in working memory are compared to the number of pulses stored in reference memory. Third, these components use a ratio derived from the pulses of working memory and reference memory to compare signals. When the number of pulses in working memory matches the number of pulses in reference memory, a response is made by the animal (i.e., pressing a lever for food or making a choice in a discrimination task). Subsequent research has expanded this model of interval timing.

Previous work has investigated the direction that time flows during interval timing tasks. The internal clock may count down much like an egg timer or count up like a stopwatch. The PI procedure has been used to investigate the direction of time by using two different stimuli (i.e., light or sound). For example, when rats are trained with a FI20 s reinforcement schedule (i.e., light) and then switched to a FI40 s reinforcement schedule (i.e., sound), the internal clock should indicate that 15 s have elapsed while 25 s remain to reach the 40 s criterion. Thus, if animals are timing up, a peak response rate should occur 25 s into the 40 s FI reinforcement schedule or until 40 s have elapsed since the beginning of the 20 s FI reinforcement schedule. Findings from this research have demonstrated rats’ peak response rate occurred around 40 s regardless of where the shift (i.e., 5, 10, or 15 s) occurred during the 20 s signal.

These findings suggest rats timed up to 5, 10, or 15 s during the 20 s signal and then continued to time up when switched to the 40 s interval by shifting the criterion time for reinforcement from 20 s to 40 s when the signal changed from a light to a sound. Three important inferences are drawn from these findings. First, the continuity of timing indicates that the internal clock added the time from these two signals together. Rats could have restarted the
internal clock during this experiment but instead continued timing the signals. Next, if rats were timing down when switched to the 40 s signal 15 s into the 20 s signal, the rat’s clock would only have 5 s left to time, which would be represented by a peak response rate 5 s into the 40 s signal. Finally, timing continued across different stimuli which suggests the internal clock is centrally located in the nervous system and readily accessed by different sensory modalities (Meck & Church, 1982). These results provide evidence that interval timing is supported by an internal clock that counts up; other work has investigated the flexibility of the internal clock model in interval timing using this temporal information.

Previous work has provided evidence that the internal clock can be stopped and started similar to a stopwatch. Rats were trained on a FI40 s schedule of reinforcement and then randomly provided 80 s probe trials to evaluate peak response time. After a peak response time was observed at 40 s during probe trials, gaps or breaks in the signal were presented during probe trials to determine the effects of the gap on interval timing behavior. For example, 10 s into a probe trial the signal would extinguish for 10 or 15 s and then come back on. A peak in response time should be observed at 40 s if the rat continued timing through the timeout. If the rat’s internal clock stopped at the beginning of the timeout and then started again once the light was back on, then a rightward shift in the peak response time would be observed that was proportional to the gap, period. Another possibility is that animals stop the internal clock when the gap begins and completely restart the clock at zero when the timeout ends. If the internal clock is reset following the gap the peak response time should be shifted far beyond 40 s since the initial signal time (i.e., 10 or 15 s) and the gap of 10 s would be added to the regular signal time of 40 s before rats reach the peak response time. In the experimental procedure, rats shifted
peak response time to the right roughly 13 s, demonstrating the ability to stop and restart the internal clock during a gap (Roberts, 1981). These experiments provide evidence that the internal clock maintains accumulated time and continues to add time when either the modality of the signal is changed or a gap in the signal is presented; this led to the investigation of the conditions that prompt resetting the internal clock.

Animals accurately time trial after trial within the same training session, suggesting the internal clock may be stopped and reset. Two stimuli serve as indicators of the end of a trial, including the introduction of a food reward and an intertrial interval, which may signal to an animal to stop and reset its internal clock. If these signals provide instructions for resetting the internal clock, omitting one of the signals should adversely affect timing on the next trial; this is known as the omission effect. Once animals are trained on a FI schedule of reinforcement, omission of reinforcement at the end of one trial leads to the acceleration of responding on the following trial (Staddon & Innis, 1969). This may be a result of the internal clock failing to completely reset between trials (Roberts, 1981).

Roberts (1981) used the PI procedure to evaluate the omission effect. Rats were trained on a FI40 s reinforcement schedule, and the effect of reward omission was observed during an empty test trial that followed the trial on which the reward was omitted. It was found that rats had a lower peak response rate and reached an earlier peak response time on empty trials following reward omission than on baseline empty control trials where there was a reward on the preceding trial. Thus, this experiment demonstrated that removing a reward at the end of a FI prevented complete resetting of the internal clock. These results provide evidence that reward, along with the intertrial interval, acts as a strong signal that normally reset the internal clock to
zero and allows for accurate timing trial after trial. These findings suggest that the internal clock is quite flexible and has many properties of a common stopwatch, although more formal theoretical development of the internal clock model is required to understand how it can accomplish timing features described thus far. These characteristics of performance suggest interval timing functions similar to a stopwatch; however, other aspects of performance indicate the limitations of the internal clock.

While the internal clock model explains some features of interval timing behavior (i.e., time multiple intervals, time up, start and stop clock) it cannot account for the variability observed in performance (Gibbon, Church, & Meck, 1984; Treisman, 1963). Most behavior is variable, including behavior that depends on processing information related to interval timing. In general, animals are better at estimating shorter amounts of time than longer amounts of time. For example, animals would be better at estimating a 20 s interval than a 40 s interval; the change in variability from a 20 s interval to a 40 s interval follows a consistent function (see Figure 3). The inability of the internal clock model to account for such characteristics of performance led to a revision of the model used to describe interval timing behavior.

Specifically, the variability observed in interval timing behavior has been described by the scalar property to response distribution in interval timing tasks. Additional evidence for the scalar property is that the peak response rate is observed in the same location for the response distribution, while the distribution of responses depends on the length of the interval being timed (see Figure 3). Peak response rate has been observed to be approximately the same across all trials in a wide range of organisms including rats, birds, and people (Cheng & Roberts, 1989; Rakitin et al., 1998; Roberts, 1981). Thus, once trained, animals still exhibit a peak response rate
Figure 3: The scalar expectancy theory characterized by the Weber law (A) and timescale invariance (B), reconstructed from Roberts, 1981. (A) Mean response rate is displayed as a function of time. (B) The scalar property is demonstrated after normalizing the temporal response functions by criterion times (i.e., timescale invariance).
at the FI schedule presented regardless of the absence of food or the length of the interval. The internal clock model of interval timing cannot make predictions about why this phenomenon is observed. Many of the concepts advanced in this model of interval timing have a dissociable neural basis; therefore, the following section reviews the neurological basis of interval timing in light of current research.

**Neurobiological Basis of Interval Timing**

A neurobiological basis of interval timing has been investigated using the PI procedure. This work has supported differential neural system involvement in the pacemaker and the memory stage of the internal clock model. The pharmacological manipulations of dopaminergic and cholinergic systems have provided dissociable evidence for each component of the internal clock in governing interval timing behavior. The effects of both agonists and antagonists on these systems are investigated in previous interval timing research.

A series of experiments has provided evidence that processing of the pacemaker is influenced by pharmacological manipulations of the dopaminergic system (see Figure 4). The dopaminergic system is altered by the administration of a dopamine agonist, such as methamphetamine. Methamphetamine can bind to and reverse dopamine transporters, resulting in both the reuptake inhibition and the release of dopamine at the mesocorticolimbic dopaminergic nerve terminals (Nakagawa & Kaneko, 2008). The administration of methamphetamine has been demonstrated to produce immediate behavioral effects in the PI procedure. For example, rats were initially trained on a FI20 s or FI40 s reinforcement schedule to provide a baseline peak response time. Following training, rats were tested in the PI procedure after acute IP injections of methamphetamine (1.5 mg/kg i.p.) to evaluate peak response time
Evidence has supported dopamine for the role of speed of the internal clock with the ability to increase or decrease the speed of durations. Evidence has supported acetylcholine for the role of memory of the internal clock with the ability to shift the memory of an interval to the left or right in time.
during probe trials. The acute administration of methamphetamine resulted in an abrupt early peak response time (i.e., peak time at 32 s instead of 40 s) that has been attributed to a mismatch in pulses. The pulses stored in memory during the initial training accumulated at a different speed than the pulses accumulated after the acute administration of methamphetamine. This mismatch between the pacemaker and the memory stage resulted in an abrupt early peak response time yet did not affect peak response rate. However, methamphetamine did not influence response rate, suggesting that changes in peak time are not attributed to motor impairments. The behavioral effects observed after acute administration of methamphetamine provide support for the role of the dopaminergic system in the pacemaker of the internal clock.

Research has evaluated dopaminergic antagonists for effects in interval timing behavior. The dopaminergic system is altered by the administration of a dopamine antagonist, such as haloperidol. Haloperidol acts by blocking the binding of D2 dopamine receptors on the post-synaptic membrane, resulting in less dopamine binding to D2 receptors in the synapse (Boyson, McGonigle, & Luthin, 1988). Haloperidol administration has also been demonstrated to produce behavioral effects in the PI procedure. For example, rats have been trained on a FI20 s or FI40 s schedule of reinforcement to provide a baseline peak response time. Following training, rats were tested in the PI procedure after acute IP injections of haloperidol (0.12 mg/kg i.p) to evaluate peak response time during probe trials. The administration of haloperidol resulted in a later peak response time (i.e., peak response at 45 s instead of 40 s) that has been attributed to a mismatch in pulses. The pulses stored in memory during the initial training accumulated at a different speed than the pulses accumulated after the acute administration of haloperidol. This mismatch between the pacemaker and the memory stage resulted in an abrupt late peak response.
time. However, peak response rate did not change at all with the administration of haloperidol, discounting the role of motor impairments. The effects observed following acute drug administration of haloperidol and methamphetamine provide support for the role of the dopaminergic system in the pacemaker of the internal clock.

Chronic administration of dopaminergic drugs has provided insight to the mnemonic aspect of interval timing. Upon repeated administration of methamphetamine, rats are able to update the speed of the pulses stored in memory across trials, such that subjective time gradually matches objective time (see Figure 5). Following the termination of repeated methamphetamine administration, rats demonstrate an abrupt late peak response time (i.e., peak time at 48 s instead of 40 s) that has been attributed to a mismatch in pulses. Rats are able to update the memory of the stored pulses across trials until subjective time matches objective time (see Figure 5; Buhusi & Meck, 2002; Heilbronner & Meck, 2014; Lake & Meck, 2013; Maricq, Roberts, & Church, 1981). This research provides another line of evidence to support the role of the dopaminergic system in the pacemaker of the internal clock and the flexibility to encode new temporal intervals.

The chronic administration of haloperidol has also been demonstrated to alter interval timing behavior. Upon repeated administration of haloperidol, rats are able to update the speed of the pulses stored in memory across trials, such that subjective time gradually matches objective time (see Figure 5). Following the termination of repeated haloperidol administration, rats demonstrate an abrupt early peak response time (i.e., peak time at 32 s instead of 40 s) that has been attributed to a mismatch in pulses. Rats are able to update the memory of the stored pulses.
Figure 5: Pacemaker patterns, recreated from Meck, 2002. Mean peak times are displayed for four different groups of rats initially trained on either 40 s (triangle symbols) or 20 s (circle symbols) peak-interval procedures and then tested after the administration of dopaminergic drugs that affect pacemaker speed (A) or after the termination of the drugs (B). The open symbols represent rats treated with haloperidol (0.12 mg/kg i.p.) while the closed symbols represent rats treated with methamphetamine (1.5 mg/kg i.p.).
across trials until subjective time matches objective time (see Figure 5; Buhusi & Meck, 2002; Lake & Meck, 2013). These effects of haloperidol on interval timing behavior provide additional support for the role of the dopaminergic system in the pacemaker of the internal clock and the flexibility to encode new temporal intervals. Recall that chronic administration of dopaminergic drugs causes a progressive change in peak time which supports the role of a flexible memory process. Cholinergic drugs have been observed to influence the performance in the PI procedure consistent with modifying the processing of the memory stage and the internal clock (see Figure 4). Physostigmine naturally occurs in the Calabar bean; it is a reversible cholinesterase inhibitor that increases the effective levels of acetylcholine (Schatzberg & Nemeroff, 1995).

Repeated administration of physostigmine has been demonstrated to produce gradual behavioral effects in the PI procedure, such that the speed at which information is transferred from the pacemaker into reference memory is increased in a dose-dependent manner. The interval timing model assumes that content in temporal memory is dependent on both the number of pulses initially accumulated by the pacemaker and the amount of time required transferring the pulses into memory (Meck, 1996). For example, rats have been trained on a FI20 s or FI 40 s reinforcement schedule to provide a baseline peak response time. Following training, rats were tested in the PI procedure after systemic injections of acute physostigmine (0.01 mg/kg i.p.). The administration of acute physostigmine does not produce abrupt effects in interval timing behavior; however, chronic administration of physostigmine across trials leads to an early peak response time (i.e., peak time at 32 s instead of 40 s; see Figure 6). This effect has been attributed to an increase in acetylcholine and, thus, memory storage speed of the internal clock. Following the termination of repeated physostigmine administration, rats demonstrated a gradual
shift from subjective time to objective time (i.e., peak time at 40 s instead of 32 s; see Figure 6; Meck, 1996). This research supports the role of the cholinergic antagonist, physostigmine, in increasing the memory storage speed of the internal clock. Additional evidence for the role of the memory stage in the internal clock is provided by research examining the effects of cholinergic antagonists.

The administration of acute atropine does not produce abrupt effects in interval timing behavior; however, chronic administration of atropine across trials leads to a late response time (i.e., peak time at 48 s instead of 40 s; see Figure 6). This effect has been attributed to a decrease in acetylcholine and, thus, the memory storage speed of the internal clock. Following the termination of repeated atropine administration, rats demonstrated a gradual shift from subjective time to objective time (i.e., peak time at 40 s instead of 48 s; see Figure 6). This research further supports the role of the cholinergic system in the memory stage of the internal clock.

Pharmacological manipulations of dopaminergic and cholinergic systems have provided evidence that pacemaker and memory stage are dissociable processes. First, the changes observed in performance in the PI procedure associated with the administration of dopamine agonists and antagonists are consistent with the dopaminergic system in the pacemaker of the internal clock. Second, the changes observed in performance in the PI procedure associated with the administration of acetylcholine agonists and antagonists are consistent with the cholinergic system in the memory stage of the internal clock. Traditional assessments of neurobiology in interval timing behavior are investigated using tasks that require extensive training, such as the PI procedure; however, spontaneous behavior may provide an alternative way to investigate the neurobiological role of interval timing processes in animals. If interval timing phenomena
Figure 6: Memory patterns, recreated from Meck, 2002. Mean peak times from four different groups of rats trained on a 40 second (square symbols) or 20 second (circle symbols) peak-interval procedure and tested after the administration of cholinergic drugs that affect memory storage speed (A) or after the removal of the drugs (B). The closed symbols represent rats treated with physostigmine (0.01 mg/kg i.p) and the open symbols represent rats treated with atropine (0.05 mg/kg i.p.). Further research has supported a role for the cholinergic system in the memory stage of the internal clock by evaluating cholinergic antagonists. Atropine is a muscarinic cholinergic receptor antagonist which blocks acetylcholine receptors and results in the breakdown of acetylcholine by acetylcholinesterase (Meyer & Quezner, 2005). Repeated administration of atropine has been demonstrated to produce behavioral effects in the peak-interval procedure. For example, rats were trained on a FI20 s or FI40 s reinforcement schedule to provide a baseline peak response time. Following training, rats were tested in the PI procedure after systemic injections of acute atropine (0.05 mg/kg i.p.) to evaluate peak response time during probe trials.
translate to spontaneous behavior, the behavior may be investigated to determine if interval
timing is mediated by similar neurotransmitter systems as traditional interval timing techniques.

**String-Pulling Behavior**

String-pulling in rats may be an alternative way to investigate the processes related to
interval timing. String-pulling is similar to interval timing in that it is a highly conserved
behavior across different animal species. Dogs (Osthaus, Lea, & Slater, 2005), cats (Whitt et al.,
2009), and rats (Alem et al., 2016; Kolb, Cioe, & Comeau, 2008; Whishaw, Tomie & Kolb,
1992) all have the ability to pull up a string to retrieve a food reward tied at the end. Wild birds
and rats have been observed to engage in the spontaneous behavior of string-pulling in natural
environments (Heinrich, 1995). For example, when a string is tied to a branch with a piece of
meat at the end, crows have successfully retrieved the meat by alternating between an upward
pulling motion of the beak and stepping on the string with the foot to hold it in place; the motion
continues until the end of the string is reached and the bird receives the meat reward (Taylor et
al., 2010). This demonstrates the potential to investigate factors that organize string-pulling
behavior.

String-pulling has been combined with discrimination techniques to investigate cognitive
processes (Koppen et al., 2015; Whishaw, Tomie & Kolb, 1992). For example, when two
different-scented (i.e., lemon and vanilla) strings were presented to rats with only one reinforced
with food, rats learned to pull in the scented string with the food reward across testing days.
These studies provide evidence for the role of string-pulling behavior in investigating cognitive
processes. Some advantages to string-pulling behavior are reducing the length of training time to
less than a week, low cost and possibly to investigate the neural basis of information processing.
that may be more difficult to assess in highly trained behaviors (Koppen et al., 2015). Further, different neural circuits may be recruited for the processing of temporal information for spontaneous behaviors compared to more heavily trained behaviors. Most current behavioral analyses (i.e., PI procedure) require extensive training in order to produce a temporal interval (Balci et al., 2008; Buhusi & Meck, 2007; Kaiser, 2008; Matell & Portugal, 2007; McClure et al., 2014; Pleil et al., 2011; Swantan & Matell, 2011). In addition, string-pulling behavior may rely on alternative neural systems to mediate interval timing processing compared to more heavily trained operant conditioning techniques (i.e., PI procedure). Gross lesions usually result in deficits in performance in traditional assessments of behavior (Whishaw & Kolb, 2004); however, traditional assessments of more selective lesions may not reveal deficits in performance (Koppen et al., 2012). Possible compensatory mechanisms may not allow researchers to detect changes in performance following neural manipulations. However, spontaneous behaviors (i.e., exploration, food hoarding, string-pulling) may be more sensitive in detecting deficits following brain damage (i.e., stroke; Koppen et al., 2015; Martin & Wallace, 2007). String-pulling behavior may be similar to other spontaneous behaviors in the ability to detect changes in performance after more selective manipulations and therefore provide additional information on interval timing processing that may not otherwise be detected using traditional assessments. Thus, the spontaneous nature of string-pulling behavior may provide novel insights to information processing related to interval timing.

The set of experiments reported in this thesis evaluated the potential use of string-pulling behavior to investigate temporal processing and dissociate possible motivational processes. Experiment 1 examined the effects of temporal factors in string-pulling behavior elicited by
training with strings of varied lengths (i.e., short and long). Experiment 2 investigated the effects of motivational factors on string-pulling behavior by varying reward contingencies (i.e., high and low). Finally, Experiment 3 examined the effects of temporal factors by determining whether time to reach the reward was sufficient to elicit discriminative responding. The results of these studies have provided evidence for string-pulling behavior as an assessment of interval timing.
Chapter II

EXPERIMENT 1

Rationale

This experiment was designed to evaluate the hypothesis that temporal factors influence the organization of string-pulling behavior. The organization of movement (i.e., pull time, stops) was assessed during probe trials to determine if retrieving the cashew from a short or long string was sufficient to differentially influence performance. The results of this study build a foundation for future work examining interval timing processing in string-pulling behavior.

Methods

Subjects

Male rats (Rattus norvegicus), 8-10 months old, were randomly assigned to two groups, either a short string (n=7) or a long string (n=6). Rats were pair housed, unless any of the combined weights of rats (N=2, one rat from each group) was above 1,000 grams and then rats were single housed in clear cages. Rats that failed to pull strings during habituation (n=3) were dropped from the study. Vivarium temperature (20 to 21 °C) and light (12-hr light-dark cycle) conditions were consistent throughout testing. Rats were food deprived, maintained at 85% of their free-feeding body weight, and provided water ad libidum. The Institutional Animal Care and Use Committee at Northern Illinois University approved all procedures described in this experiment and all guidelines set by the Office of Laboratory Animal Welfare were followed.
Apparatus

The string-pulling apparatus was a transparent, rectangular cage (46 cm x 26 cm). The apparatus sat on a table that was located 1.3 m above the floor in a room with many cues, including posters, a chair, and a door. In between each trial, the rat was transferred to an opaque holding cage (46 cm x 26 cm) while the testing apparatus was thoroughly cleaned and prepared for the subsequent trial.

Multiple strings (i.e., 1.5 m or 3.0 m respectively) were provided to rats during testing to minimize familiarity to rewarded strings during standard trials and non-rewarded strings during probe trials. In addition, completely new strings were provided every other day during testing to prevent rats from becoming accustomed to the same strings and associating them to standard or probe trials.

Procedures

Habituation

Once food deprived, rats were single housed overnight to provide exposure to unscented strings and cashews. Twenty strings of varying lengths were draped in cages to be spontaneously pulled in by rats. Half of the strings were baited with equal-sized cashews while the other half was not baited with a cashew.

Pretraining

The day following habituation, pretraining began which exposed rats to the testing apparatus. A 1 m string was presented with a cashew tied to the end. Rats were required to pull in eight trials in a day before graduating to a 2 m string the next day. Pretraining criterion was met when rats successfully pulled in eight trials per day for two days.
Training

Once pretraining was complete, rats were randomly assigned to two groups which received one string for eight trials per day over 10 days. Both groups received a 5.0 m string. One group received a food reward at 1.5 m of the string while the other group received a food reward at 3.0 m of the string. In addition, all rats received one probe trial, consisting of a string 5.0 m in length randomly presented throughout the eight trials for each of the 10 testing days.

Data Analysis

Standard Trials

The time it took for rats to approach the string to initiate pulling once placed in the testing apparatus was averaged across the standard trials for each of the 10 days of testing. In addition, the time it took for rats to reach the cashew was averaged across the standard trials for each of the 10 days of testing.

Probe Trials

The time it took for rats to approach the string to initiate pulling once placed in the testing apparatus was averaged across the probe trials for each of the 10 days of testing. The percentage of probe trials that were pulled in the entire way was compared between groups by collapsing percent pulled across all testing days. A probe trial was terminated if the rat stopped pulling in the string and did not resume after 30 s. In addition, the amount of time rats pulled in the string during probe trials was averaged across the probe trials for each of the 10 days of testing.

Exploratory Analysis of Probe Trials

The Peak Modus motion capture system was used to conduct microlevel analysis of left and right hand movement organization during string-pulling behavior. Motion capture software
used x-y coordinates to evaluate kinematic organization of the rats’ hands at 30 frames per second. Several measures were evaluated using the Peak Modus motion capture system, including peak speed and total distance travelled by hands. For each measure, every 30 frames, or 1 s, were averaged from probe trials to determine the expectation of reward. Further analyses of probe trials were purely exploratory.

**Statistical Analysis**

Repeated-measures ANOVAs were conducted across days (i.e., 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10) to evaluate main effects and interactions on each dependent measure. The Greenhouse-Geisser (G-G) correction was used in the analyses where Mauchly’s test indicated significant departure from the assumption of sphericity. Partial eta squared ($\eta^2_p$) was used as a measure of effect size for each main effect and interaction. Linear trend and Tukey HSD post hoc analyses were used to further investigate significant main effects and interactions.

**Results**

**Standard Trials**

**Approach Time**

Results from the first study evaluated group differences in string-pulling organization. Recall that one group experienced short (1.5 m) strings and the other group experienced long (3.0 m) strings with food during standard trials, while both groups received longer (5.0 m) strings without food during probe trials. The amount of time rats took to approach the string was evaluated for standard trials across testing (see Figure 7a). The G-G correction ($\varepsilon=0.196$) was used to adjust the degrees of freedom associated with the lack of sphericity in approach time. Repeated-measures ANOVA conducted on approach time revealed a significant main effect of Day [$F (1.760, 21.126) = 6.155, p = 0.010, \eta^2_p = 0.339$] yet failed to reveal a significant main
Figure 7: Approach time and pull time for standard trials, Study 1. The amount of time it took rats in the first study to approach strings during standard trials (A) is plotted across all testing days. Pull time to reach the cashew for standard trials (B) is plotted across all testing days.
effect of Group \( F(1, 12) = 1.306, p = 0.275, \eta_p^2 = 0.098 \) or Group by Day interaction \( F(1.760, 21.126) = 0.190, p = 0.801, \eta_p^2 = 0.016 \). Post hoc analysis revealed a significant decreasing linear trend in approach time across blocks \( F(1, 12) = 9.396, p = 0.01, \eta_p^2 = 0.439 \).

**Pull Time**

The amount of time rats took to pull in standard trials was evaluated across testing (see Figure 7b). The G-G correction \( (\varepsilon=0.323) \) was used to adjust the degrees of freedom associated with the lack of sphericity in pull time. Repeated-measures ANOVA conducted on pull time revealed a significant main effect of Group \( F(1, 12) = 70.888, p < 0.001, \eta_p^2 = 0.855 \), Day \( F(2.907, 34.881) = 7.914, p < 0.001, \eta_p^2 = 0.397 \), and Group by Day interaction \( F(2.907, 34.881) = 3.197, p = 0.037, \eta_p^2 = 0.210 \). Post hoc analysis revealed a significant decreasing linear trend in approach time across blocks \( F(1, 12) = 29.375, p < 0.001, \eta_p^2 = 0.710 \). Further post hoc analysis revealed that groups differed across all testing days (HSD < 0.05).

**Probe Trials**

**Approach Time**

Results from the first study evaluated group differences in string-pulling organization. Recall that one group experienced short (1.5 m) strings and the other group experienced long (3.0 m) strings during standard trials, while both groups received longer (5.0 m) strings without food during probe trials. First, the amount of time rats took to approach the string was evaluated for probe trials across testing (see Figure 8a). The G-G correction \( (\varepsilon=0.297) \) was used to adjust the degrees of freedom associated with the lack of sphericity in approach time. Repeated-
Figure 8: Approach time and pull time for probe trials, Study 1. The amount of time it took rats in the first study to approach strings during probe trials (A) is plotted across all testing days. The amount of time rats pulled in the string during probe trials (B) is plotted across testing days.
measures ANOVA conducted on approach time failed to reveal a significant main effect of Group \( F(1, 11) = 0.003, p = 0.958, \eta^2_p < 0 \), Day \( F(2.673, 29.399) = 2.221, p = 0.113, \eta^2_p = 0.168 \), or Group by Day interaction \( F(2.673, 29.399) = 0.955, p = 0.418, \eta^2_p = 0.080 \).

### Pull Time

The amount of time rats pulled in strings during probe trials was evaluated across testing (see Figure 8b). The G-G correction \((\epsilon=0.320)\) was used to adjust the degrees of freedom associated with the lack of sphericity in probe pull time. Repeated-measures ANOVA conducted on pull time revealed a significant main effect of Day \( F(2.878, 31.657) = 6.226, p = 0.002, \eta^2_p = 0.361 \) yet failed to reveal a significant main effect of Group \( F(1, 11) = 2.832, p = 0.121, \eta^2_p = 0.205 \) or Group by Day interaction \( F(2.878, 31.657) = 1.316, p = 0.286, \eta^2_p = 0.107 \). Post hoc analysis revealed a significant decreasing linear trend in approach time across blocks \( F(1, 11) = 41.972, p < 0.001, \eta^2_p = 0.792 \).

### Percent of Probes Pulled

Probe trials were further analyzed to evaluate the percent of strings pulled in all the way across the entire testing session (see Figure 9). An independent-samples \( t \) test revealed a significant difference in percent of strings pulled in entirely during probe trials for the short \((M=45.71, SD=24.40)\) and long \((M=90.00, SD=11.55)\) groups \( t(12) = 4.314, p = 0.001 \). All rats that received short standard trials were more likely to stop engaging in string-pulling behavior before the end of the probe string was reached, and rats that received long standard trials were more likely to pull in the entire probe string until the end was reached.

### Exploratory Analysis of Probe Trials

Before further analyses were conducted between groups, the left and right hands were compared for peak speed and total distance travelled during probe trials for the short and long
Figure 9: Percent of probe trials pulled in entirely, Study 1. The percent of probe trials pulled entirely is collapsed across all testing days for rats that received 1.5 m and 3 m during standard trials.
groups respectively. Kinematic characteristics of movement did not differ between left and right hands for the short or long group throughout testing; therefore, all further analyses were averaged across hands for each group.

**Peak Speed**

Peak speed was evaluated to determine if there were any differences between the short and long groups (see Table 1; see Figure 10a). The G-G correction ($\varepsilon=0.347$) was used to adjust the degrees of freedom associated with the lack of sphericity in peak speed. Repeated-measures ANOVA conducted on peak speed failed to reveal a significant main effect of Group [$F(1, 9) = 1.750, p = 0.219, \eta^2_p = 0.163$], Day [$F(3.119, 28.069) = 1.150, p = 0.347, \eta^2_p = 0.113$] or Group by Day interaction [$F(3.119, 28.069) = 1.031, p = 0.396, \eta^2_p = 0.103$]. Short and long groups did not differ in peak speed across testing.

**Total Distance**

Total distance travelled during probe trials was evaluated for the short and long groups (see Table 1, see Figure 10b). The G-G correction ($\varepsilon=0.364$) was used to adjust the degrees of freedom associated with the lack of sphericity in total distance. Repeated-measures ANOVA conducted on total distance revealed a significant effect of Group [$F(1, 9) = 9.064, p = 0.015, \eta^2_p = 0.502$] yet failed to reveal a significant main effect of Day [$F(3.278, 29.501) = 0.523, p = 0.685, \eta^2_p = 0.055$] or Group by Day interaction [$F(3.278, 29.501) = 2.402, p = 0.083, \eta^2_p = 0.005$]. The total distance the hands travelled differed for the short and long groups across testing, such that the short group travelled a shorter total distance on average compared to the long group.
Table 1: Kinematic measures, Study 1. Peak speed and distance statistical values are displayed for probe trials for rats provided with either short or long standard trials in Experiment 1.

<table>
<thead>
<tr>
<th>Kinematic measures</th>
<th>$F$</th>
<th>$p$</th>
<th>$\eta^2_p$</th>
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<tbody>
<tr>
<td><strong>Peak Speed</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Group</td>
<td>1.750</td>
<td>0.219</td>
<td>0.163</td>
</tr>
<tr>
<td>Day</td>
<td>1.150</td>
<td>0.347</td>
<td>0.113</td>
</tr>
<tr>
<td>Group X Day</td>
<td>1.031</td>
<td>0.396</td>
<td>0.103</td>
</tr>
<tr>
<td><strong>Distance</strong></td>
<td></td>
<td></td>
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<tr>
<td>Group</td>
<td>9.064</td>
<td>0.015</td>
<td>0.502</td>
</tr>
<tr>
<td>Day</td>
<td>0.523</td>
<td>0.685</td>
<td>0.055</td>
</tr>
<tr>
<td>Group X Day</td>
<td>2.402</td>
<td>0.083</td>
<td>0.005</td>
</tr>
</tbody>
</table>
Figure 10: Peak speed and total distance, Study 1. Peak speeds averaged across rats’ hands are plotted for each group across testing (A). Total distance traveled (B) by hands is plotted across testing days for each group.
Discussion

This study investigated the organization of string-pulling behavior using short (i.e., 1.5 m) and long (i.e., 3 m) strings. All rats developed a consistent time to approach standard trials across testing demonstrating similar motivation to engage and perform in the string-pulling task. However, groups differed in pull time for standard trials, such that rats that received long strings during standard trials took longer to pull in the string than rats that received short strings during standard trials. In addition, the time it took rats in the short group to pull in standard trials did not change across testing, while the time it took rats in the long group to pull in standard trials decreased across testing days. Temporal, kinematic and topographic characteristics of movement were evaluated during probe trials for evidence of the organization of interval timing behavior. Short and long standard trials were shown to elicit different temporal and kinematic characteristics during probe trials across testing. Rats that received short strings during standard trials were also more likely to disengage in string-pulling behavior before the end of the probe string compared to rats that received long strings during standard trials. This suggests rats in the short group may have expected the food reward earlier compared to rats in the long group. The differences observed in responding during probe trials after receiving short or long strings suggests a role for interval timing processes in the organization of string-pulling behavior. Time to reach the cashew elicited a specific pattern of responding in this experiment, whereas the next experiment was designed to examine whether varying motivation aspects of the string-pulling task would produce a different pattern of results.
Rationale

This experiment examined the hypothesis that motivational factors influence the organization of string-pulling behavior. The organization of movement (i.e., pull time, stopping) was assessed during probe trials to determine if retrieving the cashew with high or low reinforcement was sufficient to differentially influence performance. According to the matching law, animals match behavioral responses to the rate of reinforcement presented in a test session (Davison & McCarthy, 2016). The matching law has been observed in the PI procedure, such that altering reinforcement rate produced behaviorally different responses between the high and low group that affected the rate of response (i.e., speed) during probe trials but not the production of the interval (i.e., stop) (Roberts, 1981). The results of the current study may build a foundation for future work investigating motivational factors in the organization of string-pulling behavior.

Methods

Subjects

Rats (*Rattus norvegicus*), 8-10 months old, were randomly assigned to two groups, either high reinforcement (n=6) or low reinforcement (n=6). These naïve rats were housed under similar conditions as Experiment 1, such that rats (N=3; two high reinforcement, one low reinforcement) with combined weights above 1,000 grams were single housed in clear cages. Rats that failed to pull strings during habituation (n=2) were dropped from the study.
Apparatus

The same string-pulling apparatus used previously in Experiment 1 was used in the second study with several changes, such that strings were all one length (1.5 m) for both groups. Further, each group received different reinforcement contingencies (i.e., high or low).

Procedures

Habituation

Habituation in Experiment 2 was the same as in Experiment 1.

Pretraining

Pretraining in Experiment 2 was the same as in Experiment 1.

Training

Once pretraining was complete, rats were randomly assigned to two groups that received training of eight trials per day over 10 days. The two groups received different reinforcement rates: the high reinforcement group received one probe trial (i.e., 5.0 m) and seven standard trials (i.e., 1.5 m); the low reinforcement group received four probe trials (i.e., 5.0 m) and four standard trials (i.e., 1.5 m) that were randomly presented throughout the eight trials across the 10 days of testing. A cashew was tied as a reward at the end of the string. To account for weight, 3.5 m of string was added to the end of each 1.5 m string, making the length equivalent to the probe string.

Data Analysis

Standard Trials

The time it took for rats to approach the string to initiate pulling once placed in the testing apparatus was averaged across the standard trials for each of the 10 days of testing. In
addition, the time it took for rats to reach the cashew was averaged across the standard trials for each of the 10 days of testing.

**Probe Trials**

Only the first probe trial presented to the low reinforcement group was used for analysis. The time it took for rats to approach the string to initiate pulling once placed in the testing apparatus was averaged across the probe trials for each of the 10 days of testing. The percentage of probe trials that were pulled in the entire way was compared between groups by collapsing percent pulled across all testing days. A probe trial was terminated if the rat stopped pulling in the string and did not resume after 30 s. In addition, the amount of time rats pulled the string was averaged across the probe trials for each of the 10 days of testing.

**Exploratory Analysis of Probe Trials**

The Peak Modus motion capture system was used to conduct microlevel analysis of left and right hand movement organization during string-pulling behavior. Motion capture software used x-y coordinates to evaluate kinematic organization of the rats’ hands at 30 frames per second. Several measures were evaluated using the Peak Modus motion capture system, including peak speed and total distance travelled by hands. For each measure, every 30 frames, or 1 s, were averaged from probe trials to determine the expectation of reward. Further analyses of probe trials were purely exploratory.

**Statistical Analysis**

Repeated-measures ANOVAs were conducted across days (i.e., 1,2,3,4,5,6,7,8,9, and 10) to evaluate main effects and interactions on each dependent measure. The Greenhouse-Geisser (G-G) correction was used in the analyses where Mauchly’s test indicated significant departure from the assumption of sphericity. Partial eta squared ($\eta^2_p$) was used as a measure of effect size.
for each main effect and interaction. Linear trend and Tukey HSD post hoc analyses were used to further investigate significant main effects and interactions.

**Results**

**Standard Trials**

**Approach Time**

Results from Experiment 2 evaluated the effect of varying reinforcement rate on the organization of string-pulling behavior. First, the amount of time it took rats to approach the string during standard trials was evaluated across testing (see Figure 11a). The G-G correction (ε=0.367) was used to adjust the degrees of freedom associated with the lack of sphericity in approach time between the low and high reinforcement groups. Repeated-measures ANOVA conducted on approach time revealed a significant main effect of Group \([F (1, 14) = 9.434, p = 0.008, \eta^2_p = 0.403]\) and Day \([F (3.350, 46.270) = 2.7750, p = 0.048, \eta^2_p = 0.164]\) yet failed to reveal a significant Group by Day interaction \([F (3.350, 46.270) = 1.044, p = 0.387, \eta^2_p = 0.069]\).

Post hoc analysis revealed a significant decreasing linear trend in approach time across days \([F (1, 14) = 15.867, p < 0.001, \eta^2_p = 0.531]\). Rats that received low reinforcement took less time to approach the string during standard trials compared to rats the received high reinforcement.

**Pull Time**

Second, pull time for standard trials were compared between low and high reinforcement groups across testing (see Figure 11b). The G-G correction (ε=0.167) was used to adjust the degrees of freedom associated with the lack of sphericity in pull time between groups. Repeated-measures ANOVA conducted on pull time revealed a significant effect of Group \([F (1, 14) = 13.040, p = 0.003, \eta^2_p = 0.482]\) and Day \([F (1.502, 21.028) = 6.078, p = 0.013, \eta^2_p = 0.303]\) yet
Figure 11: Approach and pull time for standard trials, Study 2. The amount of time it took rats in the second study to approach strings during standard trials (A) is plotted across testing. Pull time to reach the cashew for standard trials (B) is also plotted across testing for each group.
failed to reveal a significant Group by Day interaction \([F (1.502, 21.028) = 2.512, p = 0.116, \eta_p^2 = 0.152]\). Post hoc analysis revealed a significant decreasing linear trend in pull time across blocks \([F (1,14) = 38.818, p < 0.001, \eta_p^2 = 0.735]\); further, rats which received low reinforcement took significantly more time to pull in the string to reach the cashew than rats that received high reinforcement.

**Probe Trials**

**Approach Time**

The amount of time it took rats to approach the string during probe trials was evaluated across testing (see Figure 12a). The G-G correction \((\varepsilon=0.343)\) was used to adjust the degrees of freedom associated with the lack of sphericity in approach time between the low and high reinforcement groups. Repeated-measures ANOVA conducted on approach time revealed a significant main effect of Group \([F (1, 14) = 11.748, p = 0.004, \eta_p^2 = 0.456]\) yet failed to reveal a significant main effect of Day \([F (3.089, 43.247) = 0.790, p = 0.509, \eta_p^2 = 0.053]\) or Group by Day interaction \([F (3.089, 43.247) = 0.784, p = 0.513, \eta_p^2 = 0.053]\). Rats that received low reinforcement took longer to approach standard trials than rats that received high reinforcement.

**Pull Time**

The amount of time rats spent pulling the string during probe trials was analyzed for the low and high reinforcement groups across testing (see Figure 12b). First, repeated-measures ANOVA conducted on pull time revealed a significant main effect of Day \([F (9, 126) = 7.810, p < 0.001, \eta_p^2 = 0.358]\) and Day by Group interaction \([F (9, 126) = 2.016, p = 0.043, \eta_p^2 = 0.126]\) yet failed to reveal a significant main effect of Group \([F (1, 14) = 2.079, p = 0.171, \eta_p^2 = 0.129]\). Post hoc analysis revealed a significant decreasing linear trend in pull time across blocks \([F (1, 14) = 25.275, p < 0.001, \eta_p^2 = 0.644]\). Rats did not differ between Days 4, 6 or 8 of
Figure 12: Approach and pull time for probe trials, Study 2. Approach time (A) and the amount of time rats pulled in strings during probe trials (B) is plotted across testing.
testing, whereas rats that received low reinforcement spent less time pulling in the probe string on Days 7, 9 and 10 compared to rats that received high reinforcement.

Then, a single repeated-measures ANOVA ($\varepsilon=0.424$) conducted on pull time for rats that received high reinforcement failed to reveal a significant main effect of Day \( [F(9, 26.696)] = 2.180, p = 0.101, \eta^2_p = 0.237 \). Pull time for probe trials did not change across days for rats that received high reinforcement. Next, a single repeated-measures ANOVA ($\varepsilon=0.399$) conducted on pull time for rats that received low reinforcement revealed a significant main effect of Day \( [F(3.588, 25.114) = 1815.700, p < 0.001, \eta^2_p = 0.539 \]. Therefore, amount of time spent pulling in the string during probe trials changed across days for rats that received low reinforcement.

**Percent of Probes Pulled**

Probe trials were further analyzed to evaluate the percent of probes that were pulled in entirely collapsed across testing days (see Figure 13). An independent-samples $t$ test revealed a significant difference in percent of probes pulled in the entire way for the low (M= 13.75, SD= 10.61) and high (M= 35.00, SD= 19.27) reinforcement groups \( [t (14) = -2.732, p = 0.016] \). All rats that received low reinforcement were more likely to stop engaging in string-pulling behavior before the end of the probe string was reached, and rats that received high reinforcement were more likely to pull in the entire probe string until the end was reached.

**Exploratory Analysis of Probe Trials**

Before further analyses were conducted between groups, the left and right limbs were evaluated for peak speed and total distance of hand movement for probe trials for the low and high reinforcement groups respectively. After analyses of left and right hand use was evaluated for the low and high reinforcement group respectively and no differences were found between the left and right hands, all further analyses averaged across hands for each group.
Figure 13: Percent of probes pulled in entirely. Study 2. The percent of probe trials pulled in entirely is collapsed across all testing days for each group.
Peak Speed

Peak speed was evaluated to determine if there were any differences between the low and high reinforcement groups (see Table 2; see Figure 14a). Repeated-measures ANOVA conducted on peak speed revealed a significant main effect of Day \( [F(9, 126) = 2.086, \ p = 0.035, \ \eta^2_p = 0.130] \) yet failed to reveal a significant main effect of Group \([F(1, 14) = 0.361, \ p = 0.558, \ \eta^2_p = 0.025]\) or a significant Group by Day interaction \([F(9, 126) = 1.007, \ p = 0.438, \ \eta^2_p = 0.067]\). Post hoc linear trend analysis revealed peak speed decreased across days for both low and high reinforcement groups \([F(1, 14) = 0.434, \ p = 0.521, \ \eta^2_p = 0.030]\).

Total Distance

Total distance travelled was evaluated to determine if there was a difference between the low and high reinforcement groups (see Figure 14b). The G-G correction \((\varepsilon=0.333)\) was used to adjust the degrees of freedom associated with the lack of sphericity in total distance between groups. Repeated-measures ANOVA conducted on total distance revealed a significant main effect of Day \([F(3.001, 42.021) = 4.457, \ p = 0.008, \ \eta^2_p = 0.241]\) yet failed to reveal a significant main effect of Group \([F(1, 14) = 0.510, \ p = 0.487, \ \eta^2_p = 0.035]\) or Group by Day interaction \([F(3.001, 42.021) = 2.335, \ p = 0.088, \ \eta^2_p = 0.143]\). Post hoc linear trend analysis revealed that the total distance travelled decreased for both the low and high reinforcement groups across testing days \([F(1, 14) = 7.684, \ p = 0.015, \ \eta^2_p = 0.354]\).

Discussion

This study demonstrated that reinforcement rates influenced the organization of string-pulling behavior. Different reinforcement rates (i.e., low or high food reward) produced distinct patterns of string-pulling behavior, such that low reinforcement resulted in a longer time to approach the string and a longer time to pull in standard trials compared to high reinforcement.
Table 2: Kinematic measures, Study 2. Peak speed and distance statistical values are displayed for probe trials for rats provided with either high or low reinforcement during standard trials in Experiment 2.

<table>
<thead>
<tr>
<th>Kinematic measures</th>
<th>$F$</th>
<th>$p$</th>
<th>$\eta^2_p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak Speed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td>0.361</td>
<td>0.558</td>
<td>0.025</td>
</tr>
<tr>
<td>Day</td>
<td>2.086</td>
<td>0.035</td>
<td>0.130</td>
</tr>
<tr>
<td>Group X Day</td>
<td>1.007</td>
<td>0.438</td>
<td>0.067</td>
</tr>
<tr>
<td>Distance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td>0.510</td>
<td>0.487</td>
<td>0.035</td>
</tr>
<tr>
<td>Day</td>
<td>4.457</td>
<td>0.008</td>
<td>0.241</td>
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<tr>
<td>Group X Day</td>
<td>2.335</td>
<td>0.088</td>
<td>0.143</td>
</tr>
</tbody>
</table>
Figure 14: Peak speeds and total distance, Study 2. Peak speeds averaged across rats’ hands are plotted for each group across testing (A). Total distance traveled by hands (B) is plotted across testing days for each group.
Further, rats that received low reinforcement were less likely to pull in the entire string during probe trials, while rats that received high reinforcement were more likely to pull in the entire string during probe trials. These results demonstrate changes in motivation to engage and perform in the string-pulling task are dependent on reward contingencies. Yet, pull time did not differ for probe trials between low and high reinforcement groups across testing. This suggests that string-pulling may be an online process that rats are already highly efficient at performing from the very beginning of testing. In addition, no differences were found in peak speed between groups; however, performance was related to groups with varied motivation, suggesting that temporal and motivational factors are independent processes in string-pulling behavior. Performance in the string-pulling task was influenced by factors other than time (i.e., motivation).

Experiments 1 and 2 involved presenting only one string at a time. The PI procedure has been adapted for rats to associate two temporal intervals (i.e., FI20 vs. FI40) with distinct stimuli (i.e., light vs. tone). Experiment 3 evaluated whether string length was sufficient to elicit odor discrimination.
Chapter IV

EXPERIMENT 3

**Rationale**

This experiment was designed to evaluate the hypothesis that temporal characteristics were sufficient to elicit odor discrimination (i.e., percent of short string chosen first). The organization of movement (i.e., pull time, stopping) was assessed during probe trials to determine if retrieving a cashew from a predicted or unpredicted odor/length pairing was sufficient to differentially influence performance. The results of this study build a foundation for future work examining temporal discrimination with odors in string-pulling behavior.

**Methods**

**Subjects**

Rats (*Rattus norvegicus*), 8-10 months old, were randomly assigned to two groups, either predicted (n=6) or unpredicted (n=6). All rats in the current study were pair-housed. Rats that failed to pull strings during testing (n=2, predicted; n=2, unpredicted) were dropped from the study. Temperature (20 to 21 °C) and light (12-hr light-dark cycle) were maintained in housing rooms. Rats were food deprived, maintained at 85% of their free-feeding body weight, and provided water ad libidum. The Institutional Animal Care and Use Committee at Northern Illinois University approved all procedures described in this experiment and all guidelines set by the Office of Laboratory Animal Welfare were followed.

**Apparatus**

The same string-pulling apparatus used previously was used in the third study with several changes. First, two strings were presented side by side. Second, strings were soaked in
flavor extract, lemon or vanilla, for 3 minutes and let dry for 24 hours. Finally, strings were stored separately in jars and rescented every other day to ensure no contamination from other odors.

**Procedures**

**Habituation**

Habituation in Experiment 3 was the same as in Experiment 1.

**Pretraining**

Pretraining in Experiment 3 was the same as in Experiment 1.

**Training**

Once pretraining was complete, rats were randomly assigned to two groups for training occurring over 15 days. The predicted group received a consistent odor/length pairing, such that the 1.5 m string was always scented lemon or vanilla and the 3.0 m string was always scented vanilla or lemon; thus, the odor-length pairings were counterbalanced for rats in the predicted group. In contrast, the unpredicted group did not receive a consistent odor/length pairing, such that the 1.5 m string was either lemon or vanilla and the 3.0 m string was either vanilla or lemon, respectively.

First rats received eight trials per day of scented strings, vanilla and lemon, of varying lengths (1.5 and 3.0 m) for 10 days. For the last 5 days of testing rats received 10 trials with the addition of two probe strings (5.0 m) or non-rewarded trials where the probe trial was presented for considerably longer than the previously learned duration. One 5.0 m vanilla string and one 5.0 m lemon string were randomly presented throughout each testing day. String weight and length were controlled for by making all strings 5.0 m in length with the cashew either presented
at 1.5 or 3.0 m. In addition, the side of apparatus (left or right) the string was presented on was controlled by randomly switching strings throughout trials and across days.

**Data Analysis**

**Standard Trials**

Quantification of the percentage of the selection of the short string chosen first was evaluated and plotted across the 15 days of testing to determine if rats developed a significant preference in string selection. The time it took for rats to approach the string to initiate pulling once placed in the testing apparatus was averaged across the standard trials for each of the 15 days of testing. In addition, the time it took for rats to reach the cashew was averaged across the standard trials for each of the 15 days of testing.

**Probes Trials**

The time it took for rats to approach the string to initiate pulling once placed in the testing apparatus was averaged across the probe trials for each of the final 5 days of testing. The percent of trials the entire string was pulled in for each rat was compared between groups by collapsing percent of trials pulled across the final 5 testing days. A probe trial was terminated if the rat stopped pulling in the string and did not resume after 30 s. In addition, the amount of time rats pulled in the string was averaged across the probe trials for each of the final 5 days of testing.

**Exploratory Analysis of Probe Trials**

The Peak Modus motion capture system was used to conduct micro level analysis of left and right hand movement organization during string-pulling behavior. Motion capture software used x-y coordinates to evaluate kinematic organization of the rats’ hands at 30 frames per second. Several measures were evaluated using the Peak Modus motion capture system,
including peak speed and total distance travelled by hands. For each measure, every 30 frames, or 1 s, were averaged from probe trials to determine the expectation of reward. Further analyses of probe trials were purely exploratory.

**Statistical Analysis**

Repeated-measures ANOVAs were conducted across days (i.e., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15) to evaluate main effects and interactions on each dependent measure for standard trials. The G-G correction was used in the analyses where Mauchly’s test indicated significant departure from the assumption of sphericity. Partial eta squared ($\eta^2_p$) was used as a measure of effect size for each main effect and interaction. Linear trend and Tukey HSD post hoc analyses were used to further investigate significant main effects and interactions. In addition, paired-samples $t$ tests were used to evaluate each dependent measure collapsed across the final 5 days (i.e., 11, 12, 13, 14 and 15) of testing for probe trials. Paired-samples $t$ tests were used to compare performance within groups in the current study, as the counterbalancing of odor/length pairings between groups does not allow for between group comparisons.

**Results**

**Standard Trials**

**Approach Time**

Results from Experiment 3 evaluated rats’ ability to use different length strings to elicit odor discrimination (see Figure 15a). First, the amount of time it took rats to approach the first string was evaluated for all standard trials across testing. The G-G correction ($\varepsilon=0.184$) was used to adjust the degrees of freedom associated with the lack of sphericity in approach time between the predicated and unpredicted group. Repeated-measures ANOVA conducted on approach time revealed a significant main effect of Day [$F (2.574, 25.739) = 8.530, p = 0.001, \eta^2_p = 0.460$] yet
Figure 15: Approach time and percent of short strings chosen first is displayed for standard trials, Study 3. The amount of time it took rats in the third study to approach strings during standard trials (A) is plotted across all 15 days of testing. The percentage of short strings chosen first (B) is plotted across all 15 days of testing.
failed to reveal a significant main effect of Group \( F (1, 10) = 0.003, p = 0.960, \eta^2_p < 0.001 \) or Group by Day interaction \( F (2.574, 25.739) = 1.259, p = 0.307, \eta^2_p < 0.112 \). Post hoc analysis revealed a decrease in linear trend, such that rats in both groups took less time to approach the string across days \( F (1, 10) = 11.970, p = 0.006, \eta^2_p < 0.545 \).

**Percent Short Chosen First**

The percentage of short strings chosen first was evaluated between groups across all testing days (see Figure 15b). The G-G correction (\( \varepsilon=0.411 \)) was used to adjust the degrees of freedom associated with the lack of sphericity. Repeated-measures ANOVA conducted on percentage of short strings chosen first revealed a significant main effect of Group \( F (1, 10) = 339.421, p < 0.001, \eta^2_p = 0.971 \) yet failed to reveal a significant main effect of Day \( F (5.754, 57.535) = 2.238, p = 0.055, \eta^2_p = 0.183 \) or Group by Day interaction \( F (5.754, 57.535) = 5.754, p = 0.072, \eta^2_p = 0.172 \). Rats in the predicted group were more likely to select the short string first compared to rats in the unpredicted group.

**Pull Time**

The amount of time it took rats to pull in short and long strings during standard trials was evaluated across testing. Pull time of short strings was first compared between predicted and unpredicted groups (see Figure 16a). The G-G correction (\( \varepsilon=0.247 \)) was used to adjust the degrees of freedom associated with the lack of sphericity in pull time for short standard trials between the predicted and unpredicted groups. Repeated-measures ANOVA conducted on pull time failed to reveal a significant main effect of Group \( F (1, 10) = 1.765, p = 0.214, \eta^2_p = 0.150 \), Day \( F (3.460, 34.597) = 4.175, p = 0.010, \eta^2_p = 0.295 \) or Group by Day interaction \( F (3.460, 34.597) = 1.497, p = 0.119, \eta^2_p = 0.130 \). Pull time for short standard trials did not differ between the predicted and unpredicted groups across testing.
Figure 16: Pull time for short and long standard trials, Study 3. Pull time for short (A) and long (B) standard trials is plotted across all 15 days of testing.
Pull time of long strings was compared between predicted and unpredicted groups (see Figure 16b). The G-G correction ($\varepsilon=0.313$) was used to adjust the degrees of freedom associated with the lack of sphericity in pull time for long standard trials between the predicted and unpredicted group. Repeated-measures ANOVA conducted on pull time revealed a significant main effect of Group [$F (1, 10) = 4.994, p = 0.049, \eta^2_p = 0.333$] and a significant main effect of Day [$F (4.381, 43.811) = 8.247, p < 0.001, \eta^2_p = 0.452$] yet failed to reveal a significant Group by Day interaction [$F (4.381, 43.811) = 2.290, p = 0.070, \eta^2_p = 0.186$]. Pull time for long standard trials decreased linearly for both the predicted and unpredicted groups across testing [$F (1, 10) = 45.728, p < 0.001, \eta^2_p = 0.821$]. Groups significantly differed across testing such that rats in the unpredicted group took more time to pull in the long string during standard trials.

**Probe Trials**

Between-groups comparisons of probe trials were not possible, since the odors with short and long strings were counterbalanced for the predicted group. Therefore, the subsequent analyses were restricted to within-group examination of results. Odor/length pairings for the predicted group compared short and long string lengths during probe trials, while odor/length pairings for the unpredicted group compared lemon and vanilla string lengths during probe trials.

**Approach Time**

The amount of time it took rats to approach probe trials was collapsed across the last 5 days of testing. First, approach time was evaluated for probe trials for the predicted group (see Figure 17a). A paired-samples $t$ test revealed a significant difference in approach time for the short ($M= 2.322, SD= 0.424$) and long ($M= 3.704, SD= 0.988$) odor/length pairings.
Figure 17: Approach time for all probe trials, Study 3. Approach time for predicted (A) and unpredicted (B) probe trials is plotted collapsed across the last 5 testing days for each group.
[t (6) = -3.977, p = 0.011]. Approach time differed by probe trial for the predicted group across the last 5 testing days.

Second, approach time was evaluated for probe trials for the unpredicted group (see Figure 17b). A paired-samples t test failed to reveal a significant difference in approach time for the lemon (M= 3.41, SD= 1.164) and vanilla (M= 5.012, SD= 1.75) odor/length pairings [t (6) = -2.448, p = 0.058]. Approach time did not differ by probe trial for the unpredicted group across the last 5 testing days.

Percent of Probe Pulled

Percent of probe trials pulled in entirely by rats in the predicted group were collapsed across the final 5 testing days (see Figure 18a). A paired-samples t test failed to reveal a significant difference in percent of probes pulled in entirely for the short (M= 80.00, SD= 17.89) and long (M= 93.33, SD= 10.33) strings [t (6) = -1.348, p = 0.235]. Percent of probe trials pulled in entirely did not differ by string odor/length pairing for the predicted group for the last 5 days of testing.

Percent of probe trials pulled in entirely by rats in the unpredicted group was analyzed across the final 5 testing days (see Figure 18b). A paired-samples t test failed to reveal a significant difference in percent of probes pulled in entirely lemon probes for the lemon (M= 80.00, SD= 10.33) and vanilla (M= 80.00, SD= 5.164) strings [t (6) < 0.00, p = 1.00]. Percent of probe trials pulled in entirely did not differ by string scent/length association for the unpredicted across the last 5 days of testing.

Pull Time

The amount of time rats pulled in the string during probe trials was collapsed across testing. First, pull time for short and long probe trials was evaluated for the predicted group
Figure 18: Percent of probes pulled in entirely, Study 3. Percent of probes trials pulled in entirely by rats in the predicted group (A) and percent of probe trials pulled in entirely by rats in the unpredicted group (B) collapsed across the last five days of testing for each group.
see Figure 19a). A paired-samples $t$ test revealed a significant difference in pull time for short (M = 12.37, SD = 2.354) and long (M = 16.12, SD = 3.423) strings \[ t(6) = -3.098, p = 0.027 \]. Pull time during probe trials significantly differed by string odor/length association for the predicted group across testing days, such that rats spent less time pulling in the probe string associated with the short standard trial and more time pulling in the probe string associated with the long standard trial.

Second, pull time for lemon and vanilla probe trials were evaluated for the unpredicted group (see Figure 19b). A paired-samples $t$ test failed to reveal a significant difference in pull time for lemon (M = 17.628, SD = 4.714) and vanilla (M = 16.701, SD = 2.267) strings \[ t(6) = 0.713, p = 0.507 \]. Pull time during probe trials did not differ by string odor/length association for the unpredicted group across testing days.

**Exploratory Analysis of Probe Trials**

Before further analyses were conducted between groups, the left and right hands were evaluated for peak speed and total distance for probe trials for the predicted and unpredicted groups respectively. After analyses of left and right hand use was evaluated for the predicted and unpredicted groups respectively and no differences were found between the left and right hands, all further analyses averaged across left and right hands for each group.

**Peak Speed**

Peak speed was evaluated for probe trials across testing. First, peak speed for short and long probe trials was evaluated for the predicted group (see Table 3; see Figure 20a). A paired-samples $t$ test failed to reveal significant difference in peak speed for short (M = 37.65, SD = 2.26) and long (M = 37.66, SD = 2.4) strings \[ t(6) = -0.014, p = 0.990 \]. Peak speed did not differ by string odor/length association for the predicted group across testing days.
Figure 19: Pull time for probe trials, Study 3. Pull time for predicted (A) and unpredicted (B) probe trials is collapsed across the last 5 testing days for each group.
Table 3: Kinematic measures, Study 3. Peak speed and distance statistical values are displayed for probe trials for rats provided with either predicted or unpredicted odor/length pairing during standard trials in Experiment 3.

<table>
<thead>
<tr>
<th>Kinematic measures</th>
<th>M</th>
<th>SD</th>
<th>t</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td><strong>Peak Speed</strong></td>
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</tr>
<tr>
<td>Predicted</td>
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<tr>
<td>Short</td>
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<tr>
<td>Long</td>
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<td>2.4</td>
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</tr>
<tr>
<td>Unpredicted</td>
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<tr>
<td>Lemon</td>
<td>35.16</td>
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<td>Vanilla</td>
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</tr>
<tr>
<td>Predicted</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>0.405</td>
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<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>Lemon</td>
<td>188.632</td>
<td>46.131</td>
<td>-1.147</td>
<td>0.303</td>
</tr>
<tr>
<td>Vanilla</td>
<td>210.45</td>
<td>19.343</td>
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</tr>
</tbody>
</table>
Figure 20: Peak speeds of hands during probe trials, Study 3. Peak speeds averaged across rats’ left and right hands are collapsed across the last 5 testing days for predicted (A) and unpredicted (B) probe trials.
Second, peak speed for lemon and vanilla probe trials was evaluated for the unpredicted group (see Figure 20b). A paired-samples \( t \) test failed to reveal significant difference in peak speed for lemon (M= 35.16, SD = 5.759) and vanilla (M= 36.941, SD = 2.137) strings [\( t(6) = -0.978, p = 0.373 \)]. Peak speed did not differ by string odor/length association for the unpredicted group across testing days.

**Total Distance**

Total distance travelled by limbs was evaluated for probe trials across testing. First, total distance for short and long probe trials was evaluated for the predicted group (see Figure 21a). A paired-samples \( t \) test failed to reveal significant difference in total distance for short (M= 178.34, SD = 23.242) and long (M= 197.06, SD = 49.294) strings [\( t(6) = -0.909, p = 0.405 \)]. Total distance did not differ by string scent/length association for the predicted group across testing days.

Second, total distance for lemon and vanilla probe trials was evaluated for the unpredicted group (see Figure 21b). An independent samples \( t \) test failed to reveal significant difference in total distance for lemon (M= 188.632, SD = 46.131) and vanilla (M= 210.45, SD = 19.343) strings [\( t(10) = -1.147, p = 0.303 \)]. Total distance did not differ by string scent/length association for the unpredicted group across testing days.

**Discussion**

Experiment 3 demonstrated that string length was sufficient to elicit odor discrimination supporting the role of time in organizing string-pulling behavior. Rats that received a predicted string length (i.e., 1.5 m and 3 m) and odor (i.e., lemon and vanilla) pairing developed a preference to choose the short string first over the long string once placed in the apparatus across testing days. However, rats that received an unpredicted string length (i.e., 1.5 m and 3 m or 3 m
Figure 21: Total distance traveled of hands during probe trials for each group, Study 3. Total distance hands traveled is plotted for predicted (A) and unpredicted (B) probe trials collapsed across the last 5 days of testing.
and odor (i.e., lemon and vanilla or vanilla and lemon) pairing did not develop a preference to choose the short string first once placed in the apparatus across testing. In addition, rats that received a predicted odor/length paring during standard trials demonstrated differential responding in pull time during probe trials. Rats in the predicted group spent less time pulling the string during probe trials associated with the short odor/length paring and more time pulling in the string during probe trials associated with a long odor/length paring. Further, pull time during probe trials did not differ for rats in the unpredicted group, suggesting that an odor/length association may have not developed. Time may be contributing to the differential pull times observed for probe trials in the predicted group. These findings are consistent with using temporal information to guide the organization of string-pulling behavior; however, other factors may also be influencing the organization of string-pulling behavior.

Several other factors may have contributed to performance differences observed in Experiment 3. First, motivation to engage in the string may have influenced performance. Although approach time did not differ between groups for the first string chosen once placed in the apparatus, differences were observed during probe trials. The predicted group (short vs. long) approached the short string quicker relative to the long string. In contrast, no differences in approach time were observed in the unpredicted group (i.e., lemon vs. vanilla). This suggests that motivation to approach the first string was similar between groups throughout testing. However, the predicted group differed in approach time for probe trials (i.e., short vs. long). The unpredicted group did not differ in approach time for probe trials (i.e., lemon vs. vanilla). When the short string is cued by an odor it is chosen over the long string, demonstrating a preference or greater motivation to engage in an early vs. late reward. These findings suggest that within- and
between-subjects experimental designs influence performance to engage in the string-pulling task differently.

Next, motor learning may be contributing to performance observed in the string-pulling task. The spontaneous aspect of string-pulling may limit the influence of motor learning observed in traditional timing tasks across days. For example, the speed of hand over hand movement did not differ between short vs. long or lemon vs. vanilla strings during probe trials.

These results suggest that motor learning is not mediating group differences observed in the string-pulling task. String-pulling may be a highly organized spontaneously occurring behavior, and the movement observed during string-pulling behavior may be an online process that rats are already highly efficient at performing from the very beginning of testing. Finally, string novelty may also be contributing to group differences for probe trials unrelated to odor/length discrimination. The novelty of a string may reduce rats’ motivation to engage with the string or complete the string-pulling task; however, a variety of old and new strings were used for standard trials throughout testing to control for the addition of the string during probe trials. The findings from Experiment 3 demonstrate that one factor other than time, motivation, influenced performance in string-pulling behavior during odor discrimination; however, motor learning and string novelty are not likely factors influencing the organization of string-pulling behavior.
Chapter V

GENERAL DISCUSSION

The current set of experiments used string-pulling behavior to investigate and dissociate temporal and motivational processes. Experiment 1 provided evidence that string length was sufficient to elicit differences in performance consistent with using temporal information to organize behavior. Experiment 2 demonstrated that modification of reinforcement elicited differences in performance that were distinct from varying string length and reflect the influence of motivational factors on performance. Finally, the results of Experiment 3 demonstrated that consistent odor/length pairing was sufficient to elicit odor discrimination, providing further evidence that temporal and motivational factors influence the organization of string-pulling behavior. The results of these studies demonstrate the ability of the string-pulling task to assess interval timing and motivation under various conditions.

**Temporal Factors Influence String-Pulling Behavior**

Temporal factors influenced the organization of string-pulling behavior. Results from Experiment 1 demonstrate that different length strings (i.e., 1.5 m or 3 m) presented during standard trials influence performance on probe trials. Rats that received long strings during standard trials were more likely to pull in the entire string and travel greater total distances with the hands during probe trials compared to rats that received short strings during standard trials. Previous work has demonstrated that temporal factors influence the organization of behavior in the PI procedure similarly (Catania, 1970; Roberts, 1981). For example, Fl 20 s vs. Fl 40 s schedules result in the peak response rates occurring at 20 s or 40 s after the stimulus is presented, respectively. However, the peak response rate is maintained across both intervals.
(Roberts, 1981). Peak speed is also maintained across short and long strings during probe trials in string-pulling behavior. Together this research demonstrates that temporal factors influence the organization of interval timing behavior in both well-trained (i.e., PI procedure) and spontaneous (i.e., string-pulling) tasks.

Temporal factors were shown to further influence performance when consistent odor/length pairings were presented in Experiment 3. Rats presented with an odor/length pairing for standard trials were more likely to select the short string first across testing compared to rats that received an unpredicted odor/length pairing during standard trials. From the onset of testing, rats in the predicted group exhibited a tendency to organize behavior to select the shortest string first. In addition, rats that received a predicted odor/length paring during standard trials demonstrated differential responding in pull time during probe trials. Rats in the predicted group spent less time pulling in the string during probe trials associated with the short odor/length paring and more time pulling in the string during probe trials associated with a long odor/length paring. Previous research has shown that rats use temporal information to discriminatively respond to a light and tone. For example, rats exhibit differential responding when a stimulus (i.e., tone or light) is consistently paired with a specific schedule of reinforcement (i.e., FI20 or FI40; Gibbon, Church, & Meck, 1984). Specifically, rats’ peak response time during a probe trial where a light stimulus was presented would be at 20 s vs. peak response time during a probe trial where a tone stimulus was presented would be at 40 s. This type of discriminative responding to stimuli (i.e., tone or light) was based on processing temporal information. These observations support a role for temporal information processing in organizing string-pulling behavior; however, other possible explanations are considered.
Rats may be using information other than time to organize performance. Other factors that may be influencing behavior in the current set of string-pulling tasks include distance, the weight of the string, and reinforcement rate. First, rats could be using distance estimation to influence performance. For example, rats may encode the distance their hands travelled while pulling in the string. Previous work has demonstrated that rats use distance estimation to organize food hoarding behavior. During a food hoarding trip under dark conditions, an animal must search an open environment for a food reward. Upon finding the food item, rats follow a path directly to the home base (Whishaw & Kolb, 2004). The direct path back to the home base is characterized by peak speed that occurs at the mid-point of the path and is scaled to the Euclidean distance (Martin & Wallace, 2007). These features are evidence that rats use self-movement cues to estimate direction and distance to return to a home base. Although both temporal and distance estimates are sufficient to organize string-pulling behavior, parsimony would favor the simplest explanation that rats are using temporal information to organize behavior in the current set of string-pulling tasks.

Next, rats may use weight to differentiate between short and long strings. Previous work has evaluated response force during lever pressing with rats under various conditions, including discrimination (Noterman & Block, 1960; Noterman & Mintz, 1962) and FI performance (Gollub & Lee, 1966). Rats are not likely using weight to discriminate between strings. The weight of the string was controlled for by adding string after the presentation of the cashew for short vs. long strings, making all strings 5 m in length. Finally, reinforcement rate varied between groups (i.e., Experiment 1) and scented strings (i.e., Experiment 3) and that may have influenced the level of motivation to engage in string-pulling behavior. This is consistent with the different approach times observed in Experiment 3. In contrast, no differences in approach
time were observed in Experiment 1. Reinforcement rate is not sufficient to account for the entire pattern of results; however, these observations are consistent with a role for motivation in organizing string-pulling behavior. Further, Experiment 2 showed that high and low reinforcement rates elicited different levels of performance to engage in the string-pulling task yet similar performance during probe trials, suggesting temporal processing may be distinct from motivational factors. The next section discusses the role of motivation in the organization of string-pulling behavior.

**Motivational Factors Influence String-Pulling Behavior**

Motivation has been shown to be a critical factor in organizing animal behavior. There are many outcomes that motivate animal behavior, including environmental resources such as food, reproduction, and exploration. Through experience, multiple stimuli can function to signal access to these outcomes. Varying the magnitude of these outcomes (i.e., food reinforcement) has been observed to influence performance. For example, increases in reinforcement magnitude are associated with a stronger response rate (Ratliff & Ratliff, 1971; Roberts, 1969). Varying food reinforcement has been used extensively to investigate the effects of motivation on performance in multiple behavioral paradigms. Previous research has demonstrated that varying the amount of food reward has consequences on motor learning in alleyway performance and lever pressing. Alleyway running speed has been shown to vary based on reward magnitude (Crepsi, 1942; Fountain & Hulse, 1981; Hulse & Dorsky 1977; Roberts, 1969; Wallace & Fountain, 2003). Rats will run faster down a runway for a larger food reward and slower down a runway for a smaller food reward. Further, Ratliff and Ratliff (1971) demonstrated that rats’ alleyway running speed and rate of improvement across training were positively related to reward magnitude (i.e., 2, 4, 8, or 16 food pellets). Alleyway running speed was shown to
increase as food reward increased. Thus, varying the amount of food reward alters response rate. This type of relative rate of responding is equal to the relative rate of reinforcement according to the matching law (Davison, 2016; Herrnstein, 1970).

The current set of string-pulling studies demonstrates that anticipated reinforcement drives response intensity during the task similarly to earlier work. For example, rats within the low reinforcement group in Experiment 2 took more time to pull in reinforced strings and were less likely to pull the string in all the way to the end during probe trials. Further, rats within the predicted group of Experiment 3 showed differences in approach time for short and long strings; however, rats in Experiment 1 that experienced either short or long strings did not demonstrate any differences in approach time. String length was found to influence measures of performance (i.e., approach time) only when a comparison was being made with at least two string lengths (i.e., short and long). These findings suggest that motivation is influencing the organization of string-pulling behavior to engage in short vs long strings within a group of rats. Differences in approach time between the predicted group (i.e., short and long) in Experiment 3 and the short and long groups in Experiment 1 further suggest that it may not be the absolute differences but relative differences in reinforcement that influence performance in string-pulling behavior. The next section will consider the effects of changing reinforcement magnitude on rate of improvement, or learning.

Motor learning refers to how quickly or slowly performance changes across time, and reinforcement magnitude has been shown to influence this learning rate. These changes have been demonstrated in the PI procedure with rats trained on a FI20 s schedule. Rats given a large food reward demonstrated a higher response rate compared to rats that were provided a small food reward (Roberts, 1981). Roberts (1981) further demonstrated that the manipulation of a
food reward has differential effects on peak time and peak rate, such that peak response rate changes but not peak response time. Rats also vary the response rate of bar presses depending on the FI schedules of reinforcement (Stevenson & Black, 1985). The FI10 s schedule produced a faster overall response rate than the FI60 s schedule. These traditional assessments of motivation suggest that response rate, or motor learning, is influenced by manipulating reward contingencies; however, motor learning was not found to be influenced by differential levels of motivation. Specifically, the peak speed of rats’ hand movement in the current string-pulling tasks did not differ between groups for any condition, even when varying reward contingencies. Peak speed of rats’ hand movement is another measure of response rate. Changes in peak speed may suggest that motor learning has occurred. However, altering the length of the string during standard trials resulted in differential responding during probe trials, whereas peak speed was not influenced. The lack of group differences in peak speed discounts different rates of motor learning acquisition mediating group differences in other measures (i.e., pull time, stops).

A complete dissociation of temporal and motivational factors may not be possible within the scope of the current set of studies. For example, in both Experiment 1 and Experiment 2, group differences were observed during probe trials related to the percentage of strings pulled in completely. In Experiment 1 the short group pulled a lower percentage of strings during probe trials relative to the long group. Experiment 2 demonstrated that the low reinforcement group pulled a lower percentage of strings during probe trials relative to the high reinforcement group. The consistent findings with lower percentage of strings pulled during probe trials for the short group in Experiment 1 and the low reinforcement group in Experiment 2 may suggest that temporal and motivational factors are not completely independent processes. Both temporal and motivational factors have been demonstrated to influence the organization of string-pulling
behavior; however, future work should further investigate the role of temporal and motivational factors in string-pulling behavior.

**Future work**

Research has long debated the organization of temporal processing across various behaviors. Performance in tasks assessing interval timing behavior has shown to be influenced by temporal processing and motivational factors. The results from the current study are consistent with dissociating both processes. Pharmacological manipulations have been used to investigate temporal and motivational factors. Specifically, previous work has evaluated the effects of manipulating the dopaminergic system on temporal and motivational processing. This work is compared to the findings in the current set of experiments on time and motivation in string-pulling behavior.

Manipulations of the dopaminergic system have been demonstrated to influence performance consistent with changes in processing temporal information (i.e., pacemaker speed) and motivation (i.e., enhancing reward salience). This has been demonstrated in rodents using the PI procedure (Balci et al., 2008). First, the acute administration of methamphetamine resulted in an abrupt early peak response time (i.e., peak time at 32 s instead of 40 s) yet did not affect peak response rate. Peak response rate was not affected, suggesting that changes in peak time are not attributed to changes in motivation or motor impairments. If these pharmacological methods were applied to string-pulling behavior, rats would be expected to have shorter pull times during probe trials and stop pulling in the string during probe trials sooner compared to control rats. Therefore, peak response time would be earlier with the administration of methamphetamine; however, peak response rate, or peak speed, in the string-pulling task would not be expected to change as observed in previous research. Further manipulation of the dopaminergic system
provides more evidence for the distinct roles of time and motivation in the organization of behavior.

Haloperidol administration has also been demonstrated to produce differential behavioral effects in the PI procedure on temporal processing and motivational factors. The administration of haloperidol resulted in a later peak response time (i.e., peak response at 45 s instead of 40 s) yet peak response rate did not change with the administration of haloperidol, discounting the role of motor impairments and motivation in the organization of behavior (Meck, 1996). If these pharmacological manipulations were applied to string-pulling behavior, rats would be expected to have later pull times during probe trials and stop pulling in the string later, or possibly pull in the string entirely, compared to control rats. Response time would be later with the administration of haloperidol. Yet, peak response rate (i.e., peak speed) would not be expected to change in the string-pulling task as observed in previous research. The effects observed following acute drug administration of haloperidol and methamphetamine provides further support for the distinct organization of temporal and motivational processing. Future work should investigate the effects of pharmacological manipulations on the organization of temporal processing in string-pulling behavior. String-pulling may provide a novel tool to investigate how these systems interact to influence animal behavior.

Conclusions

The string-pulling task can dissociate temporal and motivational processes across a range of various conditions with limited training. The results from this series of studies demonstrated that string-pulling has the potential to investigate the organization of temporal processing. The current study provides evidence for a novel assessment to dissociate temporal and motivational factors through the natural behavior of string-pulling. The spontaneity of string-pulling behavior
may result in performance being mediated by a different set of neural structures that are recruited during traditional tasks (i.e., PI procedure). As a result, string-pulling can be used to assess temporal deficits and motivational factors associated with rodent models of neurological disorders. Deficits may be detected earlier in disease onset and tracked throughout disease progress using string-pulling. Detecting changes in performance at an early time point may be crucial in intervening, stopping, or slowing the progression of diseases. String-pulling behavior may also have a therapeutic use to improve cognitive function or fine motor control.
BIBLIOGRAPHY


