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PAVLOVIAN TO INSTRUMENTAL TRANSFER IN THE PEAK PROCEDURE: INSIGHTS INTO TIME

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Abstract Title: Pavlovian to Instrumental Transfer in the Peak Procedure: Insights into time

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Responding in the peak procedure has long been utilized as a prototypical model of timing behavior in animals (Church, 2002). Part and parcel to this approach has been the assumption that peak intervals and peak rates are representative measures of two independent constructs, timing and motivation respectively. However, recent investigations into motivational influences and the biological underpinnings of timing behavior have resulted in converging models that indicate responding in the peak procedure may actually be a combination of motivational and timing factors (Kirkpatrick, 2014). In pursuit of this, the present study utilized two experimental manipulations to examine the impact of pavlovian conditioned cues on timing and the role of fluoxetine in mediating pavlovian cue effects. The results provided evidence for pavlovian to instrumental transfer effects within the peak procedure and further outlined the role of fluoxetine in impacting response processes.

Introduction

With the rise of information theory and its integration into the field of psychology as well as advances in neuroscience, there have been a host of calls to reconcile models of learning, timing, and choice into a coherent framework (Kirkpatrick, 2014; Jensen et al. 2013; Galtress, Marshall, & Kirkpatrick, 2012; Galisteel & Gibbon, 2000). Of critical importance to the success of this endeavor will be finding common ground in integrating elements of timing models with aspects of incentive motivation. In particular, neurocomputational approaches and behavioral data have begun to outline similar neural structures and pathways that facilitate different elements of responding that are related to anticipation of when to respond, the choice to respond, and the vigor of the response. Given the power of incentive motivational approaches to describe reward valuation processes (Berridge, 2000; Dayan& Balliene, 2002; Dickinson & Balliene, 2002) and the well documented impact of incentive cues on instrumental response rates (Holmes, Marchand, & Coutureau, 2010; Balliene& Ostlund, 2007;), it is clear that integration with timing models that specify rate estimation processes and decision rules (Galistel & Gibbon, 2000) will result in better models of choice behavior.

Recent reports (Kirkpatrick, 2014; Galtress, Marshall, & Kirkpatrick, 2012; Balsam et al., 2009) have indicated that motivational factors may alter timing processes within the peak procedure, a behavioral task widely used to investigate temporal learning in animals. Of particular interest, within these reports the motivational manipulations utilized all have been demonstrated to impact aspects of incentive salience process, and as such incentive salience may play a critical role in time estimation. As a means of elucidating the probable role of incentive salience processes in peak interval timing behavior, an experiment with two manipulations was conducted. In the first manipulation, a classically conditioned excitatory cue was presented

during probe and fixed interval trials of a peak procedure task as a means of detecting a pavlovian instrumental transfer effect mediated by incentive salience processes. Building off of these results, the second manipulation sought to further tease apart the impact of the classically conditioned cue on timing behavior by utilizing the drug fluoxetine which has been demonstrated to alter motivational and motoric aspects of learned behavior.

The peak procedure

Ferster and Skinner (1957) provided one of the first extensive descriptions of interval responding in an operant procedure. In examining the impact of fixed interval schedules, in which the first response after the specified interval duration has elapsed is reinforced, Skinner and Ferster noted a typified pattern of responding in which response rates steadily increased as the time of reinforcement availability approached with a brief pause in responding after reinforcement delivery which produces the now famous scallop shape pattern. Schneider (1969) further analyzed fixed interval schedule patterns of responding in pigeons and noted that at about half way through the elapsed interval pigeons began a burst of responding. Building off of this work Catania (1970) developed the peak procedure in which reinforced FI trials are interspersed with unreinforced probe trials in which the stimulus signaling the fixed interval remains on past the normal duration in order to examine the timing behavior of animals.

Roberts (1981) provided one of the first extensive analyses of peak procedure responding as a means of isolating an internal clock. In particular, Roberts replicated across five experiments the now standard pattern of responding during peak trials (see figure 1) typified by a gradual increase and decrease in responding following a Gaussian distribution with a mean around the normal time of reinforcement during the fixed interval trial and spread that scales to the duration being timed (Church, 2002; Church & Broadbent, 1990; Roberts, 1981). Furthermore in follow

up examinations of factors influencing peak responding, Roberts (1981) provided evidence that the peak rate, the point of highest responding, was representational of motivational influences as prefeeding resulted in decreases in response rates; while the peak interval, the center of the distribution of responses, was representative of timing properties, as introducing gaps by turning off and then on the timed signal resulted in shifts in the peak interval. From these results Roberts (1981) concluded that the two main factors of responding in the peak procedure, peak rate and peak interval, were representative of independent constructs, potentially controlled by a clock with a comparator process and a motivational drive component related to the probability of reinforcement.

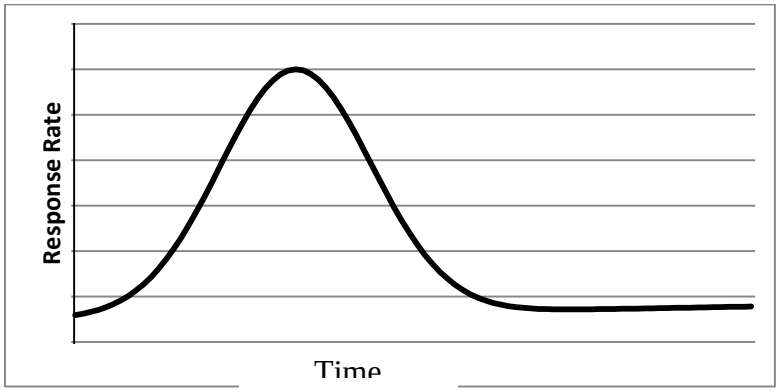

Figure 1. Prototypical pattern of responding within the peak procedure. The dashed line represents the normal time of reinforcement.

Following the framework of Church & Broadbent (1990) and Gibbon and Church (1984), theoretical conceptualizations of responding in the peak procedure have typically utilized a similar set of constructs to explain the pattern of consistent data (Sanbaria & Killeen, 2007; Church, 2002). Principally, the typical response function is thought to be controlled by a pacemaker that sends beats to an accumulator at a constant rate. With the eventual arrival of reinforcement the beats collected by the accumulator are then stored in a memory bank from which a comparator process samples the stored beat counts and compares it to the current

accumulator value and according to a decision rule either initiates or terminates responding. Lastly to account for the scalar timing properties observed, stochastic noise is specified to occur either in the random sampling of the beat counts from the memory store or in the accumulation of beats from the pacemaker.

Factors that impact responding in the peak procedure

In the time since Roberts (1981), an extensive body of research regarding the behavioral factors involved in peak procedure responding has accrued. These results have provided insight into how responding within the experimental task is impacted by trial factors, such as the ratio of probe to fixed intervals, and motivational factors related to reward shifts and reward devaluations.

Trial factors A variety of factors related to the structure of the peak procedure have been demonstrated to impact performance within the task. With respect to the response function as a whole Kaiser (2009, 2008) demonstrated that the ratio of peak trials to fixed interval trials drastically impacted both the acquisition and spread of the response function in the peak procedure, with lower ratios of peak trials to fixed interval trials producing the fastest acquisition rates and smaller spreads of responses around the peak interval. In line with this effect, Matell & Kurti (2014) demonstrated that altering the probability of reinforcement within a bi-peak task resulted in shifts in responding according to the interval with the highest density of reinforcement. If the ratio of peak trials to fixed interval trials is taken as a manipulation of reinforcement densities around the timed interval, as reinforcement is only available during the fixed interval trials; then the results of both Kaiser (2009, 2008) and Matell & Kurti (2014) indicate that responding within the task is dependent upon a process that tracks the probability of reinforcement in relation to the elapsed time in order to initiate and inhibit responding. Likewise

examinations of peak response rates have been demonstrated to be impacted by a pattern of switching from low to high to low states of activity that are altered by the probability of reinforcement within the timed interval (Harris, 2015; Matell & Kurti, 2014; Swanton & Matell, 2011); thus further indicating that both the height and spread of the response function in the peak procedure are impacted by a timing and probability density tracking process.

In addition to the ratio of probe to fixed interval trials and reinforcement densities, the peak of responding within the peak procedure is also dependent upon external cue elements related to the signaled interval. For example, inserting gaps into the timed interval by turning off the timing signal produces shifts in the entire response function to the right in accordance to the relative duration of the gap (Brown, Richer, & Doyere, 2007; Church, 2002; Roberts, 1981). Furthermore, intruding signals in the form of compound stimuli signaling different interval values (Matell & Kurti, 2014; Swanton & Matell, 2011) or associated with different schedule contingencies (Aum, Brown, & Hemmes, 2007; Brown, Richer, & Doyere, 2006; Aum, Brown, & Hemmes, 2004) produce shifts in the timing function that are neither additive nor multiplicative. This later difference between gaps producing scalar differences in timing shifts while intruding events produce non additive or multiplicative shifts indicates that the centering of the response function is part of both timing clock components but also a response selection decision component.

Motivational factors Contrary to Roberts (1981) initial conclusion that motivational factors do not impact timing behavior a variety of studies have indicated that manipulations of motivational states can impact the entire response function from response rates to the center and spread of the function. Balci, Ludvig, & Brunner (2010) demonstrated that within the peak procedure as trial sessions increased the response rate decreased and the spread of the response function began to

flatten, thus indicating a potential effect of satiety. In line with this observation Kirkpatrick (2014) and Galtress & Kirkpatrick (2009) demonstrated that prefeeding subjects before experimental sessions resulted in rightward shifts in the response function and lowered response rates. Furthermore, motivational shifts in the form of increased or decreased reward magnitudes also produce shifts in the timing function and response rate, with shifts from low magnitudes to high magnitudes producing leftward shifts and increased response rates (Kirkpatrick, 2014; Galtress, Marshall, & Kirkpatrick, 2012; Galtress & Kirkpatrick, 2009; Ludvig et al., 2011; Ludvig et al. 2007). Likewise, reinforcer devaluation with lithium chloride has also been demonstrated to produce decreased response rates and right ward shifts in the timing function (Kirkpatrick, 2014).

In light of these findings, it is clear that motivational manipulations do impact more than just the response rate in the peak procedure. Furthermore when compared to the effects of trial manipulations in the form of reinforcement densities or stimulus gaps, motivational shifts may function to alter multiple components or processes that determine responding in the peak procedure. For example, the produced rightward shifts in timing and decreased response rates in studies of prefeeding (Kirkpatrick, 2014) and reward magnitude decreases could be accomplished through altering the effect of reinforcement densities in the encoding of the temporal interval or through changes in the inhibition threshold for initiating responses. Adding a further level of complication, it is also possible that the motivational shifts may alter the clock rate or perception of the elapsed time as indicated by the similarity of leftward shifts in the timing function by reward magnitude increases (Kirkpatrick, 2014; Ludvig, 2007) and administration of amphetamines (Hellbronner & Meck, 2014). However, to date no clear evidence has been provided that can precisely account for how motivational shifts impact the

response function or the underling mechanisms that motivation impacts (Kirkpatrick, 2014; Galtress, Marshall, & Kirkpatrick, 2012; Balsam et al., 2009). As such, further research is needed to elucidate the mechanisms by which motivational manipulations influence responding in the peak procedure.

Incentive Salience and Pavlovian Instrumental Transfer

The theory of incentive salience provides one avenue of explanation that may provide some insight into how motivational manipulations impact responding in the peak procedure. At its heart, incentive salience theory states that unconditioned stimuli have natural hedonic properties that interact in a reward valuation process with current deprivation levels of homeostatic drives to produce an activated state of "wanting" (Berridge, 2000). Furthermore, when an unconditioned stimulus is paired with a neutral stimulus the incentive value of the unconditioned stimulus is transferred to the neutral stimulus such that presentations of the now conditioned stimulus serves to activate both a cognitive representation of the unconditioned stimulus, as well as seeking behavior for the unconditioned stimulus (Dickinson & Balliene, 2002; Berridge, 2000). When applied to instrumental tasks, the incentive salience account predicts that the presentation of the conditioned stimulus will produce changes in instrumental responding that is contingent on unconditioned stimulus attainment, a phenomena known as Pavlovian to instrumental transfer or PIT (Holmes, Marchad, & Coutureau, 2010).

Diagrammatically, PIT can be represented as a pairing of a stimulus S with an unconditioned stimulus S*, such that when the S is presented in the context of the instrumental response R producing the outcome O, the rate of R is altered (Balliene & Ostlund, 2007). Research into the nature of PIT has indicated that the phenomena can be divided into two types, general and selective. In general PIT the conditioned stimulus is paired with an unconditioned

stimulus that is not utilized as the reinforcer in the R-O contingency. In appetitive preparations, this has the effect of increasing the rate of responding with CS presentations independent of the produced outcomes (Holmes, Marchad, & Coutureau, 2010; Corbit & Balliene, 2005; Lovibond, 1989). However in selective PIT, in which the unconditioned stimulus also serves as the outcome in the R-O contingency, presentations of the CS will only increase responding on the instrumental response for the associated outcome. For example Colwill & Rescorla (1988) demonstrated that when subjects are trained on different R-O contingencies, PIT will only occur for the R when the US is the same as the O in the R-O contingency, a finding that has been well replicated in the literature (Delamater & Holland, 2007; Holland, 2004; Rescorla, 1997; Colwill & Motzkin, 1994).

Theories of PIT differ in the exact means by which the noted transfer effects on instrumental responding are accomplished while all generally relying on some form of the incentive salience process. Early theories of PIT (Estes, 1943; Rescorla & Solomone, 1967), argued for a general motivating role of the conditioned stimulus in increasing instrumental responding, however this account fell out of favor due to its in ability to specify why selective PIT should occur. In response to this Baliene & Ostlund (2007), argued for a two process account of PIT in which the similar S-O relation functions to prime the O-R relation which results in performance of the R-O contingent behavior. Thus, in situations where the O is the same stimulus in the R-O contingency the relation of the S-O simply elevates the responding of the organism to the R-O contingency. However when different outcomes, O_1 and O_2, are made contingent on different responses, R_1-O_1 and R_2-O_2, pairings of S-O_1 will selectively elevate responding to the R_1-O_1 contingency due to the S having taken on the incentive values of the O_1 and thus producing an expectancy of O_1 which prompts the seeking behavior of initiating the

response contingency R_1-O_1. As such, the S-O relation mediated by incentive salience processes plays a critical role in the control of instrumental responding.

Incentive motivational processes in the peak procedure

Viewed within the peak procedure, the reported motivational manipulations on the response function can be analyzed as variations of incentive salience motivational processes and provide evidence for potential PIT effects by intruding cues. For example during initial training trials in peak procedure experiments, the subjects are trained to perform the instrumental action of lever pressing in the presence of a stimulus, typically a house light or tone, that will later serve as the timing cue for the fixed interval duration. As such, the presence of the timing stimulus comes to signal the availability of the unconditioned stimulus reward (S-O) as well as the response outcome (R-O) contingency. Within the framework of incentive motivation, the presentation of the time cue (S) can be conceptualized as priming the subject to respond to the instrumental response via activation of the seeking pathways. In situations where the drive state of the organism is altered, via prefeeding or deprivation, the produced changes in the response function within the task are the resultant of the drive state interacting with the hedonic value of the timing stimulus which has taken on the incentive properties of the unconditioned stimulus. Thus in studies of prefeeding or reinforcer devaluation with lithium chloride (Kirkpatrick, 2014), the arousing properties of the timing stimulus are decreased which results in a decrease in the amount of responding and lessens the impact of the timing stimulus in controlling behavior. In a similar manner, reinforcer magnitude shifts serve to increase the saliency of the timing stimulus and produce increases in response rates as well as shifts in the response function through anticipatory responding produce by the same drive state incentive value interaction.

Stated plainly, an incentive salience account of motivational influences in peak procedure responding argues that any manipulation that changes the strength of the S-O relation will result in alterations in responsiveness to the R-O contingency, and as such produce changes in the temporal control of behavior. Furthermore, if it is the S-O relation that prompts responding to the R-O contingency in the timing tasks then two facts follow: 1) the temporal information is encoded as part of the S-O relation, and 2) when presented with multiple S-O relations responding should be biased towards the pair with the most incentive value. Given this conceptualization, motivational impacts likely exert their effects through incentive salience valuation processes that guide anticipatory response processes related to the S-O priming of the R-O contingency.

Interestingly, no direct test of the above account has been conducted. Perhaps the closest lines of evidence are data from Brown, Richer, and Doyere (2007) that demonstrated rightward shifts in peak responding and decreased rates of responding with presentations of inhibitory conditioned stimulus that was paired with a foot shock, and temporal integration results from Lessing, Sawa, & Blaisdell (2007) that demonstrated alterations in peak responding according to the timing of unconditioned stimulus presentations. In particular Lessing, Sawa, & Blaisdell (2007) provided evidence that when a short conditioned stimulus A was embedded into a second longer conditioned stimulus B, and food presentation was set to occur at the end of A, rats produced peaks of responding to the stimulus B in accordance to the time when stimulus A would normally occur, thus implying a temporal encoding within the S-O relation.

Drawing from these results, in a peak procedure task in which two stimuli are presented, a time cue stimulus A and a conditioned excitatory cue stimulus B that has been paired with a larger amount of reward than normally available, the presentation of A should produce one level

of anticipatory responding which produces the usual response function. However in compound presentations of A and B, the addition of B should serve to: one increase the anticipatory responding on the instrumental component of the task through incentive motivational processes related to PIT, and two shift the time function to center around the point at which reinforcement would be normally obtained after presentation of stimulus B. However, it is not entirely clear behaviorally whether these two effects can be conceptualized under changes to reward density tracking processes or response selection and initiation processes, or some combination of both which an analysis of serotonergic pathways may help differentiate between.

Role of Serotonin

The monoamine serotonin has been implicated as playing a role in the mediation of impulsivity (Crockett et al., 2010), response inhibition (Carli & Sammin, 2000; Soubrie, 1986), and reward sensitivity (Nonkes, van de Vandervoort, & Homber, 2014; Homberg, 2012). However, isolating the exact role of serotonergic pathways within the above domains has proven to be elusive with depletions and elevations of serotonergic levels often providing opposing results (Homberg, 2012). Behaviorally, serotonergic manipulations produce impacts on both reward valuation processes as well as motor inhibition processes which may underlie the wide variety of reported effects (Cools, Nakamura, & Daw, 2011). With respect to the neural systems involved in these processes, serotonin's influence is likely through action on dopaminergic activity within the Substantia Nigra and Ventral Tegmental area (Dalley & Roisser, 2012; Winstanely et al., 2006), structures that have all been implicated in mediating reward valuation processes related to incentive salience (Eisenreich & Szalda-Petree, 2015; Berridge, 2000; Ciccopio, 1998) as well as aspects of timing behavior (Kirkpatrick, 2014; Galtress, Marshall & Kirkpatrick, 2012). As such serotonin pathways represent a prime candidate for exploring

possible neural circuits that underpin motivational influences in timing behavior and their impacts on different computational processes.

Serotonin in time based tasks

When to respond is a question that features prominently in delay discounting tasks and the peak procedure. Traditional conceptualizations of delay discounting argue that when to respond is a function of the reward magnitude and the delay to the reward, such that a short immediate reward can have a higher subjective value than a larger reward that is greatly delayed (Mazur, 2001). Thus, delay discounting tasks principally examine the sensitivity of subjects to relative delays of reinforcement. Likewise, the peak procedure is primarily concerned in examining the relative expectation of when the trained reward is perceived to occur, such that the greatest amount of responding centers about the typical time of reinforcement. As such, manipulations of neurotransmitters within both tasks provide insight into how temporal information is utilized in deciding when to respond.

Delay Discounting Studies examining serotonin's impact on rates of impulsivity within delay discounting tasks have revealed a general trend of across species of lower levels of serotonin being associated with higher levels of impulsivity (Homberg, 2012; Crockett et al, 2010; Soubrie, 1986). In addition to this, increases of serotonin levels utilizing SSRI's, such as fluoxetine, produce decreases in the rate of impulsivity (Denk et al., 2005; Winstanely et al., 2004), and tonic firing rates of serotonergic raphe nuclei have been demonstrated to correspond with the delay to the reward (Miyazaki et al., 2011). Furthermore, the serotonin 1a and 2a/c receptors has been associated with increased rates of impulsivity after agonist administration (Homberg, 2012), however lesion studies of the nucleus acumbens shell have been demonstrated to block these effects (Winstanely et al., 2005). As such, the apparent role of serotonin function

within delay discounting appears to be in modulating the relative sensitivity of the subject to time delays and likely involves action through dopamine pathways.

Peak Procedure Within the peak procedure serotonergic manipulations have been demonstrated to exert mixed effects upon the timing components of the task. Morissey et al. (1994) demonstrated that lesions of the serotonin system produced increased spread in the response function and altered the response rate, while the peak interval remained the same, thus indicating intact timing in subjects. Likewise, up regulation of the serotonin system with the SSRI fluoxetine has been demonstrated to reduce the rate of responding while leaving the spread and peak interval of responding unimpaired (Heilbroner & Meck, 2014; Ho et al., 2002; Ho et al., 1996). In conjunction with this trend, Heilbroner & Meck (2014) also demonstrated that when given a deflection lever that produced an immediate reward within a bi-peak procedure containing a small peak and a large peak, fluoxetine reduced impulsive responding on the deflection lever. However, administrations of serotonin 1a agonists have been demonstrated to shift the response function to the left of baseline similar to the effects of amphetamines (Asgari et al., 2005). Thus, as with delay discounting tasks the serotonergic system appears to influence the sensitivity of subjects to temporal durations.

Serotoninergic function: an arousal activation account

In synthesizing the findings of serotoninergic manipulations in the peak procedure and delay discounting tasks a few trends become apparent: 1) serotonin 1a receptors do play a role in timing behavior processes 2) serotonin does appear to impact elements of reward valuation processes when time is a factor, 2) serotonin may have a role in response inhibition for engaging in impulsive responding, and 3) serotonin effects in delay discounting and potentially the peak procedure are mediated by dopamine pathways. Based on these facts, serotonergic function

likely serves as an arousal activation pathway that serves to either impact incentive motivational processes through attentional processes, as has been proposed by (Eisenreich & Szalda-Petree (2015) and Homberg (2012) or through motor inhibition pathways.

Evidence for the arousal attentional-activation account is provided by reports indicating that SSRIs block the acquisition of conditioned escape responses (Nelson et al, 1997; Beulig and Fowler, 2009), appetitive stimulus discriminations (Eisenreich & Szalda-Petree, 2015), and reward reinforced lever pressing (Frick et al., 2014). Applied within the incentive salience framework, the reduction in responding to reinforcement and the blocking of stimulus discriminations can be conceptualized as a disruption in the incentive transfer from the unconditioned stimulus to the conditioned stimulus, thus reducing the control of the stimulus on behavior. However, the reduction in impulsivity reported in delay discounting tasks (Winstanely et al., 2004) and the failure of SERT knockout to block pavlovian incentive transfer in mice (Nonkes, van de Vondervoort, & Homberg, 2014), provides evidence that serotonin pathways may also modulate motor inhibition pathways. Conceptually, an increase in motor inhibition would result in lessoned responding and could masquerade as an incentive transfer effect, thus a task in which attentional and motor performance processes can be disassociated will aid in the evaluation of these two hypothesizes.

Rationale

Converging evidence has indicated that: 1) motivational processes may impact timing behavior, and 2) these processes may be controlled by serotonin pathways. Furthermore, serotonin pathways have been implicated in modulating aspects of motivated responding and timing through potential attentional and motor inhibition effects. In order to examine the impact of motivational processes within timing behavior and the role of serotonin an experiment was

conducted using two successive manipulations. The first manipulation consisted of a behavioral manipulation utilizing a modified version of the peak procedure that included the introduction of a classically conditioned incentive cue. Drawing from the literature on pavlovian to instrumental transfer, the peak procedure was modified to include both cued FI and probe trials in addition to the standard un-cued trials. If incentive processes served to influence responding within the timing task, then response rates should be elevated during cued trials in accordance with pavlovian to instrumental transfer effects as well as exhibiting shifts in the centering of the response function in accordance to the duration of the conditioned cue.

Building off of the pattern of data from the behavioral manipulation, a pharmacological manipulation consisting of an IP injection of fluoxetine was conducted to assess the role of fluoxetine in mediating the impact of the conditioned cue on response rates and timing behavior. Critically if fluoxetine served to alter pavlovian incentive transfer through attentional processes, then both timing functions and response rates should be equal between cued and un-cued trials. However if fluoxetine functions through motor inhibition pathways, then an overall reduction in response rates to both cue and un-cued trials should occur with no change in the effect of the cue on timing behavior.

Methods

Subjects

Eight male experimentally naïve Sprague dawley rats were housed in pairs inside standard rat cages containing wood chip bedding and a wood block. Subjects were kept on a 12

L:D cycle and given free access to water, as well as being restricted to 85% of their free feeding weight. Subjects were labeled by placing marks their tails to indicate their assigned research ID.

Apparatus and Materials

Operant Chambers All training occurred in an operant chamber (28 cm X 22 cm X 27 cm, LxWxH) housed inside a sound attenuating box. The operant chambers contained a pellet dispenser hooked up to a food hopper, a lever press located 8 cm to the right of the food hopper, a stimulus light located 9 cm above the food hopper, and a fan to produce a constant masking background noise.

Drug Protocol Fluoxetine HCL was obtained from TCI America. Dosages were selected based upon previous research demonstrating no impact on the timing of interval responses but a clear reduction in response rates in a peak procedure (Cordes et al., 2008; Hellbroner & Meck, 2014). The dosage used was 5 mg/Kg delivered in saline vehicle at 2 mL/ Kg via an intraperitoneal (IP) injection. Sham injections of the saline solution, 2 mL/ Kg, were also administered on non-drug test days. Administration of the sham or drug injections occurred on the following schedule, 1 day of IP injections followed by a two day period. Following this schedule all subjects received 3 sham injections followed by 3 injections of fluoxetine.

Experimental Procedures

Pavlovian Conditioning and Interval Schedule Training	Peak Procedure Training	Modified Peak Procedure	Modified Peak Procedure (Extinction)	Modified Peak Procedure Saline Baseline	Modified Peak Procedure Fluoxetine
12 Sessions	15 Sessions	7 Sessions	3 sessions	3 Sessions	3 Sessions

Figure 2. Diagram of experimental time course across experimental manipulations. Light gray shading denotes the behavioral manipulations while dark gray denotes pharmacological manipulations.

Pavlovian conditioning In order to create an incentive motivational cue, rats were classically conditioned to a tone food association. Classical conditioning training occurred within the operant chamber and consisted of a 15 s presentation of a 440 Hz tone followed immediately by access to 3 45 mg sugar pellets. Classical conditioning training occurred daily for five trials before fixed interval training trials begin with 5 intermittent days of retraining trials occurring randomly throughout fixed interval and peak procedure training. In total all subjects received a total of 50 15s tone and sugar pellet pairings.

Fixed Interval Training Fixed interval training consisted of training the rats to lever press for 45 mg sugar pellets on a CRF schedule for 3 days and then transitioning to a VI 30 schedule for 4 days. After consistent responding was established on the VI 30 schedule on day 4, rats were then trained on an FI 30 schedule for 5 days. Trials within the three schedules consisted of the insertion of the lever and the illumination of the stimulus light. During the CRF schedule trials the light was turned off and the lever retracted after each lever press for each of the 60 trials. For VI 30 and FI 30 trials the light was turned off and the lever retracted on the first response after the scheduled interval had elapsed.

Peak Procedure Training on the peak procedure occurred over the course of 19 days and consisted of 60 trials each day, of which 10 were probe trials. Fixed interval trials consisted of the stimulus light illuminating for 30 s and the lever being inserted into the chamber. On all fixed interval trials the first lever press after the 30 s interval resulted in a 45 mg sugar pellet and the retraction of the lever. Probe trials consisted of the illumination of the light for 90s and the insertion of the lever. On probe trials the light remained illuminated and the lever available until the 90s interval had elapsed at which point the lever was retracted and the light turned off. During probe trials no reinforcement was given.

Modified Peak Procedure with Incentive Cues After establishing a steady state of responding to the peak procedure, the pavlovian incentive cue in the form of the 15 s presentation of a 440 hz tone was introduced into half of the probe trials and 5 of the standard fixed interval trials, thus producing the modified peak procedure. The onset of the tone occurred at the beginning of the trial with the illumination of the light and insertion of the lever. This schedule created a total of 10 probe trials (5 cued, 5 un-cued) with no reinforcement being given, imbedded within 50 FI 30 trials (5 cued, and 45 un-cued) in which reinforcers were delivered. During both the fixed and probe trials the rate of responding as well as the elapsed time was recorded on a computer.

Modified Peak Procedure with Incentive Cues under extinction After 7 days of initial testing under the modified peak procedure, it was noted that there was no apparent effect of the classically conditioned incentive cue on response rates and the timing function of rats, indicating a failure of producing pavlovian to instrumental transfer. Previous reports in the literature (Cartoni et al., 2015, Cartoni et al., 2013; Dickinson et al., 2000) have noted that pavlovian to instrumental transfer is strongest when tested under extinction. Thus in order to amplify any

effect the conditioned incentive cue may be having on response rates and timing functions, the modified peak procedure was placed under extinction, that is no sugar pellets were delivered during the 60 trials, and a new schedule of test days and recovery days was implemented. In particular, subjects were placed on a schedule of one test day followed by two recovery days. Furthermore, in order to ensure an adequate number of response trials the ratio of probe and fi schedules was changed to the following: 22 probe trials (11 cued, 11 un-cued), and 38 fixed interval trials (8 cued, 30 un-cued). Following this procedure data was collected across 3 test sessions.

Impact of Fluoxetine on Incentive cues in the modified peak procedure Following the completion of 3 test sessions under the modified peak procedure on an extinction schedule, subjects were then injected with a 2ml/kg saline vehicle 1 hour before beginning testing on the same modified peak procedure task previously described. Injections followed the same testing schedule utilized above, that is 1 injection/test session followed by a two day rest/recovery sessions. After the completion of 3 test sessions, subjects were then injected with a 5mg/kg dose of fluoxetine delivered in the 2ml/kg saline vehicle and data was once again collected for 3 test sessions spaced apart by 2 day recovery sessions.

Analysis

In order to analyze alterations in peak interval timing and peak rates, responses were collapsed into 3 s bins and averaged across cued and un-cued peak trials for either the last 3 days of testing (initial modified peak procedure sessions) or the three test sessions under extinction and within the pharmacological manipulations. Due to the change in total number of peak trials with the introduction of the extinction schedule, the average rate of response per 3s bin was adjusted for the total number of trials, thus producing an average rate of response per 3s bin per

trial. Following the standard practice, peak time and peak rates were determined by fitting an estimated function to the data using the equation provided by Buhusi, Perra, and Meck (2005). This produced three main response factors which were analyzed using a multiple repeated measures ANOVA: peak rates, peak times, and 3s response bins.

Analysis 1 Effect of Extinction Ruling out a potential confound Due to the use of extinction and the possibility of response rates decreasing as a function of increasing exposure to extinction test sessions obscuring changes due to fluoxetine injections within the pharmacological manipulation, a preliminary analysis of both response rates and times was conducted using a 4 x 2 multiple repeated measures ANOVA. In particular the two main repeated factors were entered as follows: the effect of experimental manipulations was broken into four blocks (block 1= under reinforcement, block 2= under extinction, block 3= saline injections, and block 4= fluoxetine injections), and trial type (cued or un-cued). If exposure to extinction had any effect on response rates or timing then there should be a significant main effect of experimental manipulations. Furthermore, when broken down via simple contrasts an effect of extinction would manifest as a continuous decrease in responding across the four experimental blocks (Block 1> Block 2>Bock 3> Block 4).

Analysis 2 Impact of Experimental Manipulations and Incentive cues on Peak times and Rates Stemming from the analysis above the peak times and peak rates were also analyzed using the same 4x2 multiple repeated measures ANOVA, with particular interest paid to the main effects of the factors trial type and experimental manipulation, as well as the interaction between experimental manipulations and the trial type. Of particular note was whether the following pattern would hold for either peak times or peak rates for the main effect of experimental manipulations (Block 1 =/= Block 2, Block 2 = Block 3, Block 3 =/= Block 4) and

trial type (Cued =/= Un-cued), in addition to an interaction effect between trial types across Blocks 1 and 2, as well as Blocks 3 and 4.

Analysis 3 Impact of Experimental Manipulations and Incentive Cues on 3s Bins In addition to the analysis of peak times and rates, a final analysis was conducted on the change in response rates across the 3s bins by extending the above 4x2 multiple repeated measures ANOVA into a 4x2x30 multiple repeated measures ANOVA with the addition of the 3s bin factor with 30 levels. The particular aim of this analysis was to assess whether the spread of responding changed across experimental manipulations (an experimental manipulation x bin interaction) or across trial types and experimental manipulations (a trial type x experimental manipulation x bin interaction), either of which would indicate a change in the response rule for starting and stopping responses.

Analysis 4 Graphical Analysis of Cued and Un-cued FI trials In addition to the standard analyses performed in peak interval timing experiments, a graphical analysis was also conducted on the standard FI 30 trials from each portion of the four experimental blocks. The main thrust of this analysis was to further elucidate the impact of the conditioned incentive cues on response rates and as a secondary check on the presence or absence of pavlovian to instrumental transfer effects. In order to conduct this analysis response rates on both cued and un-cued FI 30 trials were average across the three experimental sessions and then adjusted for the total number of FI trial types that occurred during each session. This produced an average response rate per second within a single trial for each of the eight subjects. These were then averaged together to produce a group response rate per second per trial which was then plotted on a cumulative response graph for the two trial types for each of the four experimental blocks.

Results

Training Training on the FI30 schedule produced a relatively high and stable pattern of responding on the lever, per session $M = 1432$, $SD = 74$. Furthermore, initial training on the peak interval procedure produced a stable response function with an average peak interval of 30s, $M = 30.1$ $SD = 2.87$, and a peak rate of 39 lever presses, $M = 38.91$ $SD = 5.34$.

Effect of Extinction The analysis of peak times using the 4x2 multiple repeated measures ANOVA described above revealed no significant main effects for the factor of experimental manipulations or type, as well as no significant interactions. Contrarily, the analysis of peak rates using the 4x2 multiple repeated measures ANOVA did reveal a significant main effect for the factor of experiment, $F(3,21) = 31.9891$, $p < .001$, $\omega^2 = .757$. A post hoc analysis using a Bonferoni test revealed the following pattern of significant differences between peak rates: Block 1> Block 2, Block 2 = Block 3, Block 3 > Block 4. Applied to the experimental procedure, these results indicate that the extinction procedure did reduce rates however the reduction in rates was relatively stable and potentially discernible from the impact of fluoxetine between Blocks 3 and 4.

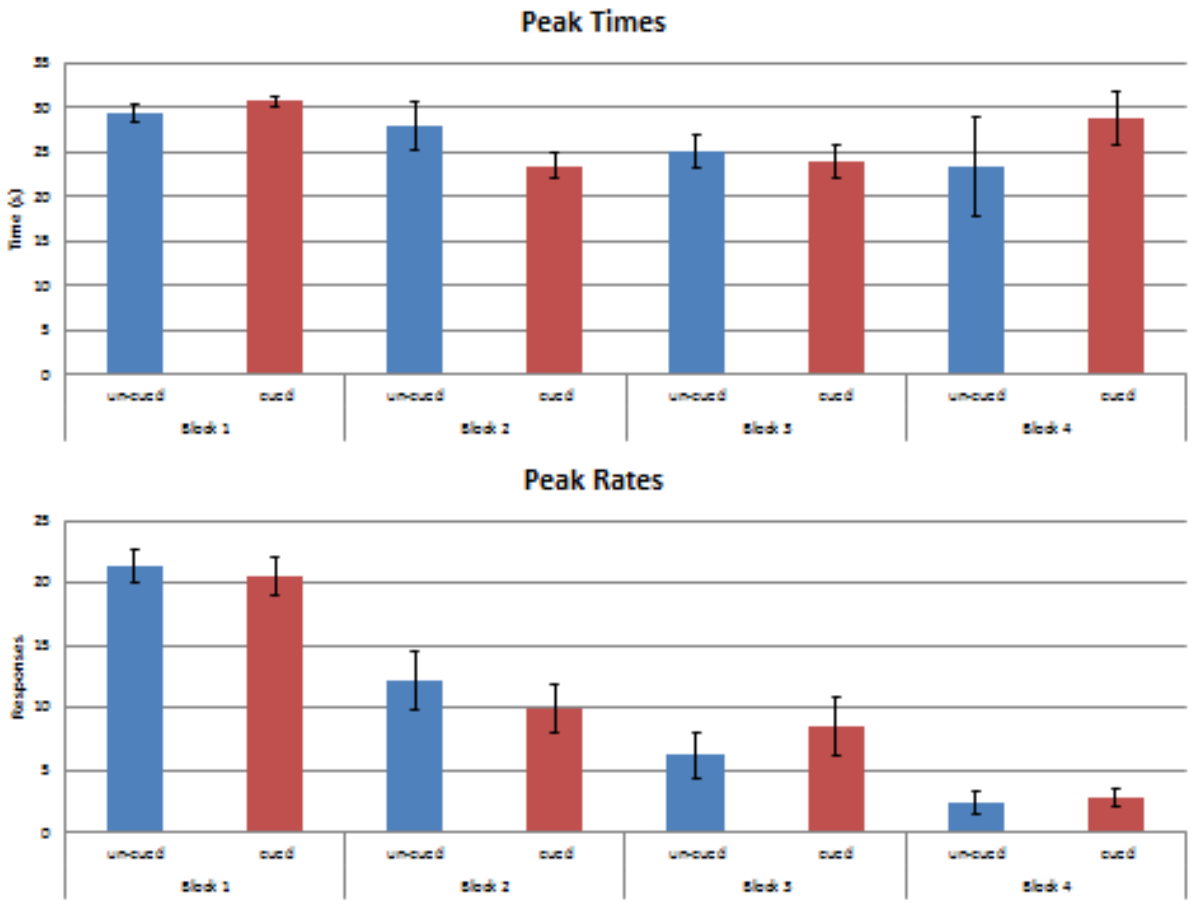

Figure 3. Average peak times and peak rates across the experimental manipulations, blue bars are for un-cued trials and red bars are for cued trials. Error bars are the standard error of the mean.

Impact of Incentive Cues and Fluoxetine

Peak Times An analysis of peak times using a 4x2 multiple repeated measures ANOVA revealed no significant interactions, or significant main effects for the factors of experimental manipulation and trial type for the dependent measure of peak times; thus indicating no effect of the incentive cues (Cued = Uncued), extinction (Block 1= Block 2 =Block 3) or of fluoxetine (Block 3= Block 4) on the peak timing of subjects.

Peak Rates An analysis of peak rates revealed a significant interaction between experimental manipulations and trial types, $F(3,21) = 15.568$, $p = .019$, $\omega^2 = .001$, as well as a significant main effect for the factor of experimental manipulations, $F(3,21) = 31.9891$, $p < .001$, $\omega^2 = .757$. A follow up planed comparison conducted on the interaction effect revealed a significant difference between Block 2 and Block 3 for the two trial types, $F(1,7) = 6.386$, $p = .039$, $\omega^2 = .064$, thus indicating PIT of increased response rates for cued trials. As stated above a post hoc analysis of the main effect for experimental manipulations revealed significant groupings between Block 1 and 2, $F(1,7) = 21.749$, $p = .002$, $\omega^2 = .32$, and Block 3 and 4, $F(1,7) = 7.857$, $p = .026$, $\omega^2 = .069$. Applied to the impact of fluoxetine, the disappearance of the significant interaction effect from Block 3 to 4 as well as the decrease in overall response rates indicates that fluoxetine did alter responding between cued and un-cued trials, however this effect may be an artifact of a floor effect (figure 4).

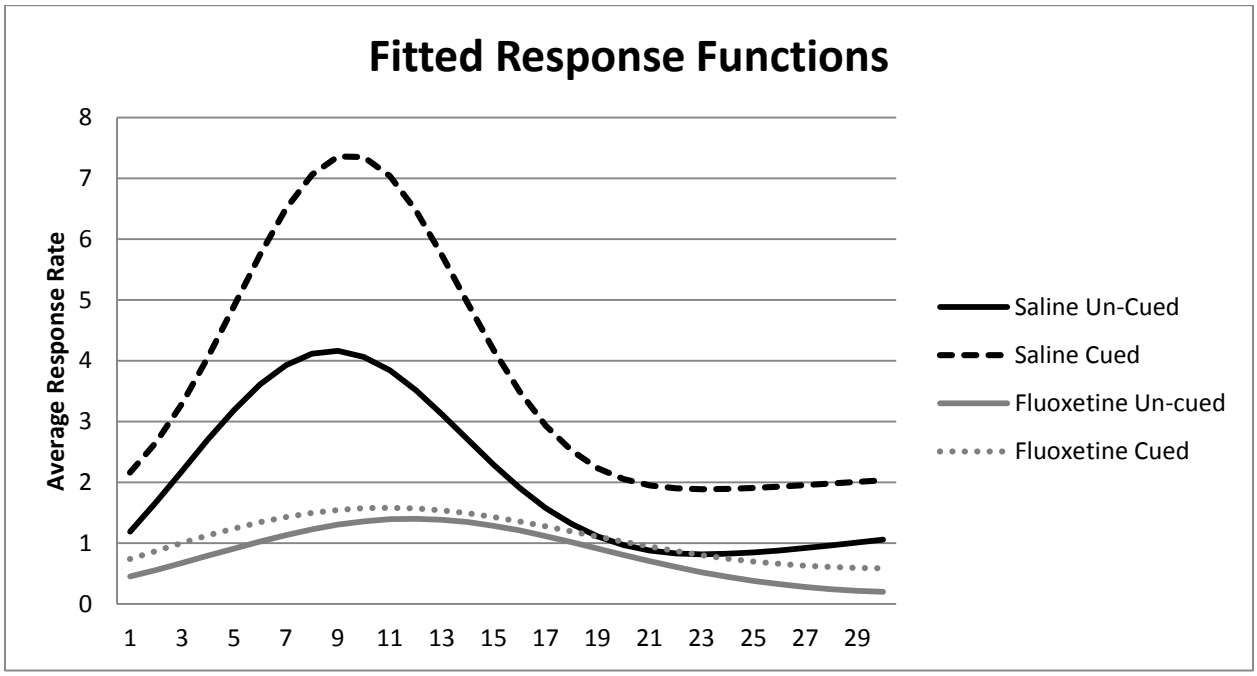

Figure 4. Change in response functions from saline injections (black lines) to fluoxetine injections (gray lines) for cued and un-cued trials.

Impact of Experimental manipulations and Incentive cues on 3 Bins Similar to the previous analyses, the 4x2x30 multiple repeated measures ANOVA revealed a significant interactions between the factor of experimental manipulation and trial type, $F(3,21) = 3.285$, $p = .041$, $\omega^2 = .007$, as well as a significant interaction between the factors of experimental manipulation and bins, $F(87, 609) = 10.545$, $p < .001$, $\omega^2 = .131$. In addition to these interactions significant main effects were found for the factors experimental manipulations, $F(3, 21) = 31.088$, $p < .001$, $\omega^2 = .307$, and bin, $F(29, 203) = 28.366$, $p < .001$, $\omega^2 = .226$. Overall the significant interaction between experimental manipulation and trial type as well as the main effect of experimental manipulations, provide further support for the role of incentive cues in increasing response rates and fluoxetine in reducing the overall pattern of responding noted in the previous analysis of peak rates. However the experimental manipulation by bin interaction is unique from the overall decrease in response rates and may provide insight into how fluoxetine and extinction may be altering response processes.

In order to assess the experimental manipulation by bin interaction the average response rates within each experimental block for each bin were divided by the maximal average rate of responses for that block. This produced 30 bins corresponding to the proportion of maximal response within each experimental block. From this changes in start and stop times corresponding to the shift from low to high states of responding were determined by looking at the corresponding bin in which the proportion of maximal response was greater than 50 %. Doing so indicated a relatively stable pattern of start and stop times across experimental blocks 1-3, but a distinct spread between start and stop times within block 4, thus indicating that fluoxetine not only decreased overall response rates but also impacted the response function for initiating and inhibiting lever pressing as can be seen in figure 5.

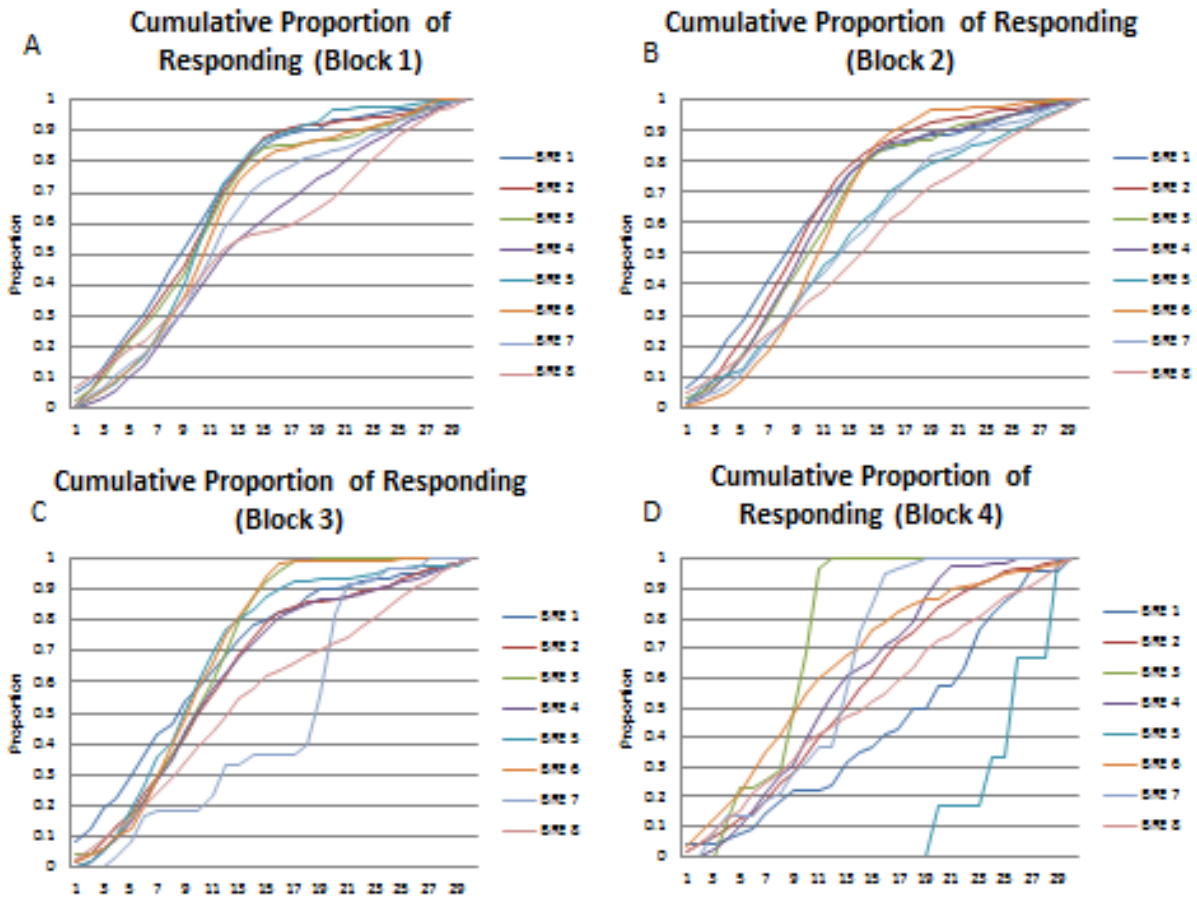

Figure 5. Cumulative proportion of average trial responding within peak trials for all eight subjects across the four experimental manipulations.

Assessment of PIT in Cued and Un-cued FI 30 trials Using the procedure described above four graphs were produced showing the cumulative rate of response for the entire group of eight subjects over the 30 second interval. For the first experimental block in which reinforcement was available after the 30 second interval had elapsed, the conditioned incentive cue produced no discernible change in the overall responses rates of rats. However when placed under extinction (Blocks 2 and 3), the conditioned incentive cue produced a clear increase in responding. Interestingly, with the addition of fluoxetine in Block 4 the effect of the conditioned cue was abolished (figure 6 A-D).

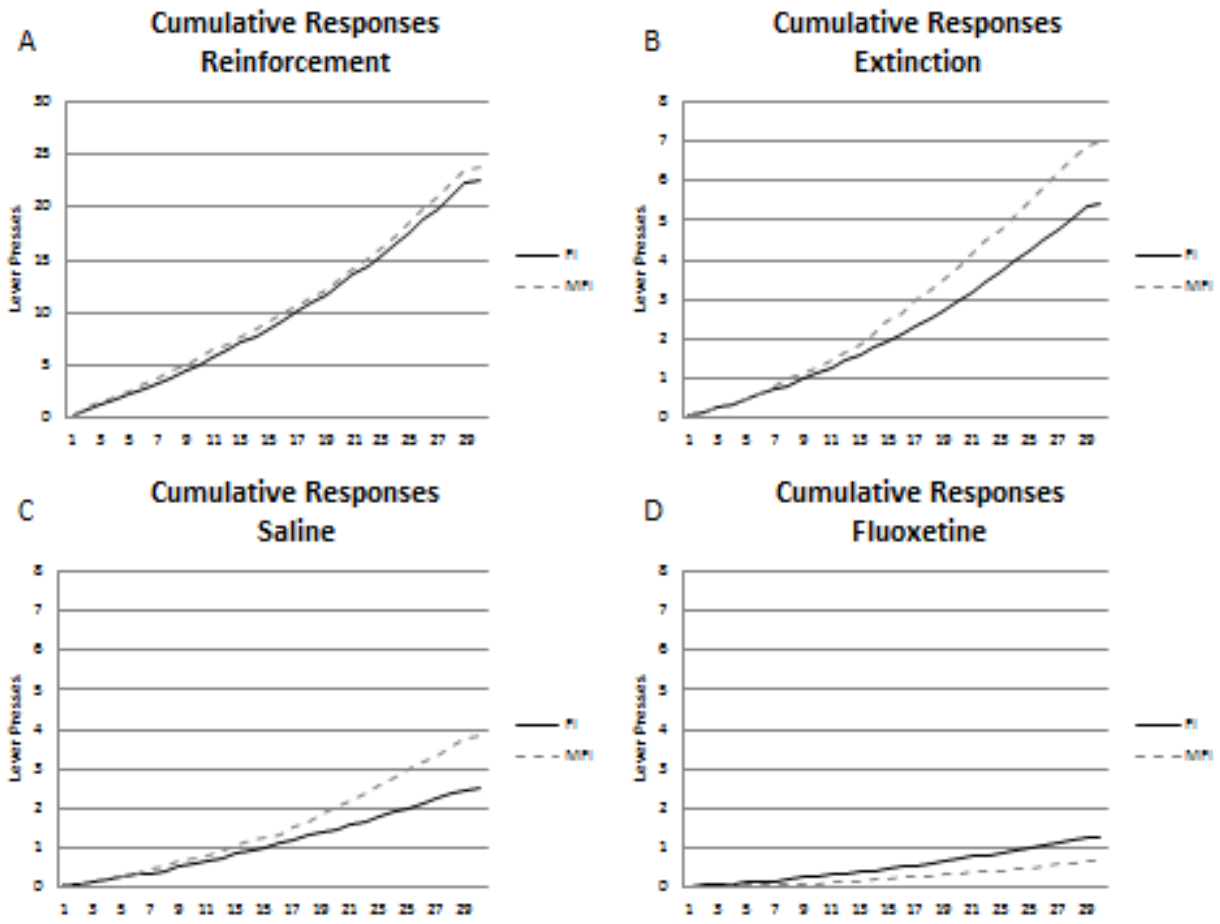

Figure 6. Average cumulative responses per trial for cued (dotted line) and un-cued (solid line) FI 30 trials.

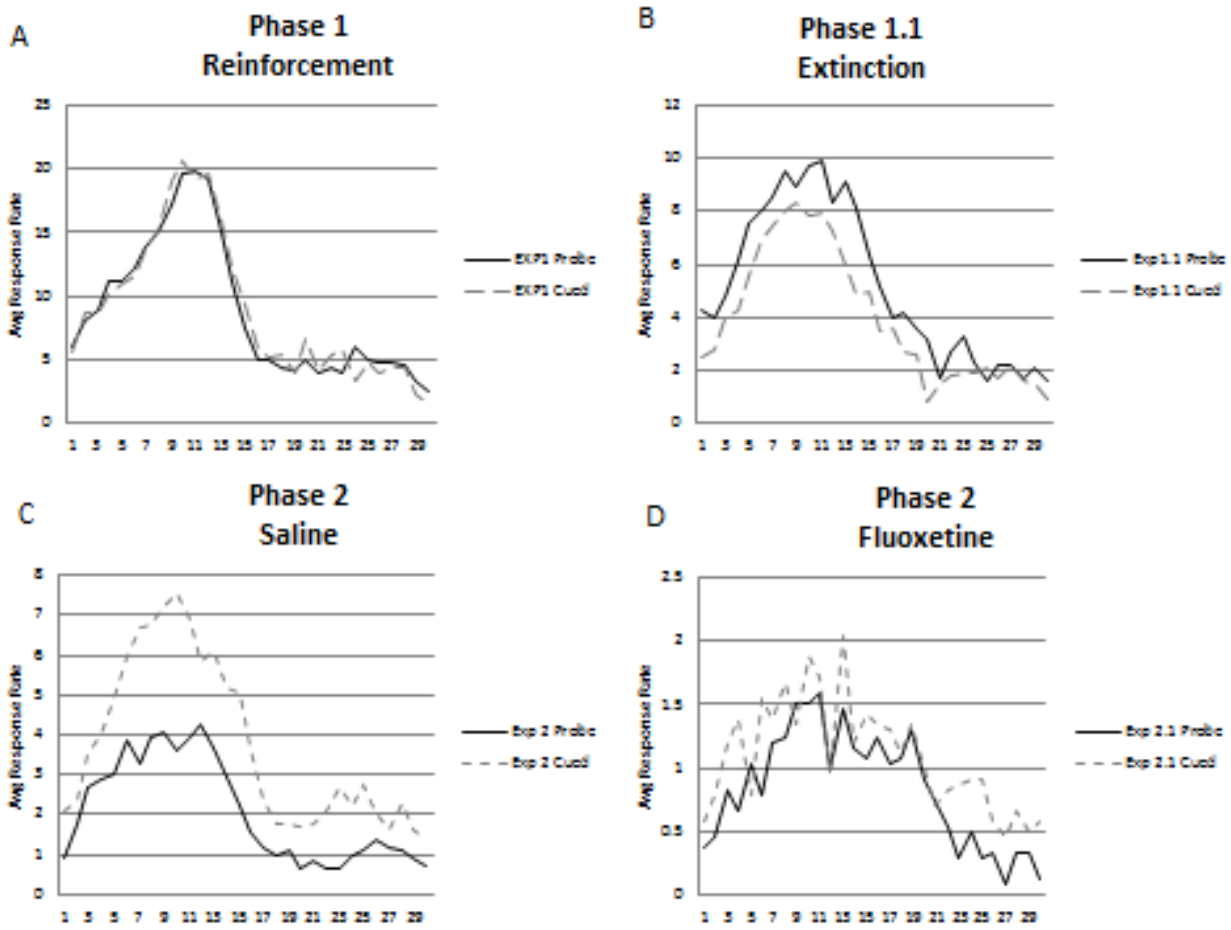

Figure 7. Group response rates for peak trials across the four experimental conditions. Dotted lines are for cued trials, solid lines are for un-cued trials.

Discussion

Behavioral effects of incentive cues

Stemming from growing interest in the role of motivational factors on timing behavior and a growing body of evidence suggesting that motivational cues may produce shifts in timing within the peak procedure, the present study sought to directly assess whether classically conditioned incentive cues would produce alterations in responding on the peak interval

procedure. Of particular interest was whether the pavlovian to instrumental transfer (PIT) effect could be produced within a peak procedure task and how this transfer effect would alter interval timing. With respect to the former question, initial attempts at inducing a PIT effect under a condition of reinforcement failed to produce any distinguishable differentiation between cued and un-cued FI and Peak trials (Figure. 6a and Figure. 7a). However when tested under extinction, differentiation between cued and un-cued FI and Peak trials emerged with a general increase in responding during cued trials (Figure. 6b-c and Figure 7b-c).

Functionally, it was proposed that responding in the peak procedure could be diagramed in a similar manner to Ballenine and Ostlund (2007) conceptualization of pavlovian to instrumental transfer. Specifically, it was stated that initial instrumental training in the peak procedure produced a stimulus outcome (S-O) and a response outcome pairing (R-O) such that with the introduction of the temporal contingency the presentation of the stimulus S served to guided responding on the R-O association due to an encoding of the temporal requirement between the S-O interval. Building on this model, it was further theorized that the incentive transfer that occurs between S-O pairings could influence the temporal aspects of a learned S-O interval, and as such the introduction of a new stimulus (S_1) paired with an outcome carrying more incentive value (O_1) would serve to shift responding on the trained O-R, R-O contingency.

With respect to proposed functional account of peak procedure performance, the results of the behavioral manipulation when tested under extinction fit partially with this model. Overall the produced PIT effect did not impact any timing aspect of responding within the task, but did increase peak rates with the introduction of the conditioned incentive cue. While this is contrary to the initial predictions of a leftward shift in peak times due to the higher incentive value S-O

interval guiding R-O responding, it is in line with an increase in responding to the R-O. However it should be noted that the failure of a temporal shift could have been due to two possible factors.

First, when analyzing responding on the FI 30 trials, it appears that the point of differentiation between the cued rate of response and the un-cued rate of response has its origin just after 15s, which corresponds to the end of the conditioned incentive cue presentation. This point also corresponds with the typical transition point into a high state of responding identified by Schneider (1969) as occurring halfway into the timed interval. Thus one possible explanation for the increase in peak rates without the corresponding leftward shift in peak times, is that the cued duration chosen was too long to produce a noticeable anticipatory shift in responding when compared to un-cued trials.

A second explanation for the overall failure to shift peak times and the conserved nature of the timing functions produced, is that the training on the two S-O intervals was not equivalent and thus the S-O interval for the conditioned cued was insufficient to alter temporal responding. Kaiser (2008) provides some support for this interpretation as he found that the overall ratio of peak trials to FI trials impacts the acquisition of temporal responding. Of particular note, Kaiser demonstrated that with lower ratios of peak trials, in which no reinforcement is available, to FI trials, when reinforcement is available, animals demonstrate both higher rates of responding and improved temporal control. With respect to the functional account, the lower peak to FI ratio is equivalent to having more S-O pairings and thus a stronger encoding of the S-O interval and incentive transfer between the stimulus and outcome, hence greater responding. Within the present experiment, subjects were given many more S-O pairings with the 30s interval than with the 15s interval, thus the addition of the conditioned cue for the 15s interval simply functioned as

a further activating prime for the O-R R-O relationships thereby increasing responding while leaving the centering of responding around the more thoroughly encoded S-O 30s interval.

In further support for this second explanation are findings from Swanton & Matell (2011) and Matell & Kurti (2014) that demonstrated when two stimuli trained with different S-O intervals and reinforcement probabilities in a peak procedure were presented simultaneously responding tended to center around the interval with the higher history of reinforcement. Likewise the findings of Lessing, Sawa & Blaisdell (2007) of responding centering around the reinforced short interval stimulus A as opposed to the long unreinforced interval stimulus B in AB compound presentations, as well as the work by Brown, Richer, & Doyere (2006) of rightward shifts in peak time that correspond to the length of a stimulus presentation paired with shock further support the notion of the amount of S-O interval pairings driving the temporal learning.

Placed within the literature the above account and the data from the behavioral manipulation fit well with a model of incentive salience effects put forth by Cartoni et al. (2013) and Cartoni et al. (2015). In analyzing the data on pavlovian to instrumental transfer, Cartoni et al. (2013) argued that pavlovian incentive cues serve three general purposes within instrumental tasks depending on the type of PIT relationship utilized. In preparations of general PIT the pavlovian stimulus functions as a motivational "utility" signal that engaging in the trained R-O contingency will result in the delivery of a reward, while preparations involving specific PIT the pavlovian stimulus functions as an "efficacy" signal that increases the subjective estimate of actual reward probability. Lastly in studies utilizing inhibitory PIT, the pavlovian stimulus functions as a "Context" signal that the R-O contingency is unavailable. With respect to specific PIT, Cartoni et al. (2015) further argued that the use of extinction increases the uncertainty of

reward delivery and thus amplifies the effect of the pavlovian stimulus that has been paired with the omitted reward in activating the O-R R-O contingent relationships.

Applied within the modified peak procedure, the failure of the incentive cue to induce PIT until the implementation of extinction can be conceptualized as a type of over training of the S-O relationship in FI30 trials. Extended further, this same over training of the 30s temporal S-O relationship may have also prevented the 15s S-O relationship from establishing temporal control. As such, the current data provide evidence for a role of incentive cues in governing overall response rates within time based tasks. However more exploration of the relationship between the number of temporal pairings of the S-O relationships, in addition to conditions of reinforcement vs. extinction are needed to further address how incentive processes may alter timing behavior.

Impact of Fluoxetine on incentive cues and timing behavior

Following up on a growing body of evidence suggesting potential motivational and motoric impacts on responding in instrumental tasks by the SSRI fluoxetine (Eisenreich & Szalda-Petree, 2015; Frick et al., 2014; Sanders et al., 2007), the pharmacological manipulation sought to tease apart motivating attentional aspects from motor response aspects of responding to incentive cues in the peak procedure using a 5mg/kg dose of Fluoxetine. Of note, was whether fluoxetine would alter the impact the conditioned cue on response rates and temporal responding in line with a reduction in motivation or simply reduce overall response rates to both cued and un-cued trials in line with motor inhibition.

Overall the results indicate that fluoxetine served to reduce response rates in all portions of the experimental task. Within FI30 and Peak trials, fluoxetine abolished any apparent increase in responding by introduction of the conditioned cue. Furthermore, while the analysis of peak

times and response functions revealed that fluoxetine had no significant effect on peak times, it did produce a significant spread in the typical break run break patterns (Figure 5).

Applied to the original hypothesis, the overall pattern of decreased responding to both cue and un-cued trials, while temporal aspects of the response pattern remained unchanged is in line with a motor inhibition explanation. For one if fluoxetine were to act solely on motivational attentional pathway, relative rates of responding in cued trials would be expected to drop due to a blocking of the PIT effect while responding on un-cued trials should remain stable, contrary to the results of experiment two. Secondly, the increased spread in peak trial responding produced by fluoxetine while the overall timing function remained similar to baseline is indicative of a disruption in the normal break run break pattern, likely due to the suppression of overall response rates. Thus, it is likely that within the functional model of peak procedure responding fluoxetine functions to reduce responding to the R-O relation not due to an effect on the subjective value of the O or relating motivational processes, but through inhibition of coordinated responding.

In support of this, are a variety of studies that have demonstrated increased motor activity with depletion of serotonin levels (Winstanely et al., 2004; Fletcher et al., 2009) and decreases of motor activity with increases in serotonin (see Homburg, 2012 for review). As well as reports by Sanders et al. (2007) and Nonkes et al. (2014) of normal PIT and incentive salience processes in rats treated with 10 mg/Kg of fluoxetine or serotonin reuptake transporter knockout genotype. Furthermore, Homberg (2012) has argued for an informational arousal model of serotonin function in which increases in serotonin concentrations promote increased vigilance and greater goal directed behavior.

Within the behavioral task of experiment two, this model would argue for a reduction in response rates while still preserving an elevation in responding for cued trials. While the results

of experiment two only confirm a reduction in response rates, the overall decrease in responding to cue and un-cued trials could be indicative of a fluoxetine by extinction interaction. In fact, Berringer (1984) demonstrated a greater attenuation of responding on an extinction schedule after injection of 5 mg/kg of fluoxetine. Furthermore, increases in serotonin (Homberg, 2012), and fluoxetine treatment specifically (Brigman et al., 2009), have been generally associated with improved reversal learning. Combined together, the improved reversal learning and attenuated responding under extinction by fluoxetine are indicative of serotonin pathways in inhibiting responding to stimuli that no longer predict reward. As such, fluoxetine may have made the rats more sensitive to the omission of reinforcement and as such produced a general decrease in responding irrespective of the presence of the conditioned cue. In either case, the important takeaway is that serotonin, and fluoxetine in particular appear to alter the responding to the functional R-O relationship within the utilized peak procedure and other operant tasks through action on a motor pathway as argued by Eisenreich & Szalda-Petree (2015). Future studies should focus on teasing apart the specific pathway through which serotonin exerts this effect.

General Discussion

Incentive Cues and Models of Timing

Over the years a variety of models have been put forth to explain how organisms encode and use time to direct responding. Mainly models of time must explain three fundamental questions: 1) how time is encoded, 2) how time is utilized to direct responding, 3) what factors impact time encoding and utilization. With respect to FI and peak interval procedure responding, the functional model presented here in and the results of the experiments along with a growing body of literature provide some basic facts in regards to questions 2 and 3 that any model of timing behavior must account for.

In particular, there is strong evidence that reinforcement rates between the stimuli to be timed impact the centering of responding, with the stimulus having the greater reinforcement density exerting the greatest control over the timing of when to respond (Matell and Kurti, 2014; Swanton and Matell, 2011; Kaiser, 2008, 2009; present experiments). In addition to this, the results of the present set of experiments along with the reports by (Kirkpatrick, 2014; Galtress and Kirkpatrick, 2009; Ludvig et al., 2007) provide further evidence that the incentive salience of the reward stimulus utilized within the timing task does impact overall intensity of responding and may serve to alter the utilization of time cues. Applied within the functional model these facts indicate that timing models must be able to explain the acquisition and utilization of S-O temporal relationships, there integration into O-R R-O relationships to control the centering of responding, and the role of incentive transfer between the S-O in activating the O-R R-O chain. What follows is an application of these facts to three commonly utilized timing models.

Scalar Expectancy theory Scalar Expectancy Theory (SET), developed by Gibbon and Church (1984), is perhaps one of the more widely cited and utilized theories in explanations of timing behavior. At its heart SET posits clock mechanism that is governed by a pacemaker that sends beats to an accumulator upon the onset of the time stimulus. With the presentation of reinforcement the accumulated beats are deposited into a memory. Responding to the time stimulus is governed within SET by a comparator process that takes a random sample from the memory store of previously accumulated beats and compares this to the current number of beats in the accumulator. If according to a response rule the current number of beats is similar to the sampled memory value responding will occur and past a second threshold value responding will stop. In the terms of the functional model, SET captures S-O relationships within the pacemaker-accumulator to memory store process, and R-O relationships within the comparator process, but

does not make any explicit predictions regarding how incentive changes in the S-O O-R relationship could impact timing. Thus, incentive impacts on timing behavior under SET could be governed by changes in the pacemaker-accumulator-memory store process, or through changes in the response rule.

With respect to the contention that differential reinforcement probabilities control the timing of responding, SET readily handles this as a time stimulus with greater reinforcement occurrence would have a greater proportion of samples in the memory store, assuming a unitary memory store for all stimuli. In regards to the effect of incentive cues two possibilities arise, either the response rule is changed or the pacemaker frequency is altered. Changes in the response rule would produce shifts in when the initiation of responding began, mainly lowering the ratio of accumulated beats to sampled beats would produce an earlier shift in responding while an increase would produce a later shift in responding. Additionally as the response rule is thought to control the cessation of responding alterations in either process (starting or stopping responding) would result in an alteration in the spread of responding. With respect to the pacemaker explanation, the increased rate of responding due to the presentations of incentive cues or alterations of incentive values would be accomplished by an increased frequency of beats. This would lead to earlier responding as the comparator process would hit the decision rule to respond earlier than the previously trained values, but it would also trigger a change in the memory store of beats if reinforcement was delivered. The practical result of this latter point is that in experiments where incentive values were increased and thus acceleration of the pacemaker occurred, subsequent shifts back to the original incentive value and thus a return to normal pacemaker rate would result in a strong rightward shift of later responding to the time stimulus due to the change in memory store sample values.

Overall the behavioral data amassed on how incentive cues impact timing behavior under SET provide no clear answer as to whether the noted changes in response patterns and rates are due to alterations in response rules or pacemaker frequency. The findings by Kirkpatrick and Ludwig of leftward shifts in timing through increase in reward magnitudes suggest an alteration in pacemaker frequency, especially Galtress and Kirkpatrick (2009) which demonstrated the predicted rightward shift as incentive value was decreased after reinforced experience with the higher valued reinforcer. However, the results of the present set of experiments do not fit with this explanation as there was no noted change in the overall timing of responding, simply changes in response rates that are better captured by a response rule shift in the comparator process. That being said, it should be noted that contrary to (Galtress and Kirkpatrick, 2009; Ludvig et al., 2011) subjects were tested under extinction and thus a shift in the pacemaker rate would not alter the memory store due to the non-delivery of reinforcement and the dumping of the accelerated beat sample into the memory store. In addition to this, the studies by Galtess and Kirkpatrick (2014) and Ludvig et al. (2011) utilized incentive shifts in reward magnitudes as opposed to the addition of a conditioned incentive cue. As such it is possible that the lack of equal S-O pairings for the conditioned incentive cue combined with the use of extinction produced the differing results, and future studies are needed to further examine these factors in relation to SET.

Learning to Time Model The Learning to Time model (LeT), developed by Macchado (1998, 2009), is another commonly invoked alternative to SET. Like SET, the LeT model posits a pacemaker type process, however instead of sending beats to an accumulator, in LeT the pacemaker serves to activate successive behavioral states. These behavioral states are conceptualized as relating to adjunctive behaviors or other possible internal changes, however

there exact specification has yet to be determined. As time progresses the cascade of behavioral states changes and associative links are formed between the states up until the time of reinforcement. With greater exposure to reinforcement at a set time, the association between behavioral states and the connecting link is strengthened for those states active at the point of reinforcement and lessened for states further away. Thus, in the LeT model the time tracking process is the current activated behavioral state and the process determining the rate of response is the associative link between the state and reinforcement history.

Applied to the role of reinforcement densities in controlling the timing of responding, the LeT model readily accounts for this. Indeed the central process that drives the time tracking process is the associative link between the behavioral state and the occurrence of reinforcement. Thus, a greater number of reinforcers at time T_1 vs T_3 will produce a centering of responding around the point of T_1. In regards to the role of incentive cues and motivational changes, it is not entirely clear how they would impact behavior under the LeT model. Galtress, Marshall & Kirkpatrick (2012) have argued that reinforcement magnitude shifts may alter the rate of change between the behavioral states. The practical effect of this change under the LeT model would be a decoupling of real time from experienced time as the model specifies the rate of behavioral state changes as the time keeping process. While this would produce an earlier pattern of responding with increases in reinforcement magnitude, the effect would only be transient as the decoupling of real time from experienced time would produce a shift in the reinforcement density towards previously unreinforced later occurring behavioral states. Along a similar vein, the presentation of incentive cues should serve to activate a similar change in the rate of behavioral state change, as they are part of a similar incentive salience process, and as such result in earlier leftward shifts in rates of responding.

As with SET, the findings of Galtress and Kirkpatrick (2009) and Ludwig et al. (2011) can be conceptualized within the LeT model and fit with the prediction of a decoupling between real and experienced time due to an increase rate in the change of behavioral states. However, the findings from the present experiment do not fit well within the LeT model. For one, the preservation of the temporal patterning of behavior and elevated rates of responding fit well with a two process time and response intensity account, which the LeT model cannot accommodate. Secondly, even if the addition of the conditioned incentive cue is conceptualized as not changing the rate of behavioral state activation, as has been argued, it is still unclear how the cue would function to produce the elevated rates of responding. One could specify that the incentive cue served to activate more associative links between the behavioral state and a global level of reinforcement; however this would still specify a change in the centering of responding unless all states were elevated equally. Thus in comparison to SET, the LeT model is able to better capture incentive changes with respect to reward magnitude processes but does not adequately explain the role of incentive cues in elevating response rates.

Multiple Time Scales Contrary to SET and the LeT model, the multiple time scales (MTS) model developed by Staddon and Higa (1999) is a pace maker free model. Instead of proposing a clock mechanism, the MTS model argues for an encoding and usage of time cues as a process of short term memory decay. The central assumptions of MTS are as follows: 1) memory strength is encoded via a leaky integrator process such that greater frequency of stimulus presentations produce stronger memory strengths than less frequent presentations, 2) memory strength decays according to a log function and follows Jost's Law such that an older memory at the same strength as a newer memory will decay slower than the newer memory, and 3) the usage of time in controlling behavior is the resultant of a comparison process that examines the memory

strengths for remembered time and current time. Applied within the peak procedure this model argues that during initial training the presentations of S and O build up strength in short term memory according to the frequency of occurrence. Within an individual trial the presentation of the S triggers a memory representation which is then compared to the remembered O representation such that responding occurs when the difference in decay strengths passes a certain threshold. Thus like SET, the MTS model contains within it a process for the encoding and usage of time and a second process for the control of response patterning by the time process.

Within the functional model, the MTS model explains the notion of S-O pairings controlling the temporal patterning of responding through the differential encoding of the frequencies of the inter-stimulus intervals in relation to each other. Applied to the noted effects of reinforcement densities, the MTS model predicts similar effects of greater reinforcement densities exerting stronger control on the temporal patterning of behavior as reinforcement densities correspond to the frequency of stimulus presentations. In addressing the role of incentive cues the MTS model is not explicitly clear, however due to the two processes involved in timing and responding a similar account to SET is likely. Staddon and Higa (1999) stated originally that reinforcement magnitude effects would serve to increase the memory strength with greater magnitudes. As the model posits a decay function of the memory strength as both the timing process and the crossing of a threshold as the response rule this would have two effects. First a shift to larger magnitude reinforcers would result in a longer pause to responding due to the heightened memory strength taking longer to decay to the threshold. This would manifest as a rightward later shift in responding when shifted to a larger reinforcer which is contrary to the results of Galtress and Kirkpatrick (2009) and Ludwig et al. (2011). Alternatively,

reinforcement magnitude shifts could also shift the response threshold such that increases raise the threshold while decreases lower the threshold. Within the MTS model this would produce the noted shifts of responding earlier for magnitude increases and later for decreases in magnitude as the decay function crossing the threshold serves as the response rule.

With respect to the role of incentive cues in elevating response rates, the MTS model is not explicitly equipped to handle the findings from the present set of experiments without additional assumptions. For one, alterations of either the threshold level for responding or the overall memory strength would produce changes in the temporal patterning of responses that are inconsistent with the conserved pattern of responding observed throughout the present experiment. This could be accounted for by arguing that the incentive salience cue served to increase both memory strength and the response threshold such that the temporal patterning was preserved but the overall activation of responding was increased. However, this requires an assumption of a third process that controls the overall vigor of responding that is impacted by memory strength. On one hand an intensity of response process controlled by memory strength seems plausible in light of standard associationist accounts of stimulus control; however more research is needed to fully outline this process.

Conclusion

Overall the results of the present experiment add to a growing body of reports that indicate, contrary to Robert's (1981) conclusion, that motivation and timing are not independent processes that models of timing can ignore. Instead future efforts into how time is utilized in controlling responding need to accommodate the influence of incentive cues and saliency changes on how temporal relations are encoded and utilized. With respect to the three common models of timing (SET, LeT, and MTS) each of them fails to capture how incentive cues alter

responding in time tasks without some form of modification. Of importance for future endeavors into timing will be how the numbers of S-O temporal pairings serve to alter the centering of responding and whether the number of pairings follows discrimination gradient similar to weber's law, as well as the role of extinction in illuminating the PIT effects in timing tasks. Currently, only SET and the MTS are equipped for handling how S-O temporal pairings serve to impact the centering of responding, while LeT is best equipped for explanations of extinction effects on response functions. Thus, future examinations along these two domains will help to refine and differentiate between current timing models and their validity.

References

Asgari, K., Body, S., Zhang, Z., Fone, K.C.F., Bradshaw, C.M., & Szabadi, E. (2006). Effects of 5ht1a and 5ht2a receptor stimulation on temporal differentiation performance in the fixed-interval peak procedure. *Behavioral Processes, 71,* 250-257

Aum, S. W., Brown, B.L., & Hemmes, N.S. (2004). The effects of concurrent task and gap events on peak time in the peak procedure. *Behavioral Processes, 71,* 43-56

Aum, S. W., Brown, B.L., & Hemmes, N.S. (2007). The effect of intruded events on peak time: the role of reinforcement history during the intruded event. *Behavioral Processes, 74,* 187-197 doi: 10.1016/j.beproc.2006.11.001

Balci, F., Ludvig, E. A., & Bunner, D. (2010). Within-session modulation of timed anticipatory responding: when to start responding. *Behavioral Processes, 85,* 204-206 doi: 10.1016/j.beproc.2010.06.012

Balleine, B. W., & Ostlund, S.B. (2007). Still at the choice-point: action selection and initiation in instrumental conditioning. *Annals of the New York Academy of Science, 1104,* 147-171

Balsam, P., Sanchez-Castillo, H., Taylor, K., Vlkinburg, H. V., & Ward, R. D. (2009). Timing and anticipation: conceptual and methodological approaches. *European Journal of Neuroscience, 30,* 1749-1755 doi: 10.1111/j.1460-9568.2009.06967.x

Beninger, R. (1984). The effects of quipazine and fluoxetine on extinction of a previously-reinforced operant response in rats. *Pharmacology, Biochemistry, and Behavior, 21,* 533-537

Berridge, K. C. (2000) Reward learning: Reinforcement, Incentives, and Expectations. *Psychology of Learning and Motivation, 40,* 223-278. Doi: 10.1016/S0079-7421(00)80022-5

Beulig, A., & Fowler, J. (2008). Fish on Prozac: effect of serotonin reuptake inhibitors on cognition. *Behavioral Neuroscience, 122,* 426-432. doi: 10.1037/0735-7044.122.2.426

Brown, B.L., Richer, P., & Doyere, V. (2007). The effect of an intruded event on peak-interval timing in rats: isolation of a postcue effect. *Behavioral Processes, 74,* 300-310 doi: 10.1016/j.beproc.2006.11.004

Brigman, J.L., Mathur, P., Harvey-White, J., Izquierdo, A., Saksida, L.M., Bussey, T.J., Fox, S., Deneris, E., Murphy, D.L., & Holmes, A. (2009). Pharmacological or genetic inactivation of the serotonin transporter improves reversal learning in mice. *Cerebral Cortext, 20,* 1955-1963. doi: 10.1093/cercor/bhp266

Buhusi, C., Perera, D., & Meck, W. (2005).Memory for timing visual and auditory signals in albino and pigmented rats. *Journal of Experimental Psychology: Animal Behavior Processes, 31,* 18-30. doi: 10.1037/0097-7403.31.1.18

Carli, M., & Samanin, R. (2000). The 5-HT1a receptor agonist 8-OH-DAPT reduces rat's accuracy of attentional performance and enhances impulsive responding in a five-choice serial reaction time task: role of presynaptic 5-HT1A receptors. *Psychopharmacology, 149,* 259-268

Cartoni, E., Puglisi-Allegra, S., & Baldassarre, G. (2013). The three principles of action: a Pavlovian-instrumental transfer hypothesis. *Frontiers in Behavioral Neuroscience, 7,* 1-11 doi:10.3389/fnbeh.2013.00153

Cartoni, E., Moretta, T., Puglisi-Allegra, S., Cabib, S., & Baldassarre, G. (2015). The relationship between specific pavlovian instrumental transfer and instrumental reward probability. *Frontiers in Psychology, 6,* 1-7 doi: 10.3389/fpsyg.2015.01697

Catania, A. C. (1970). Reinforcement schedules and psychophsical judgments: a study of some temporal properties of behavior. In: Schoenfeld, W.N. (Ed), The theory of reinforcement schedules. Appleton-Century-Crofts, New York: NY

Ciccocioppo, R. (1999). The role of serotonin in craving: from basic research to human studies. *Alcohol , 34,* 244-253

Church, R. M. (2002). Temporal Learning. In Stevens Handbook of Experimental Psychology, 3 Ed., Galistel, R. (Ed)., John Wiley & Sons, New York: NY

Church, R. M.,& Broadbent, H.A. (1990). Alternative representations of time, number, and rate. *Cognition, 37,* 55-81

Colwill, R. M.,& Motzkin, D. K. (1994). Encoding of the unconditioned stimulus in pavlovian conditioning. *Animal learning and Behavior, 22,* 384-394

Colwill, R. M., & Rescorla, R. A. (1988). Associations between the discriminative stimulus and the reinforce in instrumental learning. *Journal of Experimental Psychology Animal Behavior Processes, 14,* 155-164

Cools, R., Nakamura, K., & Daw, N. D. (2011). Serotonin and dopamine: unifying affective, actiational, and decision functions. *Neuropsychopharmacology, 36,* 98-113 doi: 10.1038/npp.2010.121

Corbit, L.H., & Balleine, B.W. (2003). Instrumental and Pavlovian incentive processes have dissociable effects on components of a heterogeneous instrumental chain. *Journal of Experimental Psychology: Animal Behavior Processes, 29,* 99-106

Crockett, M. J., Clark, L., Lieberman, M.D., Tabibnia, G., & Robbins, T.W. (2010). Impulsive choice and altruistic punishment are correlated and increase in tandem with serotonin depletion. *Emotion, 10,* 855-862

Dalley, J.W., & Roisser, J.P. (2012). Dopamine, serotonin and impulsivity. *Neuroscience, 215,* 42-58 doi: 10.1016/j.neuroscience.2012.03.065

Dayan, P., & Balleine, B.W. (2002). Reward, Motivation, and Reinforcement Learning. *Neuron, 36,* 285-298

Delamater, A. R., & Holland, P.C. (2008). The influence of CS-US interval on several different indices of learning in appetitive conditioning. *Journal of Experimental Psychology Animal Behavior Processes, 34,* 202-222

Denk, F., Walton, M.E., Jennings, K.A., Sharp, T., Rushworth, M.F.S., & Bannerman, D.M. (2005). Differential involvement of serotonin and dopamine systems in cost-benefit decisions about delay or effort. *Psychopharmacology, 179,* 587-596

Dickinson, A., & Balleine, B.W. (2002). The role of learning in motivation. In Learning, Motivation, and emotion, Volume 3 of Stevens Handbook of Experimental Psychology, 3ed. , C.R. Gallistel (Ed), John Wiley & Sons, New York: NY

Dickinson, A., Smith, J., & Mirenowicz, J. (2000). Disassociation of pavlovian and instrumental incentive learning under dopamine antagonists. *Behavioral Neuroscience, 114,* 468-483. doi: 10.1037/0735-7044.114.3.468

Eisenreich, B.R., & Szalda-Petree, A. (In press). Behavioral effects of fluoxetine on aggression and associative learning in Siamese fighting fish (Betta splendens). *Behavioural Processes*

Estes, W.K. (1943). Discriminative conditioning I. A discriminative property of conditioned anticipation. *Journal of Experimental Psychology, 32,* 150-155

Ferster, C. B., & Skinner, B. F. (1957). Schedules of Reinforcement. Prentice-Hall, Englewood Cliffs: NJ.

Fletcher, P.J., Chambers, J.W., & Chintoh, A.F. (2009). Effects of 5-HT depletion in the frontal cortex or nucleus acumbens on response inhibition measured in the 5-choice serial reaction time test and on a DRL schedule. *Behavioural Brain Research, 201,* 88-98. doi: 10.1016/j.bbr.2009.01.036

Frick, L. R., Bernardez-Vidal, M., Hocht, C., Zanutto, B. S., & Rapanelli, M. (2014). Dual role of serotonin in acquisition and extinction of reward-driven learning: involvement of the 5-ht1a, 5-ht2a, and 5-ht3 receptors. *Behavioural Brain Research,* in press doi: 10.1016/j.bbr.2014.06.025

Gibbon, J., & Church, R.M. (1984). Sources of variance in an information processing theory of timing. In: Roitblat, H.L.; Bever, T.G.; Terrace, H.S., eds. Animal Cognition. Hillsdale, NJ: Elrbaum.

Gallistel, C. R., & Gibbon, J. (2000). Time, rate, and conditioning. *Psychological review, 107,* 289-344

Gallistel, C.R., & Gibbon, J. (2002). The symbolic foundations of conditioned behavior. Lawernce Erlbaum Assoc.: London

Galtress, T., & Kirkpatrick, K. (2009). Reward value effects on timing in the peak procedure. *Learning an dmotivation, 40,* 109-131

Galtress, T., Marshall, A. T., & Kirkpatrick, K. (2012). Motivation and timing: clues for modeling the reward system. I Behavioral Processes, 90, I142-153 doi: 10.1016/j.beproc.2012.02.014

Harris, J.A. (2015). Changes in the distribution of response rates across the CS-US interval: evidence that responding switches between two distinct states. *Journal of Experimental Psychology: Animal Learning and Cognition, 41,* 217-231 doi: 10.1037/xan0000057

Heilbronner, S. R., & Meck, W. H. (2014). Dissociations between interval timing and intertemporal choice following administration of fluoxetine, cocaine, or methamphetamine. *Behavioral Processes, 101,* 123-134 doi: 10.1016/j.beproc.2013.09.013

Ho, M.Y., Al-Zahrani, S.S.A., Velazquez Martinez, D.N., Lopez Cabrera, M., Bradsha, C.M., & Szabadi, E. (1996). Effects of desipramine and fluvoxamine on timing behavior investigated with the fixed-interval peak procedure and the interval bisection task. *Psychopharmacology, 125,* 274-284

Ho, M.Y., Velazquez-Martinez, D.N., Bradshaw, C.M., & Szabadi, E. (2002). 5-Hydroxytruptamine and interval timing behavior. *Phamacology, Biochemistry and Behavior, 71,* 773-785

Holland, P.C. (2004). Relations between Pavlovian-instrumental transfer and reinforcer devalutation. *Journal of Experimental Psychology Animal Behavior Processes, 30,* 104-117

Holmes, N. M., Marchand, A. R., & Coutureau, E. (2010). Pavlovian to instrumental transfer: a neurobehavioural perspective. *Neuroscience and Biobehavioral Reviews, 34,* 1277-1295 doi: 10.1016/j.neubiorev.2010.03.007

Homberg, J. R. (2012). Serotonin and decision making processes. *Neuroscience and Biobehavioral reviews, 36,* 218-236 doi: 10.1016/j.neubiorev.2011.06.001

Jensen, G., Ward, R. D., & Balsam, P. D. (2013). Information theory, brain , and behavior. *Journal of the Experimental Analysis of Behavior, 100,* 408-431 doi: 10.1002/jeab.49

Kaiser, D. H. (2008). The Proportion of fixed interval trials to prove trials affects acquisition of the peak procedure fixed interval timing task. *Behavioral Processes, 77,* 100-108 doi: 10.1016/j.beproc.2007.06.009

Kaiser, D. H. (2009). Fewer peak trials per session facilitate acquisition of peak responding despite elimination of response rate differences. *Behavioral Processes, 80,* 12-19 doi: 10.1016/j.beproc.2008.08.005

Kirkpatrick, K. (2014). Interactions of timing and prediction error learning. *Behavioral Processes, 0,* 135-145 doi: 10.1016/j.beproc.2013.08.005

Leising, K., Sawa, K., & Blaisdell, A. P. (2007). Temporal integration in pavlovian appetitive conditioning in rats. *Learning and Behavior, 35,* 11-18

Lovibond, P. F. (1983). Facilitation of instrumental behavior by a Pavlovian appetitive conditioned stimulus. *Journal of Experimental Psychology: Animal Behavior Processes, 9*(3), 225–47.

Ludvig, E., Conover, K., & Shizgal, P. (2007). The effects of reinforce magnitude on timing in rats. *Journal of the experimental analysis of behavior, 87,* 201-218 doi: 10.1901/jeab.2007.38-06

Ludvig, E. A., Balci, F., & Spetch, M.L. (2011). Reward magnitude and timing in pigeons. *Behavioral Processes, 86,* 359-363 doi: 10.1016/j.beproc.2011.01.03

Machado, A. (1997). Learning the temporal dynamics of behavior. Psychological Review, 104, 241–265.

Machado, A., Malheiro, M.T., & Erlhagen, W. (2009). Learning to time: A perspective. *Journal of the experimental analysis of behavior, 92,* 423-458. doi: 10.1901/jeab.2009.92-423

Matell, M. S., & Kurti, A. N. (2014). Reinforcement Probability modulates temporal memory selection and integration processes. *Acta Pscyhologica, 147,* 80-91 doi: 10.1016/j.actpsy.2013.06.006

Mazur, J. E. (2001). Hyperbolic value addition and general models of animal choice. *Psychological review, 108*(1), 96.

Miyazaki, K., Miyazaki, K. W., & Doya, K. (2011). Activation of dorsal raphe serotoin neurons underlies waiting for delayed rewards. *The Journal of Neuroscience, 31,* 469-479 doi: 10.1523/jneurosci.13714-10.2011

Morrissey, G., Ho, M.Y., Wogar, M.A., Bradshaw, C.M.,& Szabadi, E. (1994). Effect of lesions of the ascending 5-hydroxytryptaminergic pathways on tiing behavior investigated with the fixed-interval peak procedure. *Psychopharmacology, 114,* 463-468

Nelson, C. J., Wesley, J. P., & Bohan, R. T. (1997). Daily flouoxetine administration impairs avoidance learning in the rat without altering sensory thresholds. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 21,* 1043-1057

Nonkes, L. J. P., van de Vandervoort, I. I. G. M., & Homberg, J. R. (2014). The attribution of incentive salience to an appetitive conditioned cue is not affected by knockout of the serotonin transporter in rats. *Behavioral Brain Research, 259,* 268-273 doi: 10.1016/j.bbr.2013.11.017

Rescorla, R. A. (1997). Response-inhibition in extinction. *Quarterly Journal of Experimental Psychology, 50,* 238-252

Rescorla, R.A., & Solomon, R.L. (1967). Two process learning theory: Relationships between pavlovian conditioning and instrumental learning. *Psychological Review, 74,* 151-182

Roberts, S. (1981). Isolation of an internal clock. *Journal of Experimental Psychology: Animal Behavior Processes, 7,* 242-268

Sanabria, F., & Killeen, P. R. (2007). Temporal generalization accounts for response resurgence in the peak procedure. *Behavioral Processes, 74,* 126-141 doi: 10.1016/j.beproc.2006.10.012

Sanders, A. C., Hussain, A.J., Hen, R., & Zhuang, X. (2007). Chronic blockade or constitutive deletion of the serotonin transporter reduces operant responding for food reward. *Neuropsychopharmacology, 32,* 2321-2329. doi: 10.1038/sj.npp.1301368

Schneider, B. A. (1969). A two-state analysis of fixed-interval responding in the pigeon. *Journal of Experimental Analysis of Animal Behavior, 12,* 677-687

Soubrie, P. (1986) Reconciling the role of central serotonin neurons in human and animal behavior. *Behavioral and Brain Sciences. 9,* 319-335. doi: 10.1017/S0140525X00022871

Staddon, J.E.R., & Higga, J.J. (1999) . Time and memory: Towards a pacemaker-free theory of interval timing. *Journal of the experimental analysis of Behavior, 71,* 215-251

Swanton, D.N., & Matell, M. S. (2011). Stimulus compounding in interval timing: the modality-duration relationship of the anchor durations results in qualitatively different response patterns to the compound cue. *Journal of Experimental Psychology: Animal Behavior Processes, 74,* 168-175 doi: 10.1037/a0014021

Winstanley, C.A., Theobald, D.E., Dalley, J.W., Cardinal, R.N., & Robbins, T.W. (2004). 5-ht2a and 5-ht2c receptor antagonists have opposing effects on a measure of impulsivity: interactions with global 5-ht depletion. *Psychopharmacology, 176,* 376-385

Winstanley, C.A., Theobald, D. E.H., Dalley, J.W., & Robbins, T.W. (2005). Interactions between serotonin and dopamine in the control of impulsive choice in rats: therapeutic implications for impulse control disorders. *Neuropsychopharmacology, 30,* 669-682