UNIVERSITY OF CALIFORNIA SAN DIEGO

Towards the Development of a Rat Model of Human Targeted Cognitive Training

A thesis submitted in partial satisfaction of the requirements
for the degree Master of Science

in

Biology

by

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2018

The Thesis of Benjamin Zalman Roberts is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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University of California San Diego

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EPIGRAPH

I have not failed. I've just found 10,000 ways that won't work.

Thomas Edison

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LIST OF ABBREVIATIONS

ASDT: Auditory Sweep Discrimination Task

LE: Long Evans

PACT: Pharmacologically Augmented Cognitive Therapy

RSDT: Rat Sweep Discrimination Task

SCZ: Schizophrenia

TCT: Targeted Cognitive Training

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ABSTRACT OF THE THESIS

Towards the Development of a Rat Model of Human Targeted Cognitive Training

by

Benjamin Zalman Roberts

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Professor Jared W. Young, Chair
Professor Susan L. Ackerman, Co-Chair

Cognitive deficits are characteristic symptoms of schizophrenia, but remain largely untreated. Targeted Cognitive Training (TCT), a computerized training regimen designed to enhance spared function, is a putative new treatment for this symptom class. Mechanisms underlying the effects of TCT are unclear, however. This thesis describes three main studies (and one supplemental study) that aimed to develop and validate the Rat Sweep Discrimination Task

(RSDT), a putative model of the Auditory Sweep Discrimination Task featured in human TCT. Nine different permutations of the basic RSDT were developed, each of which were intended to build rats' association of auditory frequency "sweeps" with requisite response outputs. Rats were unable to reliably complete the RSDT without assistance from spatial cues. In order to probe the pharmacological predictive validity of the RSDT, we assessed the effects of d-amphetamine (d-AMPH, previously demonstrated to facilitate the effects of TCT in humans; 0.1, 0.25, 0.30 mg/kg, i.p.) on performance of the one version of the task in which rats were most competent (full spatial cues). No effect of d-AMPH was observed on any measure of the RSDT, which, taken together with rats' overall poor performance in the majority of the RSDT versions developed, indicates that the RSDT requires further refinement before it can be applied as a model of human TCT. Possible limitations of the studies are discussed, and it is concluded that sub-optimal auditory stimulus specifications were the most likely cause of rats' poor performance, and that small sample sizes likely prevented the detection of d-AMPH effects.

Introduction

Schizophrenia (SCZ) is a complex psychiatric disease characterized by a diverse range of symptoms. Frontline treatment strategies prioritize management of the more overt manifestations of psychosis (hallucinations, delusions, etc.) over the disease's comparatively subtle features, e.g. impaired cognition. The current mainstay of SCZ therapy, antipsychotic medication, fails to address the disease's cognitive symptom domain, and dedicated pro-cognitive pharmacological interventions have yet to be identified and/or validated (Young and Geyer, 2015). Given that degree of cognitive impairment is a strong predictor of functional outcome for SCZ patients (Green et al., 2004), the relative neglect of such a key aspect of the disorder represents a considerable shortcoming on the part of current treatment plans.

Unfortunately, as the etiological and mechanistic complexity of SCZ becomes increasingly apparent, the identification of a single elegant pharmacological solution seems less and less likely (Swerdlow, 2012). The most striking neural abnormalities observed in SCZ are morphological and/or developmental malformations that are not likely to be overcome through medication alone (Swerdlow, 2011). For this reason, emerging treatment strategies are beginning to look to patients' intact circuitry for therapeutic targets, rather than attempting to reverse years of compounded structural and functional aberrations (Swerdlow, 2011). One such approach is targeted cognitive training (TCT), which engages spared neural circuits to induce learning-dependent neuroplasticity in brain areas implicated in the low-level processing events necessary for higher-order cognitive operations (Fisher et al., 2016).

The Posit Science Corporation's approach to TCT uses various computerized exercises designed to enhance speed and efficiency of early sensory processing over thousands of trials (Fisher et al., 2016). Most clinical assessments of the Posit Science program thus far have focused on the program's auditory component, designed to enhance accuracy of auditory processing in the service of those higher-order cognitive processes reliant upon auditory acuity, e.g., verbal working memory and long-term verbal memory (Fisher et al., 2016). Indeed, the auditory TCT suite has repeatedly conferred gains in such domains as verbal learning and memory, auditory processing speed, and global cognition to both chronic (Adcock et al., 2009; Biagianti et al., 2016; Dale et al., 2016; Fisher et al., 2009; Murthy et al., 2012; Popov et al., 2011; Thomas et al., 2018) and recent onset (Fisher et al., 2015; Ramsay et al., 2018) SCZ patients, often accompanied by normalization of certain neurological aberrations (Adcock et al., 2009; Dale et al., 2016; Popov et al., 2011; Ramsay et al., 2018). Importantly, these benefits appear to be long-lasting, with cognitive effects persisting as long as six months following cessation of training (Adcock et al., 2009; Fisher et al., 2010).

Despite significant cognitive improvements observed at the group level however, there exists substantial variability in individual patient experience (Fisher et al., 2009; Fisher et al., 2010). After approximately 50 hours of training over 8-10 weeks, as many as 45% of patients who complete this TCT module experience no appreciable cognitive enhancement (Murthy et al., 2012). Similar variability is observed in follow-up measures of functional outcome; cognitive improvements derived from TCT completion do not always translate to improvements in daily living, and those functional gains that are seen are often modest (Vinogradov et al., 2012). Considering the sizeable investment of time and effort required to complete the training regimen,

this problem of reliability must be addressed before TCT can be incorporated into mainstream treatment strategies.

The rate of response to TCT and the significance of functional gains may both be improved via precise application of specific pharmacological agents. For example, administration of a single "test dose" of a drug known to engage putatively relevant circuitry has been proposed as a way to assay individual patients' available neuroplastic resources, and to thereby judge whether or not they would be sensitive to training (Swerdlow et al., 2018); in this way, clinicians could selectively prescribe TCT to those patients who are most likely to benefit from it, while identifying and screening out probable non-responders. Additionally, TCT outcome may be strengthened through administration of pro-cognitive medication in conjunction with the training, as per the "PACT model" (Pharmacologically Augmented Cognitive Therapy) (Swerdlow, 2012). This approach was recently substantiated by a "proof-of-concept" study that found that d-amphetamine can facilitate TCT-induced gains in auditory processing speed (Swerdlow et al., 2017). Both strategies can provide much-needed reliability to the TCT regimen, but are presently hindered by a lack of knowledge regarding the mechanisms mediating training-induced cognitive enhancement. The present study sought to develop a rat model of one of the key tasks of the auditory TCT regimen, which could then be used to elucidate underlying neural mechanisms in greater resolution than can be readily provided by human studies.

The Auditory Sweep Discrimination Task (ASDT) is one of the most prominent tasks within the larger Posit Science Auditory TCT suite, and was therefore chosen for adaptation for rats. In the human task, subjects are presented with two successive sound "sweeps" – auditory stimuli that vary in frequency within a single presentation. Sweeps proceed either from a low frequency to a high frequency (an "upsweep"), or vice-versa (a "downsweep"). Subjects are then

required to report what combination of sweeps was delivered (i.e. up/down, down/up, up/up or down/down). Since the ASDT is usually administered to patients alongside other proponents of the auditory TCT suite in clinical trials, it is difficult to delineate this particular task's contribution to the cognitive benefits conferred by TCT. However, in studies in which the ASDT alone was examined, 1 hour of ASDT (in conjunction with amphetamine) improved auditory processing speed in SCZ patients (Swerdlow et al., 2017), and ASDT completion was positively correlated with improvement in verbal working memory and global cognition in SCZ patients (Adcock et al., 2009). These observations enable the development of prediction-driven paradigms, which could then provide precise mechanistic explanations for these effects.

Herein, I report on attempts to model the ASDT in rats. Specifically, three consecutive studies are described in which we attempted to develop a Rat Sweep Discrimination Task (RSDT), so that future investigations of auditory TCT may have a valuable tool with which to conduct targeted research. A supplementary study was also conducted to determine whether d-amphetamine could enhance rats' ability to complete the RSDT with the aid of spatial cues. This latter study was performed for potential validation of the paradigm (Swerdlow et al., 2017).

Chapter 1: General Materials and Methods

1.1 Apparatus

Training was conducted in 9-choice operant chambers housed in ventilated sound-attenuating cabinets (CeNeS Pharmaceuticals Inc., Norwood, MA) (**Figure 1.1A**). Auditory stimuli were delivered via paired speakers (COBY® "Mini Stereo Speaker System") positioned outside of the training chambers. Exact speaker configuration varied according to study. Auditory stimuli were confirmed via audiometer to reach the inside of the chambers at a volume of 48-50 dB prior to the beginning of each study. Single holes were opened in the center of chamber ceilings in order to optimize stimulus audibility. Auditory stimuli were generated via custom programming. Auditory stimuli were delivered in conjunction with illumination of one or two apertures located along the rear wall of the testing chamber (number of illuminated apertures varied according to study and individual trial) (**Figure 1.1B**). Apertures were spaced approximately 3 inches laterally from each other. Responses to the auditory stimuli were made via nosepoke in these apertures, and were detected by infrared beams located near the entrances. Reinforcement was 40 μL of strawberry milkshake (strawberry Nesquik® dissolved in non-fat milk) delivered via peristaltic pump into an illuminated magazine located at the front of the training chamber. Reward collection was monitored by an infrared beam at the opening of the magazine. Each training chamber contained a single house light, centered on the ceiling. Non-auditory stimulus outputs (i.e. aperture/magazine illumination) and response inputs were managed by a SmartCtrl Package 8-In/16-Out with additional interfacing by MED-PC for Windows (Med Associates Inc., St. Albans, VT) using custom programming. The same apparatus was used in in all three studies.

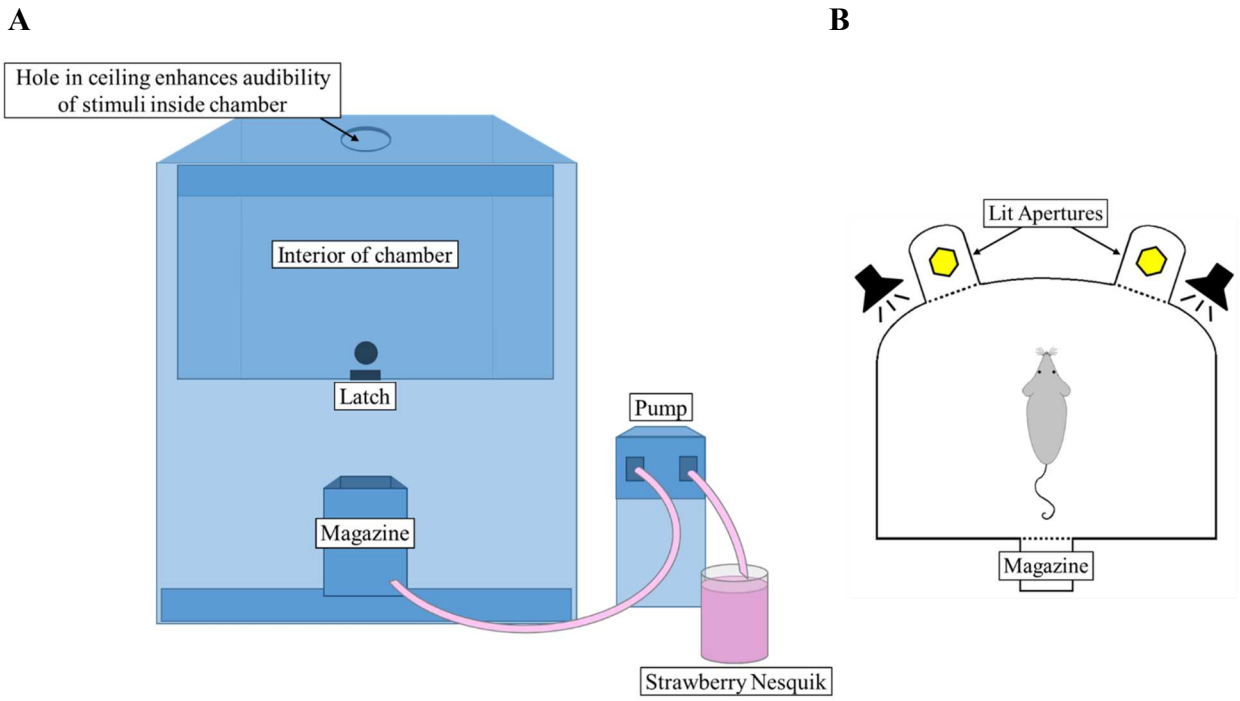


Figure 1.1. Basic Apparatus/Task Schematic. Illustration of operant chambers (exterior view) (**A**). Each chamber was housed in a sound-attenuating cabinet (not pictured). Over the course of each study, paired speakers (see Figure 2) were placed outside of the chamber, and positioned relative to each other such that full, partial, or no spatial separation was present between auditory stimuli. General task schematic (as seen from interior of chamber) (**B**). Each permutation of the RSDT followed the same basic design, in which an auditory stimulus would play through one or both speakers in conjunction with illumination of one or two lights, depending on task version. Rats would then have to respond to the auditory stimulus via nosepoke in the one lit aperture corresponding to that particular stimulus (auditory frequency upsweep vs downsweep). Speakers are pictured here fully separated, though speaker arrangement varied across studies and RSDT versions (see Figure 2). RSDT: Rat Sweep Discrimination Task.

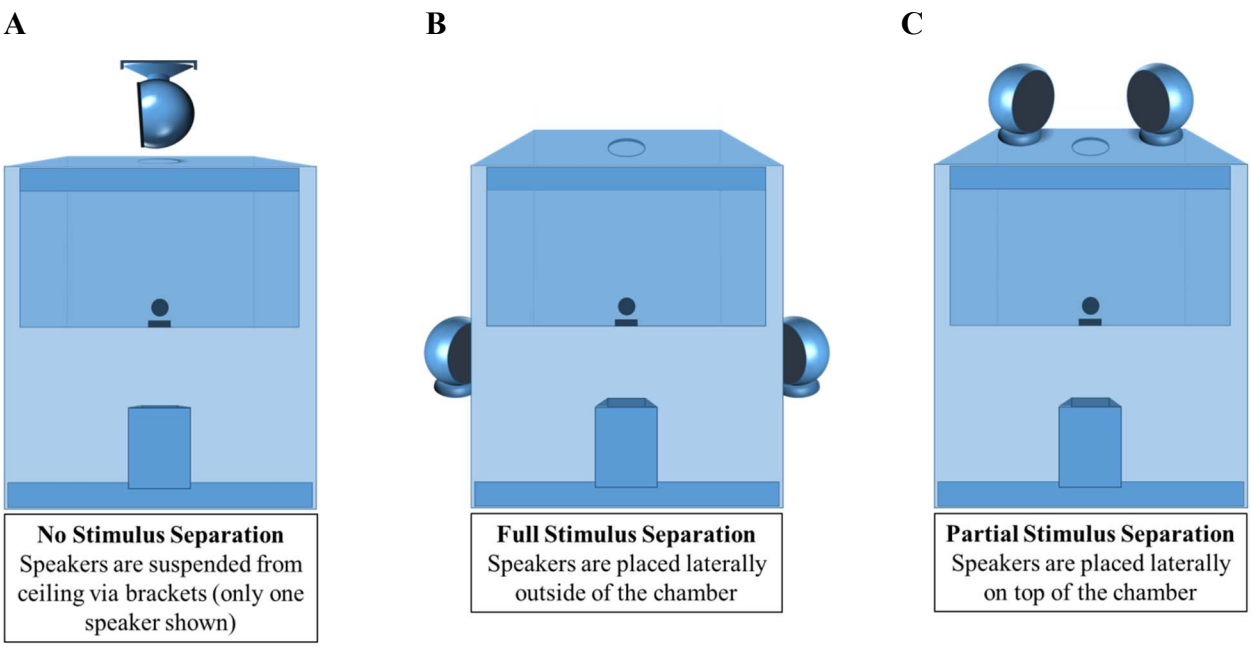


Figure 1.2. Spatial Configurations of Speakers. No stimulus separation (**A**). Paired speakers were hung directly above the chambers. Each auditory stimulus played out of both speakers simultaneously. This configuration was used in **Study 1** only. Full stimulus separation (**B**). Paired speakers were positioned laterally outside of the chamber, at approximately the height of the response apertures. The RSDT program was modified so that each auditory stimulus would play out of only the one speaker on the same side as the "correct" aperture corresponding to that stimulus. During study phases in which stimulus localization was removed, the speakers remained in the same spatial configuration, but the RSDT program was again modified so that each stimulus would play out of both speakers simultaneously. This configuration was used in **Study 2** and the end of **Study 3**. Partial stimulus separation (**C**). Speakers were placed laterally on top of the chamber, and positioned more closely to each other than in the "full separation" configuration. Each auditory stimulus would play only out of the one speaker located on the side of the "correct" aperture. This configuration was used in **Study 2** and **Study 3**.

1.2 The Rat Sweep Discrimination Task (RSDT)

Attempts to develop the Rat Sweep Discrimination Task (RSDT) spanned across three studies. Within each study, the RSDT program was modified in response to rat performance in order to facilitate task acquisition. Modifications were made to such aspects of the task as: magnitude of punishment (length of timeout period following incorrect responses), frequency and duration of auditory stimuli, presence and degree of spatial separation of auditory stimuli, individual trial characteristics, and basic training paradigms. Each of these changes is outlined in detail in below subsections.

This subsection describes the basic specifications shared by each version of the Rat Sweep Discrimination Task (RSDT) used. Each RSDT program followed a basic auditory discrimination design in which rats would be provided with a single auditory stimulus in conjunction with one or two lit apertures. Rats would then have to respond by nosepoking in the one aperture that corresponded to that particular stimulus (**Figure 1.1B**). Each version of the RSDT was designed such that the correct response to a given auditory stimulus would be in the left aperture in half of the chambers, and in the right aperture for the other half. Auditory stimuli were delivered through speakers placed outside of the training chambers, in one of the three configurations depicted in **Figure 1.2**. Prior to the beginning of each study, an audiometer was used to verify that auditory stimuli reached the inside of the chambers at 48-50 dB – well within rat auditory range (Harrison and Turnock, 1975; Kelly and Masterton, 1977).

Correct responses (defined by nosepokes in the lit aperture corresponding to the presented auditory stimulus) were rewarded by 40 μL strawberry Nesquik delivered into the magazine. In **standard trials** (**Table 2.2**), incorrect responses were punished by a timeout period, during which the house light would turn on, and no stimuli would be delivered. Duration of timeout period

varied between RSDT versions. Premature responses – responses before stimulus presentation – were punished with a timeout period and recorded in the output file for that training session. Omissions – trials in which no response is made within 10 seconds of stimulus presentation – were punished and recorded in the same fashion. Premature responses (calculated as a percentage of total responses in a session) provided a measure of motoric impulsivity (Cope et al., 2016), and % omissions provided a measure of task-related vigilance (Young et al., 2013). Training sessions lasted for 30 minutes, or until 120 trials had been completed. Session duration was constant across task versions, except where noted.

Chapter 2 Study 1

2.1. Study 1 Methods

2.1.2. Animals

Study 1 utilized 6 male and 6 female Long Evans rats (200-410 g at training, average 330 g; Envigo, San Diego). Rats were mainly housed in dyads in clear plastic cages, although fighting between cage mates often necessitated separation. Those rats that had to be housed individually were kept in clear plastic chambers identical to those that housed pairs, and were provided with environmental enrichment in addition to regular bedding. Rats were transported to and from the training room individually in smaller transport cages. All rats were kept in a climate controlled room on a 12h light/dark schedule (lights on at 7:00 PM) when not training. Training began at 10 weeks of age. Rats were food restricted to 85% of their free feeding body weight, with daily feed allotment calculated to maintain normal growth. Water was provided ad libitum in rats' home cages, but was not available in transport cages or in training chambers. Training was conducted during the dark period of rats' light/dark cycle. All rats were maintained in a dedicated animal facility approved by the American Association for Accreditation of Laboratory Animal Care; this facility met all federal and state requirements for animal care. All procedures were approved by the University of California, San Diego (UCSD) Animal Care and Use Committee.

2.1.3 Preliminary Training

Rats were initially trained in the basic behaviors necessary for operant task performance. Rats first spent 2-3 days learning to associate illumination of the magazine with food reward via the HAB1 program, in which reward delivery was paired with magazine illumination on a 15 s

fixed interval schedule. Rats were then moved to HAB2, a basic FR1 training module which rewarded single nosepokes to either of two illuminated apertures. Responses were recorded for each subject, and once all rats were performing reliably (>70 responses for at least two consecutive days), they began training in the **RSDT1** (**Table 2.1**).

2.2. Study 1 Progression

Figure 2.1. Study 1 Progression. Rats were run for 40 days, including days spent training in the basic HAB1 and HAB2 paradigms. Rats were trained using 4 different permutations of the original RSDT1 program (RSDT1-RSDT4). New RSDT programs were created in response to rat performance, and were designed to facilitate rats' acquisition of sweep discrimination based on the results from previous programs. RSDT: Rat Sweep Discrimination Task

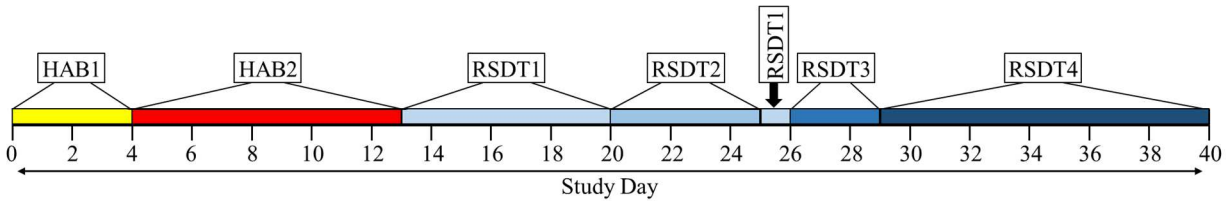


Table 2.1. RSDT Program Specifications, Study 1.

Program	Trial Types Used	Timeout Duration	Study Days
RSDT1	120 Standard Trials	4 seconds	D14-D20; D26
RSDT2	120 Forced Choice Trials	4 seconds	D21-D25
RSDT3	80 Standard, 20 Guided, 20 FCα Trials	4 seconds	D27-D29
RSDT4	80 Standard, 20 Guided, 20 FCα Trials	7 seconds	D30-D40

RSDT: Rat Sweep Discrimination Task; FCα= Alternative Forced Choice, see Table 2.

Table 2.2. Trial Type Definitions.

Trial Type	Definition
Standard	Both apertures lit Incorrect responses end the trial and elicit a timeout period
Forced Choice	Both apertures lit Incorrect responses do not end the trial Trials end only when the rat selects the correct aperture for the given auditory stimulus, or after 10 seconds of inactivity
Alternative Forced Choice (FCα)	Only the "correct" aperture lit Incorrect responses do not end the trial Trials end only when the rat selects the correct aperture for the given auditory stimulus, or after 10 seconds of inactivity

Table 2.2. continued

Guided	Only the "correct" aperture lit Incorrect responses end the trial and elicit a timeout period

Italics: Trial type used in Study 1 only

All Study 1 RSDT programs utilized only one type of auditory stimulus – the frequency "sweep". Sweeps are auditory stimuli of directionally modulated frequency. Sweeps could progress either from a low frequency to a high frequency – an "upsweep" – or from a high frequency to a low frequency – a "downsweep." The high and low frequencies used in all Study 1 RSDT versions were 7- and 4 kHz, respectively. These particular frequencies were chosen based on ¼ log scale spacing, and because both frequencies lie comfortably within human and rat audibility ranges (Kelly and Masterton, 1977). A sweep duration of 1000 ms was used for this study, the same duration initially used in the human Auditory Sweep Discrimination Task (Fisher et al., 2009; Swerdlow et al., 2017).

Visual representation of the progression of Study 1 is provided in **Figure 2.1**. The **RSDT1** operated according to the basic RSDT design described above (i.e. response in the one lit aperture corresponding to the presented sweep = correct), with a timeout duration of 4 seconds following incorrect responses (**Table 2.1**). The RSDT1 utilized exclusively **standard trials** (**Table 2.2**), in which each auditory stimulus would be accompanied by two lit apertures, and incorrect responses would be punished with a timeout before the beginning of the next trial. Accuracy was calculated via the formula:

$$\frac{\#\ correct\ responses}{\#\ correct + \#\ incorrect\ responses} \times 100\%$$

Premature responses and omissions were not included in the incorrect response tally for accuracy calculation in this, or any subsequent, RSDT version. However, premature responses and omissions were both monitored, and calculated as percentages of total responses. Rats were trained in the RSDT1 for 7 days, at which point the program underwent its first modification. The **RSDT2** (**Table 2.1**) was identical to the RSDT1 except that it utilized exclusively **forced choice trials** (**Table 2.2**), which were hypothesized to more effectively build the association between a given stimulus and its corresponding aperture. Incorrect responses *could not end* these trials; forced choice trials would only end following a nosepoke in the correct aperture, or following 10 seconds of inactivity (i.e. an omission). Since incorrect responses no longer ended trials, the accuracy formula had to be modified slightly so that only those trials that ended after a single response were counted as correct (i.e. only those trials in which the rat responded correctly on its first attempt):

$$\frac{\#\text{ "}\textbf{\textit{first try}}\text{" correct responses}}{\#\text{ "first try" correct} + \#\text{ "second, third, fourth, etc. try" correct responses}} \times 100\%$$

Rats trained in the RSDT2 for 5 days. Following this training, rats were assessed in a single day of **RSDT1** to determine whether RSDT2 training had had any effect on performance of the original task.

After analyzing rats' accuracy in the RSDT1, the **RSDT3** was developed and implemented (**Table 2.1**). The RSDT3 utilized three different trial types, each of which were designed to facilitate the association between stimuli and their requisite responses: standard trials (as in the RSDT1), **guided trials** (**Table 2.2**), and an alternate form of forced choice trials (**FCα trials**; **Table 2.2**). In guided trials, only the "correct" aperture would illuminate in conjunction with delivery of the auditory sweep; as in standard trials, incorrect responses would end the trial. In

FCα trials, only the correct aperture would illuminate (as in guided trials), but incorrect responses would not end the trial (as in regular forced-choice trials). As in the two preceding programs, each RSDT3 session consisted of 120 trials, but individual trial types were dispersed pseudorandomly across the session such that, of these 120 trials, 80 would be standard trials, 20 would be FCα trials, and 20 would be guided trials. Only the standard trial accuracy was used to gauge task performance/competence (though guided and FCα performance was also monitored in order to verify that the rats were behaving normally, e.g., low rates of non-specific response, normal attention and general task engagement):

$$\frac{\#\ \boldsymbol{standard\ trial}\ correct\ responses}{\#\ standard\ trial\ correct + \#\ standard\ trial\ incorrect\ responses} \times 100\%$$

Rats were trained in the RSDT3 for only 3 days before being moved to the **RSDT4** (**Table 2.1**), a program identical to the RSDT3 except with a more salient punishment – a timeout period of 7 seconds instead of 4. RSDT4 performance was assessed using the same formula as the RSDT3. Rats were run in the RSDT4 for 11 days. At this point, data gathered across the combined 14 days of the RSDT3 and RSDT4 were analyzed to determine whether the rats had developed side biases (i.e. whether the rats had shifted their behavior from trying to respond appropriately to the sweeps to simply choosing one aperture and preferentially nosepoking there, regardless of sweep directionality). Only data from standard trials were analyzed in this fashion, since these were the only trials in which rats were able to freely choose between two lit apertures. The calculation was made using the following formula, where the "preferred" aperture is the aperture in which a given rat responded more often:

$$\frac{\#\ responses\ in\ "preferred"\ aperture}{\#\ responses\ in\ other\ aperture}$$

A resultant value of 1 indicated that a given rat displayed no side bias whatsoever (i.e. the rat responded in both apertures exactly the same number of times), whereas larger values represented a corresponding fold preference for one aperture over the other (i.e. a value of 2 indicates that a given rat responded in one aperture twice as often as the other). To allow for natural variation of response, preference values of 1.5 and below were determined to be "normal," whereas larger values were interpreted as genuine side bias. Development of a side bias would imply that this particular cohort of rats is no longer actively trying to learn the rules of the task, and the rats have instead chosen to settle for the ~50% reward rate yielded by an arbitrary aperture. Acquisition of a given operant task relies heavily upon rats being motivated to maximize their reward rates by learning that task's reward contingencies; it was deemed highly unlikely that rats that had learned to respond arbitrarily regardless of situation would shift their behavior towards a more active strategy through subsequent training (however, this assertion was modified in subsequent studies). Therefore, the results of this analysis were considered to be predictive of whether the rats would be receptive to further training, and consequently decided whether the study would proceed. Given that there were only 6 rats per group to begin with, analyzing individual animals' preference values and deciding on a case-by-case basis whether individual rats would stay in the study or be dropped would have resulted in unfeasibly small sample sizes for subsequent phases of the study. Therefore, the analysis was performed using group averages, with the intention of ending the study altogether should the rats display side biases at a group level.

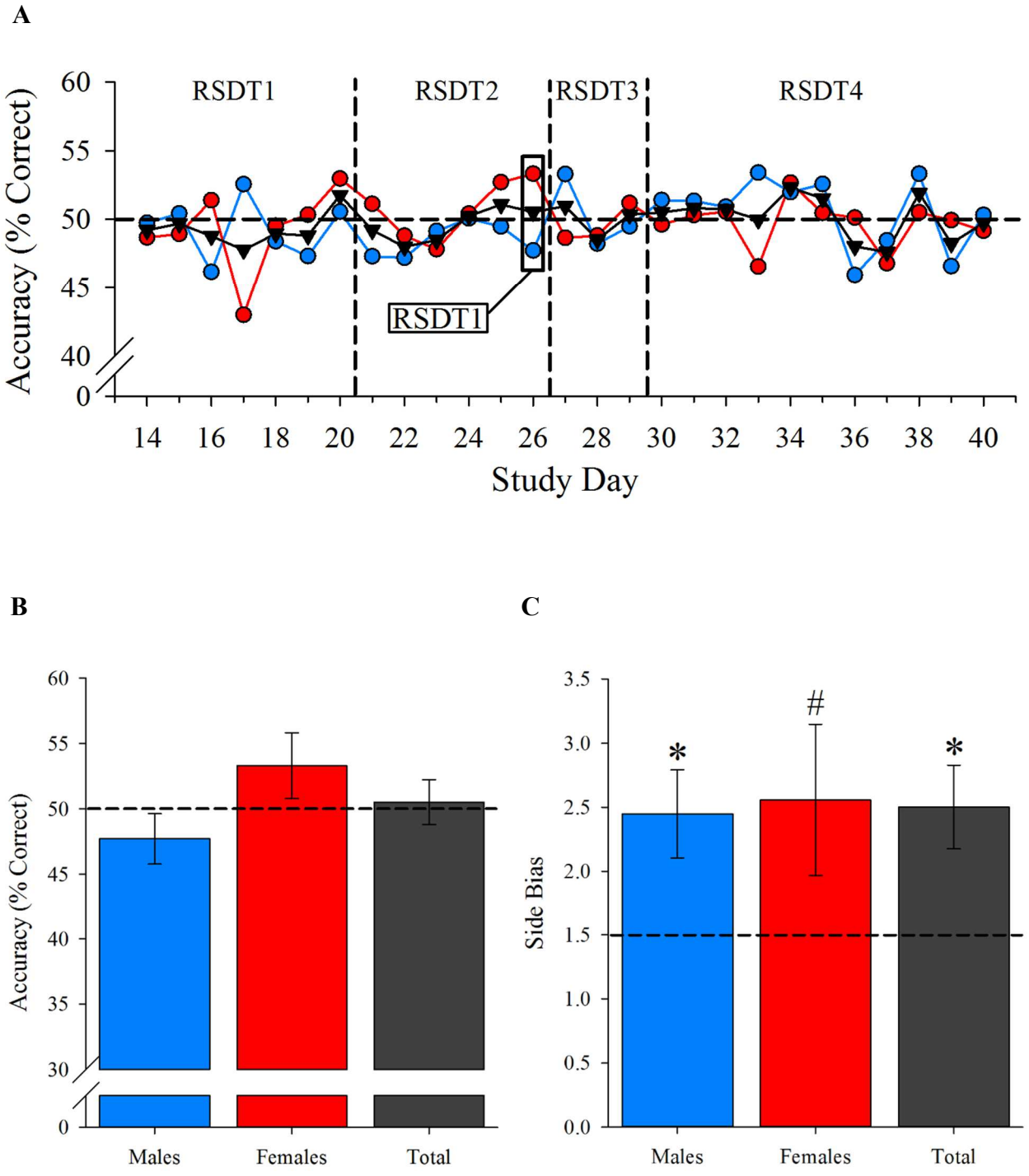
2.3. Study 1 Statistical Analyses

Primary outcome variables (% accuracy, % premature responses, and % omissions) were analyzed via two-way analysis of variance (ANOVA), one-sample T-Tests, and independent

samples T-Tests. Two-way ANOVA was used to determine differences in task accuracy across RSDT versions, with version as the within-subjects factor and sex as the between-subjects factor. Within each task version, independent samples T-Tests were used to determine whether sex exerted any effect on task accuracy. Two-tailed one-sample T-tests were then used to determine whether average rat performance was significantly different from chance (test value=50). Independent samples T-Tests were used to ascertain whether male and female rats differed in % premature responses or % omissions across the entire study. Side bias across the combined 14 days of the RSDT3 and RST4 was assessed via one-sample T-Tests for males and females separately and combined. Test value was set to 1.5 for this analysis, as 1.5 had been deemed to be a reasonable cutoff value to allow for natural variation of response. Since the study was set to end should rats' preference scores be significantly higher than 1.5, a one-tailed T-Test was used for this analysis. All data were analyzed using SPSS 24.0 (Chicago, IL) and were represented by mean and standard error of the mean. Alpha level was set to 0.05.

2.4. Study 1 Results

Graph 2.1. Study 1 outcomes. Male (blue) and female (red) rats did not significantly differ from each other in completion of any RSDT program, nor did they differ across the entire study (data from all programs analyzed together). Male, female, and total (male + female; black) performance was no different from chance during any phase of the study. Accuracy for each RSDT program was calculated according to the formulae described in the Study 1 Methods section. Chance performance is represented by a horizontal dotted line at 50% accuracy. Vertical dotted lines denote transitions between RSDT program versions (see Figure 3). RSDT program versions are indicated across the top of the graph. Boxed data points denote assessment of rats in RSDT1. Blue = male rats, Red = female rats, Black = male + female rats. Data presented as means. RSDT = Rat Sweep Discrimination Task **(A)**. **RSDT1 Re-Assessment (D26).** After 5 days of RSDT2, rats were assessed in a single session of the RSDT1. Males did not differ from females in accuracy during this assessment. Performance was no different from chance for male, female, or male + female groups. Chance performance is represented by a horizontal dotted line at 50% accuracy. Data presented as mean ±S.E.M **(B)**. **Side Bias Analysis.** Side bias over the combined 14 days of RSDT3 and RSDT4 training was calculated and assessed under the assumption that values between 1 and 1.5 denoted normal variation of response. Males and females were no different from each other in terms of side bias. Males, as well as males and females combined, displayed average preference values significantly outside of this acceptable range. Females tended to display preference values outside of the acceptable range. The upper "acceptable" limit for preference values (side bias) is denoted by a horizontal dotted line at 1.5. *=$p<0.05$ vs 1.5. #=$p<0.10$ vs 1.5. Data presented as mean preference values ±S.E.M. **(C)**.


A
RSDT1
RSDT2
RSDT3
RSDT4
RSDT1
Accuracy (% Correct)
Study Day
B
Accuracy (% Correct)
Males
Females
Total
C
Side Bias
Males
Females
Total

2.4.1. RSDT1-RSDT4 Performance

A two-way ANOVA revealed no significant effect of RSDT version [$F_{(3,30)}$=0.27, n.s.] or sex [$F_{(1,10)}$= 0.002, n.s.] on task accuracy, nor was there observed any task version/sex interaction [$F_{(3,30)}$=0.53, n.s.]. Independent samples T-Tests revealed no difference in male vs. female accuracy either across the entire study [t(52)=0.062, n.s.], or within any RSDT version [**RSDT1**: t(12)= -0.034, n.s.; **RSDT2**: t(8)=1.48, n.s.; **RSDT3**: t(4)= -0.46, n.s.; **RSDT4**: t(20)= -0.94, n.s.] (**Graph 2.1A**). One-sample T-Tests revealed that no group (males, females, or males and females combined) performed above chance level (50% accuracy) in any version of the RSDT program [**RSDT1**: t(6)=0.43, n.s. (males), t(6)=0.55, n.s. (females), t(13)=0.32, n.s. (males+females); **RSDT2**: t(4)=-2.33, n.s. (males), t(4)=0.20 (females), t(9)=-1.08 (males+females); **RSDT3**: t(2)=0.22, n.s. (males), t(2)= -0.55, n.s. (females), t(5)=-0.071, n.s. (males+females); **RSDT4**: t(10)=0.73, n.s. (males), t(10)= -0.59, n.s. (females), t(21)=0.28, n.s. (males+females)] (**Graph 2.1A**). The percentage of total trials ending in premature responses was analyzed over the entire study via independent samples T-Test, and no significant difference between males (16.57% ±3.00%; Mean ±S.E.M.) or females (16.46% ±2.01%) was found [t(10)= -0.031, n.s.]. A similar analysis was performed on % omissions, and again, there was no significant difference between males (2.98% ±3.04%; Mean ±S.E.M.) and females (1.71% ±0.47%) [t(10)=-0.96, n.s.]. When rats were assessed in the RSDT1 following 5 days of RSDT2, no difference was observed between male and female accuracy [t(10)=0.46, n.s.], and no group (males, females, or males and females combined) performed above chance levels [t(5)=-1.12, n.s. (males), t(5)=1.32, n.s. (females), t(11)=0.30, n.s. (males+females) (**Graph 2.1B**).

2.4.2. Study 1 Side Bias

Average side bias across the last 14 days of the study was analyzed in males, females, and males and females combined via one-tailed one-sample T-Test against a test value of 1.5. The male and male + female groups both displayed side preference values significantly higher than 1.5 [$t(5)=2.76$, $p<0.05$ (males), $t(11)=3.08$, $p<0.05$ (males+females)], and a similar, albeit non-significant, trend was observed in the female group [$t(5)=1.79$, $p=0.067$] (**Graph 2.1C**). An independent samples T-Test revealed no significant difference between preference values for males vs. females, however [$t(10)=0.16$, n.s.]. Based on the results of this side bias analysis, the decision was made to end the study.

Chapter 3: Study 2

3.1. Study 2 Methods

3.1.2. Animals

Given the absence of sex effects on task accuracy and measures of motoric impulsivity and task-specific vigilance (% premature responses and % omissions, respectively) observed in Study 1, it was concluded that there existed no appreciable difference between males and females in terms of RSDT performance or task-relevant behavior. Therefore, in the interest of limiting factors and thereby increasing sample sizes, only male rats were utilized in Study 2 (we intended to re-introduce the variable of sex in future studies). The study employed 15 male Long Evans rats (250-420 g at training, average 335 g; Envigo, San Diego), none of which had been used in Study 1. Rats were housed and transported in exactly the same manner as those utilized in Study 1, and were kept in the same AAALAC-approved facility. As in Study 1, rats were maintained at ~85% free-feeding body weight, and were provided with water *ad libitum*. Training began at 10 weeks of age, and rats were always trained during the dark period of their light/dark cycles. All procedures were approved by the University of California, San Diego (UCSD) Animal Care and Use Committee.

3.1.3. Preliminary Training

As in Study 1, rats were initially trained in the basic HAB1 and HAB2 programs. Rats spent 2 days in HAB1 learning to associate the magazine light with food delivery before being moved to HAB2, the basic FR1 training module. Once all rats were performing reliably in the

HAB2, they began training in the **RSDT5** (**Table 3.1**). All training took place in the same apparatus used in Study 1.

3.2. Study 2 Progression

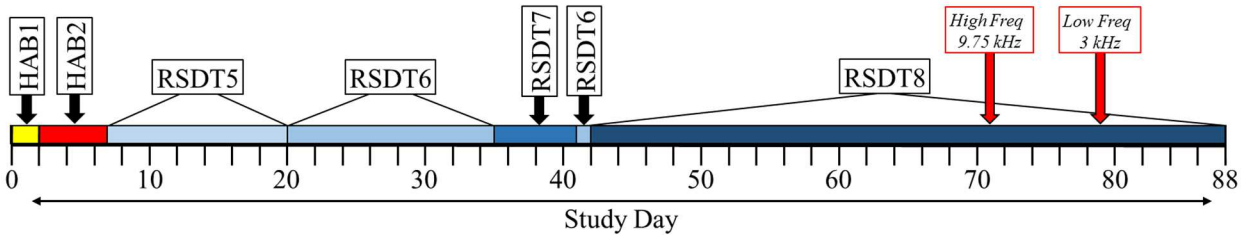


Figure 3.1. Study 2 Progression. Rats were trained for 88 days, including days spent training in the basic HAB1 and HAB2 paradigms. Rats were trained using 4 different permutations of the original RSDT1 program (RSDT5-RSDT8). New RSDT programs were created in response to rat performance, and were designed to facilitate rats' acquisition of sweep/tone discrimination based on the results from previous programs. Red arrows/boxes indicate changes of the high or low frequencies used in the RSDT. RSDT: Rat Sweep Discrimination Task.

Table 3.1. RSDT Program Specifications, Study 2.

Program	Trial Types Used	Speaker Separation	Study Days
RSDT5	40 Standard, 40 Forced, 40 Guided Trials	Full Separation	D8-D20
RSDT6	70 Standard, 40 Forced, 10 Guided Trials	Full Separation	D21-D35, D42
RSDT7	70 Standard, 40 Forced, 10 Guided Trials	No Separation	D36-D41
RSDT8	70 Standard, 40 Forced, 10 Guided Trials	Partial Separation	D43-D88

RSDT: Rat Sweep Discrimination Task
Note: See Figure 2 and Table 2, respectively, for speaker separation schematics and trial type definitions.

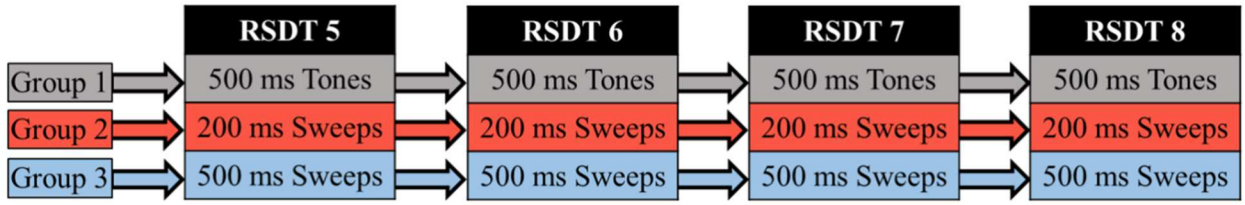


Figure 3.2. Visual Explanation of Study 2 Groups/RSDT Sub-Versions. Each RSDT version (RSDT5-8) was itself differentiated into 3 sub-versions, each of which utilized different auditory stimulus specifications (200 ms sweeps, 500 ms sweeps, or 500 ms pure tones). Prior to RSDT training, rats were assigned to one of three groups which determined the sub-version of the RSDT they would train in. Rats remained in these groups for the duration of the study, and the auditory stimulus specifications corresponding to each group remained constant across all RSDT versions. Arrows represent rats' transitioning from one RSDT program to the next. Colors indicate individual groups of rats, membership of which remained constant throughout the study. RSDT: Rat Sweep Discrimination Task.

In light of the Study 1 rats' inability to learn sweep discrimination, a primary objective of Study 2 was to identify auditory stimulus specifications most conducive to discrimination by rats. To this end, all versions of the RSDT program utilized by Study 2 were themselves differentiated into three sub-versions which differed in regards to the type and duration of presented auditory stimuli **(Figure 3.2)**. Each of the 15 rats trained in Study 2 was placed into one of three groups and then trained in the RSDT sub-version determined by its group assignment. Group assignment was counterbalanced based on time taken to achieve proficiency in HAB2. Two of the three sub-versions of the Study 2 RSDT programs utilized upsweeps and downsweeps, as in Study 1. These two sub-versions differed only in stimulus duration, with one employing 200 ms sweeps and the other employing 500 ms sweeps. The third sub-version utilized 500 ms pure tones, the frequencies of which remained constant across their presentation. Each RSDT program used in Study 2 was differentiated into the same three sub-versions, and each group of rats trained in the same sub-version across programs **(Figure 3.2)**. Study 2 used stimulus durations shorter than the 1-second duration used across Study 1 RSDT programs as it was hypothesized that the briefer stimuli would more closely resemble natural rat vocalizations, and would therefore be of greater salience to the rats. High and low frequencies were initially the same as those used in Study 1 (4- and 7 kHz), though were changed to 3- and 9.75 kHz toward the end of the study to enhance discriminability **(Figure 3.1)**.

Visual representation of the progression of Study 2 is provided in **Figure 3.1**. After demonstrating proficiency in HAB2, rats began training in the **RSDT5** (**Table 3.1**). A key difference between the RSDT5 and the RSDT programs utilized by Study 1 was the placement of the speakers; whereas the paired speakers were hung from brackets directly above the training chambers in RSDT1-4, the RSDT5 required that the speakers be placed laterally outside of the

chambers, at approximately the same level as the response apertures (**Fig. 1.2B**). At the beginning of each trial, the auditory stimulus would only play out of the one speaker on the same side as that particular stimulus' corresponding "correct" aperture. This configuration provided spatial cues which, along with the forced choice and guided trials, were intended to help rats associate auditory stimuli with their corresponding apertures. Each 30 minute session of the RSDT5 was composed of 120 trials, dispersed equally among standard, forced choice, and guided trial types (**Table 2.2**). Timeout periods for the RSDT5, and all subsequent Study 2 RSDT programs, were 4 seconds in duration. Accuracy was determined separately for standard and forced choice (FC) trials, then averaged together to yield total task accuracy:

$$Standard\ Trial\ Accuracy = \frac{\#\ \boldsymbol{standard\ trial}\ correct\ responses}{\#\ standard\ trial\ correct + incorrect\ responses} \times 100\%$$

$$FC\ Accuracy = \frac{\#\ "\boldsymbol{first\ try}"\ correct\ responses}{\#\ "first\ try"\ correct + \#\ "second,\ third,\ etc.\ try"\ correct\ responses} \times 100\%$$

$$Total\ Task\ Accuracy = \frac{Standard\ Accuracy + FC\ Accuracy}{2}$$

As in Study 1 calculations, guided trial responses were not included in accuracy calculations. Percent premature responses and percent omissions were calculated across all trial types, and premature responses and omissions were excluded from accuracy calculations. Rats were trained in the RSDT5 for 13 days before being moved to the **RSDT6** (**Table 3.1**).

The RSDT6 differed from the RSDT5 only in trial type distribution – out of 120 trials, 70 were standard, 40 were forced choice, and 10 were guided. This modification was intended as an intermediate step in the gradual removal of in-session assistance, with the ultimate goal being the removal of forced choice and guided trials altogether (a plan which was never enacted during

training phases). The task accuracy calculation had to be adjusted to take the new trial type ratios into consideration:

$$Total\ Task\ Accuracy = \left(\left(\frac{Standard\ Accuracy}{100} \times \frac{70}{110}\right) + \left(\frac{FC\ Accuracy}{100} \times \frac{40}{110}\right)\right) \times 100\%$$

The rats trained in the RSDT6 for 15 days, by which point performance had plateaued. In order to determine whether training with spatial cues had had the desired effect of building the association between auditory stimulus and correct aperture, the rats were assessed in the **RSDT7** (**Table 3.1**), a program identical to the RSDT6 except with no auditory stimulus separation (i.e. auditory discrimination with no spatial cues, but with the same trial type distribution). The speakers remained in the same configuration as in the RSDT6 (**Figure 1.2B**), with auditory stimuli played through both speakers in unison; this strategy allowed for the removal of spatial cues without a sudden commensurate change in stimulus height of origin. The rats were run in the RSDT7 for 6 days. This period was immediately followed by a single assessment in the **RSDT6**, which was intended to determine whether training in the RSDT7 had affected rats' ability to utilize spatial cues.

Rats were then run in the **RSDT8** (**Table 3.1**), a permutation of the RSDT6 that provided partial spatial cues via an intermediate speaker separation (**Figure 1.2C**). Rats trained in the RSDT8 for a total of 47 days. During this final phase of Study 2, the high and low frequencies used for auditory stimuli were changed in order to broaden the frequency ranges of the sweeps and thereby enhance discriminability. On the 30th day of the RSDT8 (D60 overall, **Figure 3.1**), the high frequency was changed from 7 kHz to 9.75 kHz – a frequency closer in pitch to natural murine vocalization (Portfors, 2007), and the upper limit of the speakers. On the 38th day of the RSDT8 (D68, **Figure 3.1**), the low frequency was decreased from 4 kHz to 3 kHz; this decision was made

in light of a recent report that Long Evans rats are able to differentiate between a 3 kHz tone and white noise (Floresco et al., 2018). This 3-9.75 kHz range was used for the remainder of the study.

Side bias was monitored across all phases of Study 2. Preference values were calculated following the same formula as in Study 1, but were analyzed and interpreted in a different manner. In Study 1, preference values were calculated only following several weeks of suspiciously poor (chance) performance (**Figure 2.1A**), at which point values from the last 14 days of the study were analyzed at once (see Study 1 Methods). Preference values were handled differently in Study 2; now cognizant of the possibility of the development of side bias, we integrated preference value calculation into the daily data analysis procedure and carefully monitored side bias across days alongside the tasks' primary outcome variables. This day-by-day tracking of side bias resulted in a far more lenient interpretation of preference values than did the first study's *post hoc* analysis of several weeks' data at once. Daily monitoring revealed that certain rats would go through phases of side bias lasting several days before modifying their behavior and returning to acceptable preference value ranges (see Results, **Figure 3.3**). The emergence of this pattern led us to reevaluate our previous assumption that rats would not instantaneously change from passive to active response strategies (see Study 1 Methods). We therefore adopted a "wait-and-see" approach in regards to individual rats, giving them time to modify their behavior before making major decisions about whether or not to keep that rat in the study, or whether the study would proceed. Given that rats would fairly regularly log values above 1.5 before quickly returning back to acceptable response patterns (see Results, **Figure 3.3**), we also increased our "acceptable" side preference cutoff from 1.5 to 2.0 to allow for greater variation of response. Ultimately, the decision was made that only the emergence of consistent bias (>10 consecutive days) in more than 50% of rats across all groups would be sufficient to end the study on the basis of side bias.

3.3. Study 2 Statistical Analyses

Primary outcome variables (% accuracy, % premature responses, and % omissions) were analyzed via one- and two-way analyses of variance (ANOVA) and one-sample T-Tests. Early in the study, a two-way ANOVA was conducted on RSDT acquisition across all 13 days of RSDT5 plus the first 4 days of RSDT6, using training day as a within-subjects factor and group (i.e. stimulus type) as a between-subjects factor. Before moving rats from the RSDT6 to the RSDT7, stability of responding in the RSDT6 was verified via a two-way ANOVA of task accuracy across the last three days of the RSDT6; training day was used as a within-subjects factor and group as a between-subjects factor. At the end of the study, two-way ANOVA was used to determine differences in task accuracy across the three different speaker configurations (full, partial, or no separation), using speaker spacing as a within-subjects factor and group as a between-subjects factor. RSDT5 data were excluded from this analysis for two reasons. First, given that the RSDT5 was essentially a primer for the RSDT6, used to ease the rats into auditory discrimination behavior, RSDT5 data would not have been representative of rats' performance using full spatial cues. Second, the RSDT5 utilized a different trial type distribution than the other Study 2 RSDT versions, and would have therefore introduced a confound into the analysis. The decision to exclude RSDT5 data from speaker configuration analysis was validated by another two-way ANOVA of accuracy using task version (RSDT5 vs RSDT6) as the within-subjects factor, and group as the between-subjects factor.

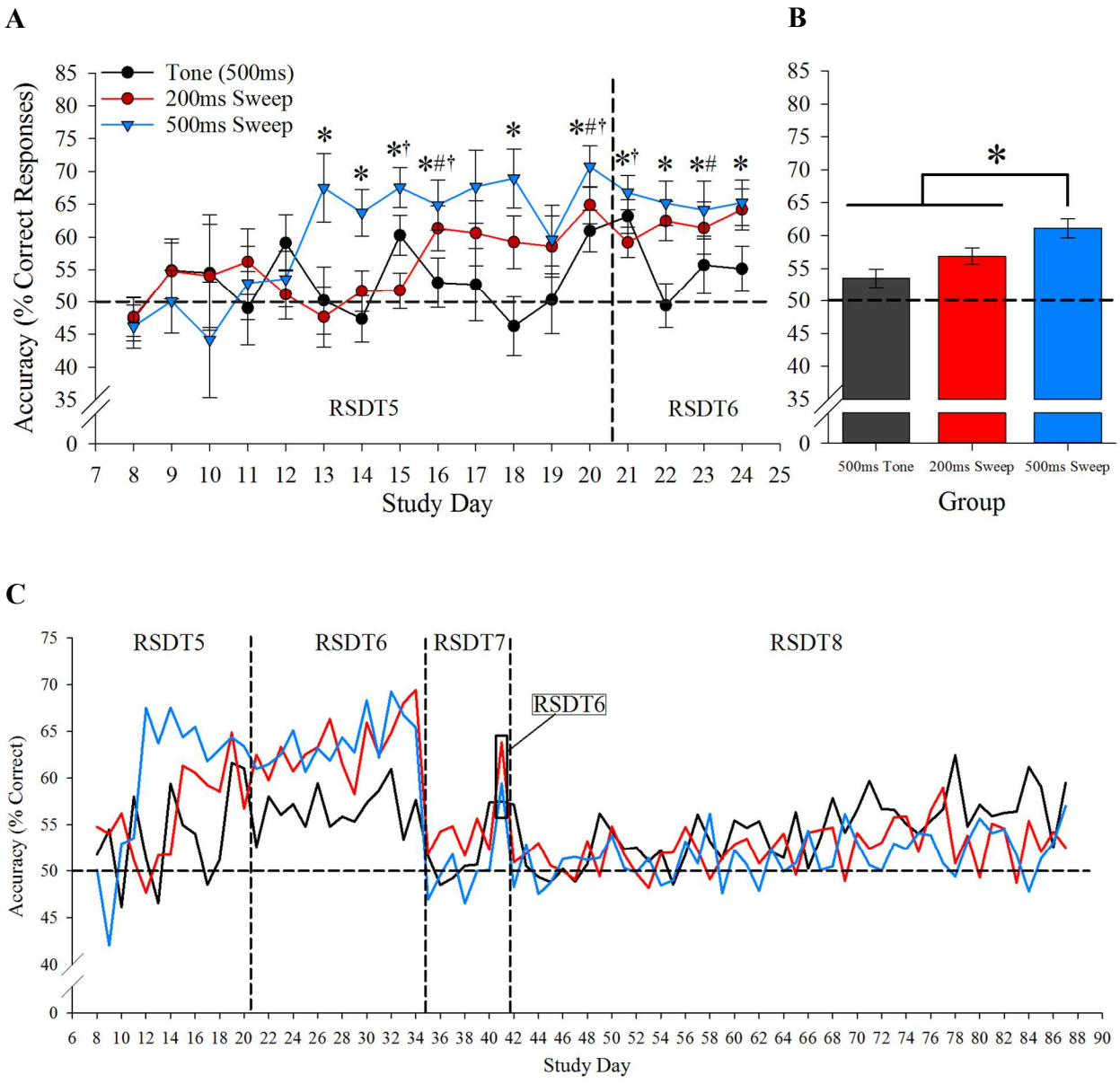
Within each task version, one-way ANOVAs were used to assess group differences in accuracy, using group as the between-subjects factor. Two-tailed one-sample T-Tests were used to determine whether rats' accuracy was significantly different from chance within RSDT versions

(test value=50). One-way ANOVAs were used to ascertain whether the three groups of rats differed in % premature responses or % omissions across the entire study. Paired samples T-Tests were conducted throughout the study on only the 500 ms tone group in order to verify that rats could respond to the high and low frequency stimuli equally well. Side bias was non-statistically assessed on a day-to-day basis (see Study 2 methods), and a *post-hoc* analysis of side bias was conducted at the end of the study to statistically verify that there was no significant side bias within any group of rats within any phase of the study. Since the results of this analysis could not decide study procession (the study had already ended by the time of the analysis), a two-tailed one-sample T-Test was used. The test value for this analysis was 2 (see Study 2 methods).

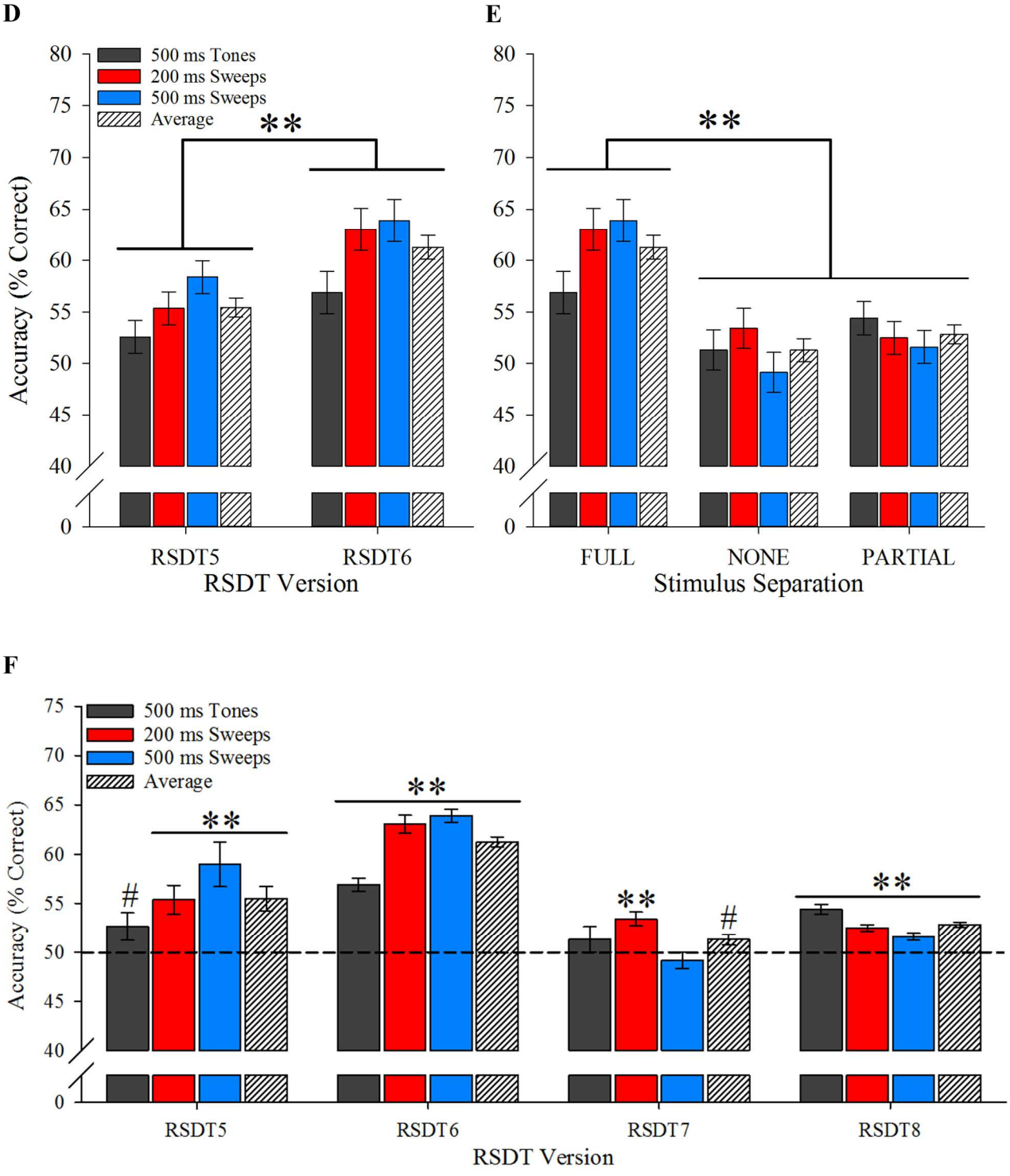
Any significant main or interactive effects revealed by above two-way ANOVAs were subjected to further analyses via Tukey *post hoc* comparisons. All data were analyzed using SPSS 24.0 (Chicago, IL) and were represented by mean and standard error of the mean. Alpha level was set to 0.05.

3.4. Study 2 Results

Graph 3.1. Study 2 Outcomes. In the earliest phases of RSDT training (Study D8-D24), rats trained using 500 ms sweeps (blue) learned the RSDT more quickly than rats trained using 200 ms sweeps (red) and rats trained with 500 ms pure tones (black), requiring only 6 days (D13) to achieve significant improvement over their performance on the first day (D8, represented by *); this group's improvement was also considerably more consistent than that of the other two groups, the performance of which fluctuated across this 17-day timeframe. Horizontal dotted line indicates chance (50%) performance. Vertical dotted line indicates transition between RSDT versions, which are indicated at the bottom of the graph. *=$p<0.05$ vs D8 (500 ms sweeps); #=$p<0.05$ vs D8 (200 ms sweeps); †=$p<0.05$ vs D8 (tones). Data presented as mean ±S.E.M. (**A**). On average, the 500 ms sweeps-trained rats (blue) performed more accurately than the rats trained with 200 ms sweeps (red) and 500 ms pure tones (grey) across the first 17 days of RSDT training (D8-D24). Horizontal dotted line indicates chance performance. *=$p<0.05$. Data presented as mean ±S.E.M. (**B**). Group (i.e. stimulus type) did not exert an effect on task accuracy within any version of the RSDT, or across the entire 90-day study. Chance performance is represented by a horizontal dotted line at 50% accuracy. Vertical dotted lines denote transitions between RSDT versions. Blue = 500 ms sweeps; Red = 200 ms sweeps; Black = 500 ms pure tones (**C**). Rats were more accurate in the RSDT6 than in the RSDT5, regardless of group. **=$p<0.01$. Data presented as mean ±S.E.M. (**D**). A main effect of auditory stimulus separation (i.e. magnitude of spatial cues) was observed on task accuracy, with rats performing with significantly higher accuracy with full stimulus separation than with partial or no separation; this effect was observed regardless of group. **=$p<0.01$. Data presented as mean ±S.E.M. (**E**). Rats trained with 500- and 200 ms sweeps, as well as all rats on average, performed significantly above chance (50% accuracy) in the RSDT5, while the pure tones group only trended toward significance. All groups performed significantly above chance within the RSDT6 and RSDT8, but only the 200 ms sweeps group performed above chance within the RSDT7. Chance performance is represented by a horizontal dotted line at 50% accuracy. *=$p<0.05$ vs 50; #=$p<0.10$ vs 50. Data presented as mean ±S.E.M (**F**).


A
B
C
Tone (500ms)
200ms Sweep
500ms Sweep
Accuracy (% Correct Responses)
Study Day
RSDT5
RSDT6
RSDT7
RSDT8
500ms Tone
200ms Sweep
500ms Sweep
Group
Accuracy (% Correct)

Graph 3.1, continued



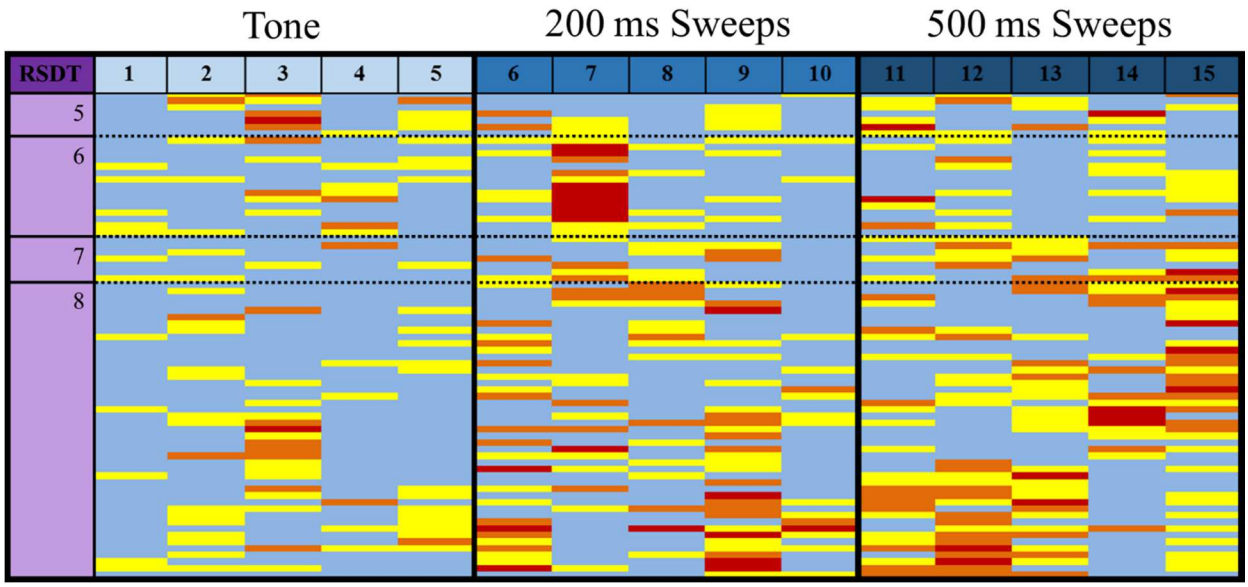


Figure 3.3. Illustration of side bias across Study 2 (RSDT5-RSDT8). Side preference values were monitored non-statistically across the entire study, with a value of "2" or greater interpreted as genuine side bias. Daily preference values for each individual rat are represented as colored bars. Individual rats are indicated by number at the top of the chart, and daily preference values are represented in descending chronological order. RSDT version transitions are delineated by horizontal dotted lines, and are labeled at left. Groups are delineated by solid vertical lines and labeled at top. Blue = preference value <1.5. Yellow = preference value >1.5 (acceptable). Orange = preference value >2. Red = preference value >3.

3.4.1. RSDT5 & RSDT6 (Full Spatial Cues) Performance

A two-way ANOVA of task accuracy across the entirety of RSDT5 and the first 4 days of RSDT6 training revealed main effects of training day [$F_{(16,160)}$=3.47, p<0.01] and group (i.e. auditory stimulus type) [$F_{(2,10)}$=7.27, p<0.05]. Further analyses revealed that accuracy improved across days (indicating learning), with average performance across all days from the 5th day of the RSDT5 onward significantly higher than the first day (p's<0.05)(data not shown). The 500 ms sweeps group learned the task more quickly than the other two groups; it took only 6 days for this group to perform significantly better than they had on the first day (D8), while it took the 500 ms tone and 200 ms sweeps groups 8 and 9 days, respectively, to achieve significant improvement over their D8 performance (**Graph 3.1A**). Task accuracy across days was also considerably more stable for the 500 ms sweeps group than for the other two groups – the 500 ms sweeps group

performed consistently better than D8 from their 6th day of the RSDT5 onward, whereas the other two groups' accuracy fluctuated across the entire 17-day time frame (**Graph 3.1A**). The main effect of group was analyzed further, and it was revealed that the 500 ms sweeps group was significantly more accurate across the 17 day window than the other two groups, which showed no difference from each other (**Figure 3.1B**). However, when the RSDT5 data were analyzed alone via one-way ANOVA (D8-D20), only a non-significant trend of group was observed on accuracy [$F_{(2,12)}$=3.23, p=0.075]. Group performance vs chance was analyzed within the RSDT5 and all subsequent versions via two-tailed one-sample T-Tests (test value = 50). Within the RSDT5, both sweeps groups performed significantly above chance [Sweeps200: t(12)=3.60, p<0.01; Sweeps500: t(12)=3.96, p<0.01], with a non-significant trend observed in the tone group [t(12)=1.87, p=0.086]. Average accuracy across all groups was also significantly higher than chance [t(12)=4.36. p<0.01] (**Graph 3.1F**).

Rats were trained in the RSDT6 for 15 days. A one-way ANOVA revealed that the initial main effect of group on task accuracy observed across the above 17-day window had disappeared – no significant difference between groups was observed across the 15 days of the RSDT6, although the 500 ms sweeps group still trended toward higher accuracy over the tone group [$F_{(2,12)}$=3.45, p=0.064]. A two-way ANOVA was used to determine stability of performance. Across the last 3 days of the task, training day failed to exert an effect on task accuracy [$F_{(2,22)}$=1.03, n.s.]. A non-significant training day X group trend was observed, with the 200 ms sweeps group apparently still learning (accuracy increasing across days) [$F_{(4,22)}$=2.34, p=0.087]; however, when analyzed alone, this group's accuracy did not significantly vary across days [$F_{(2,8)}$=2.14, n.s.]. Within the RSDT6, all groups performed significantly above chance [Tone:

t(14)=10.56, $p<0.001$; Sweeps200: t(14)=14.52, $p<0.001$; Sweeps500: t(14)=20.56, $p<0.001$; Average: t(14)=22.57, $p<0.001$] (**Graph 3.1F**).

3.4.2. RSDT7 (No Spatial Cues) Performance

Following this confirmation of stability, spatial cues were removed, and the rats were trained in the RSDT7 for 6 days. Two-way ANOVA revealed that removal of spatial cues resulted in a robust decrease in task accuracy irrespective of group [$F_{(1,12)}=115.0$, $p<0.001$], indicating that at this point in the study, rats were heavily reliant upon spatial cues to perform the RSDT. A one-way ANOVA of RSDT7 accuracy revealed no significant effect of group on accuracy [$F_{(2,12)}=1.20$, n.s]. Within the RSDT7, the 200 ms sweeps group performed significantly above chance [t(5)=4.86, $p<0.01$], with average accuracy (all groups combined) trending toward significance [t(5)=2.30, $p=0.070$] (**Graph 3.1F**); the other two groups failed to perform above chance [Tone: t(5)=1.02, n.s.; Sweeps500: t(5)=-1.03, n.s.] (**Graph 3.1F**).

3.4.3. RSDT6 Reassessment

Following these 6 days of the RSDT7, rats were assessed in a single session of the RSDT6 (D41) (**Figure 3.1**). Analysis of this single session's data via one-way ANOVA revealed no significant effect of group on accuracy [$F_{(2,12)}=1.66$, n.s.] (**Graph. 3.1C**). A two-way ANOVA of accuracy between this session (D41) and the RSDT6 data from D21-D35 was used to determine whether the intervening 6 days of RSDT7 training between the two time-points had affected rats' ability to perform the RSDT6. No effect of time-point was observed by this analysis [$F_{(1,12)}=0.68$, n.s.], indicating that rats' ability to discriminate between auditory stimuli with the aid of spatial

cues had remained intact. This result informed the decision to train the rats in the RSDT8, in which spatial cues were reintroduced, but were reduced relative to the RSDT6 configuration.

3.4.4. RSDT8 (Partial Spatial Cues) Performance

Rats were trained in the RSDT8 for the remainder of the study. A one-way ANOVA revealed no significant difference between groups within the 46 days of the RSDT8 [$F_{(2,12)}$=0.80, n.s.]. All groups performed above chance within the RSDT8 [Tone: t(45)=8.92, p<0.001; 200Sweeps: t(45)=7.04, p<0.001; 500Sweeps: t(45)=4.53, p<0.001; Average: t(45)=10.86, p<0.001] (**Graph 3.1F**). A two-way ANOVA on accuracy across the last 10 days of the RSDT8 revealed no difference in task performance across days [$F_{(9,108)}$=0.60, n.s.], with no main or interactive effect of group. This result indicated that all groups' performance had plateaued, and that no further learning was taking place. It was at this point that the decision was made to end the study. A two-way ANOVA of accuracy across the entire 90 day study revealed no significant difference in accuracy between groups [$F_{(2,12)}$=0.81, n.s.], although there was a significant group x RSDT version interaction [$F_{(6,36)}$=4.23] (**Graph 3.1C**). One-way ANOVAs (described individually above) failed to detect significant effects of group within RSDT versions [F's<3.5, *p's*>0.05].

3.4.5. Study 2 Overall Analysis

A two-way ANOVA detected a significant effect of speaker spacing (i.e. degree of auditory stimulus separation) [$F_{(2,24)}$=54.7, p<0.001], with rats performing more accurately with full stimulus separation than with partial or no separation (**Graph 3.1.E**; see **Figure 1.2 A-C** for

speaker configuration schematics); there was no significant difference between partial- and no-separation conditions (**Graph 3.1E**). RSDT5 data were not included in speaker spacing analysis (see Study 2 Statistical Analyses); this decision was substantiated by a two-way ANOVA of accuracy between RSDT5 and RSDT6 (the two RSDT versions utilizing full stimulus separation). The results of this analysis indicated that RSDT6 accuracy was significantly higher than RSDT5 accuracy [$F_{(1,12)}$=31.99, p<0.001] (**Graph 3.1D**), which thereby implied that the RSDT5 data did not accurately represent rats' ability to perform auditory discrimination with full stimulus separation.

Percent premature responses was analyzed over the entire study via one-way ANOVA, which revealed no significant difference between groups [$F_{(2,12)}$=1.38, n.s.] (Tone: 7.97% ±2.45%; Sweeps200: 13.37% ±4.27%; Sweeps500: 10.45% ±1.95%; Mean ±S.E.M.). The same analysis was run on % omissions, and again, there was no significant difference between groups [$F_{(2,12)}$=0.051, n.s.] (Tone: 2.79% ±1.03%; Sweeps200: 3.07% ±1.26%; Sweeps500: 3.08% ±4.30%; Mean ±S.E.M.).

Though all groups performed reliably above chance across nearly all phases of the study, a >50% task accuracy alone can hardly be considered competent. Each group's accuracy within each RSDT version was below 65%, with average performance in the RSDT7 and RSDT8 (the two closest approximations of the human task) failing to eclipse 55%. While these values were significantly above chance from a statistical standpoint, it was determined that the margins were not functionally meaningful, i.e. the rats' performance of the RSDT was still too low for the task to be considered a reliable model for human Auditory Sweeps Discrimination Training at the current stage of development. Given the low levels of premature responses and omissions, it was

unlikely that either measure contributed to rats' lack of performance. Given that even after 46 days in the RSDT8 rats still failed to exceed 55% accuracy (**Graph 3.1F**), the study was ended.

Paired samples T-Tests verified that rats in the 500 ms tone group did not respond differently to high vs low tones, verifying that all frequencies used throughout the study (4 & 7 kHz and 3 & 9.75 kHz) were approximately equally audible to the rats [RSDT5: t(4)=-0.73, n.s.; RSDT6: t(4)=-0.55, n.s.; RSDT7: t(4)=0.22, n.s.; RSDT8 (total): t(4)=0.38, n.s.; RSDT8 (new frequencies): t(4)=0.69, n.s.; All programs/time-points: t(4)=0.019, n.s.].

3.4.6. Study 2 Side Bias

As discussed in greater detail in the Study 2 Methods section, side bias was monitored on a day-to-day basis, rather than *post-hoc* as in Study 1. Monitoring and interpretation of preference values was done non-statistically, and rats were kept in the study even if they displayed preference values above the maximum "acceptable" value of 2. This policy enabled a pattern to emerge in which certain rats would develop strong and persistent side biases which would spontaneously disappear with further training (best illustrated by rats #14 and #15, **Figure 3.3**). Spontaneous disappearance of side biases only appeared to coincide with changes in RSDT version in three rats (see #3, #7, and #15, **Figure 3.3**). This apparent transience of side bias directly contradicts the assumption made in Study 1 that rats, once having chosen to "settle" for the 50% reward rate given by an arbitrary aperture, are not likely to adjust their response strategies. This realization led us to modify the criteria for study termination on the basis of side bias (see Methods): more than 50% of all rats would have to display 10 consecutive days of strong side bias, with the "threshold" value for genuine bias having been increased from 1.5 to 2. Since these conditions were never met, the

study was not ended on the basis of side bias. This judgment was validated by an end-of-study analysis of preference values. Two-tailed one-sample T-Tests revealed that preference values were significantly lower than the cutoff value of 2 throughout all phases of the study [RSDT5-RSDT:8 t(5-45)=(-3)-(-19), *p's*<0.005]. Two-way ANOVA of preference values across the study revealed no main or interactive effects of RSDT version [$F_{(3,36)}$=1.09, n.s.] or group [$F_{(2,12)}$=1.48, n.s.].

Chapter 4: Study 2A

4.1. Study 2A Methods

Immediately following termination of Study 2 training, the decision was made to launch a supplemental study to determine whether **RSDT6** performance (i.e. RSDT with full spatial cues, wherein highest performance was observed and was significantly above chance) could be enhanced by d-amphetamine (d-AMPH), which had been previously reported to facilitate the effects of TCT in humans (Swerdlow et al., 2017). This study utilized the same animals and apparatus as Study 2 (see Study 2 Methods). Rats were kept in the same groups as in Study 2, and were assessed in the corresponding sub-versions of the RSDT6. Study 2A training sessions used the same version of the RSDT6 used in Study 2, but with the broadened frequency range of 3-9.75 kHz employed by the latter portion of the RSDT8 (**Figure 3.1**). Modified versions of the RSDT6 (**RSDT6A**&**RSDT6B**, **Table 4.1**) were administered during testing phases; these programs were comprised entirely of "standard" trials (**Table 2.2**) in order to prevent any non-drug-induced learning from taking place within or between test days. Two separate testing phases were interceded by a six day period in which rats were run in the RSDT6 without injections. In total, the effects of three doses of d-AMPH were assessed in a within-subjects design – two doses (plus vehicle) during the first phase, and one additional dose (plus vehicle) during the second.

4.2. Study 2A Progression

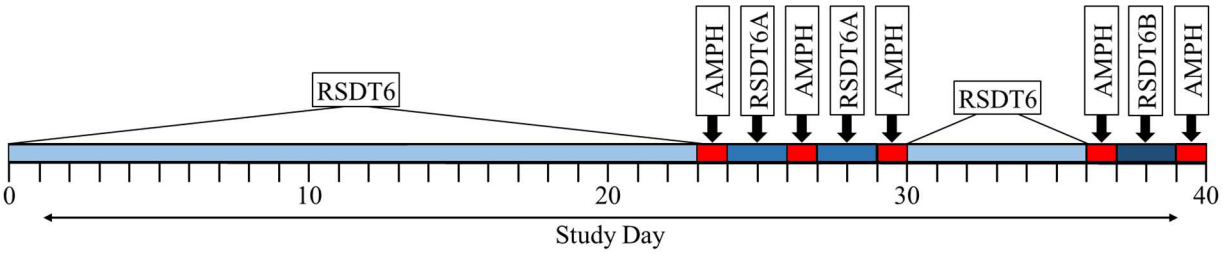


Figure 4.1. Study 2A Progression. Immediately following the termination of Study 2, the same rats were run for an additional 40 days, encompassing two phases of d-amphetamine (d-AMPH) administration separated by a 6-day period in which rats were run in the RSDT6 without injections. Days on which rats were given intraperitoneal d-AMPH are indicated in red. The study followed a within-subjects crossover design. Rats were run in the indicated RSDT program on the days between test days, and received no injections. RSDT: Rat Sweep Discrimination Task.

Table 4.1. RSDT Program Specifications, Study 2A

Program	Trial Types Used	Speaker Separation	Study Days
RSDT6	70 Standard, 40 Forced, 10 Guided Trials	Full Separation	D1-D23, D25-26, D28-29, D36, D38-39
RSDT6A	120 Standard Trials	Full Separation	D24, D27, D30
RSDT6B	210 Standard Trials	Full Separation	D37, D40

RSDT: Rat Sweep Discrimination Task
Note: See Fig. 2 and Table 2 respectively for speaker separation schematics and trial type definitions.

Visual illustration of the progression of Study 2A is provided in **Figure 4.1**. Rats were re-trained in the **RSDT6** (**Table 4.1**) for 23 days in order to reestablish stability of task performance. Task accuracy was analyzed across the last 10 days of this training period to confirm stability before initiation of the first phase of d-AMPH testing. This first phase of testing lasted 7 days, with drug assessment on the 1st, 4th, and 7th days, and no injections on the intervening days (**Figure 4.1**). On each of these 3 test days, rats were intraperitoneally administered either vehicle or d-AMPH (0.10 mg/kg or 0.30 mg/kg) in a crossover design and assessed in the **RSDT6A** (**Table 4.1**), a permutation of the RSDT6 which utilized only standard trials (120 in total). Rats were run in the RSDT6A on non-test days in order to maintain consistency in training and testing.

The first phase of testing was immediately followed by a 6-day period in which rats were again run in the RSDT6. Data from the last 5 days of this period were analyzed for stability before initiation of the second and final testing phase, during which an additional dosage of d-AMPH (0.25 mg/kg) was assessed. This second phase lasted 4 days, with drug administration on the 1st and 4th. In order to widen the window of observation of the effects of d-AMPH, rats were assessed in the **RSDT6B** (**Table 4.1**), which was comprised of 210 trials instead of 120.

Primary outcomes for the RSDT6 (accuracy, % premature responses, % omissions) were calculated following the formulae described in the Study 2 Methods section. RSDT6A and RSDT6B accuracy calculations followed the same formula used to assess RSDT1 performance (see Study 1 Methods). Given that d-AMPH has previously been demonstrated to produce differential cognitive effects in rats depending on baseline task performance (Amitai et al., 2013), data were subjected to a "median split" manipulation, such that accuracy data gathered from rats displaying low baseline performance (accuracy < median accuracy) could be analyzed separately from data gathered from higher performing rats (accuracy ≥ median). "Difference scores" were also calculated by subtracting rats' baseline accuracy from their accuracy following d-AMPH administration; difference scores were calculated for each dose of d-AMPH tested. Side bias data from the RSDT6 were collected and monitored during pre- and inter-test phase intervals, but were not collected during the test phases themselves.

4.3. Drug Treatment

Drug administration followed a cross-over design, with all rats receiving each dose of d-amphetamine (d-AMPH) over the course of the study. Long Evans rats trained in the RSDT6

received intraperitoneal injections of either vehicle or d-AMPH (0.10 or 0.30 mg/kg during the first testing phase, 0.25 mg/kg during the second) five minutes prior to assessment in either the RSDT6A (first phase) or RSDT6B (second phase). Drug solutions were prepared daily by dissolving d-AMPH into saline to a concentration of 0.30 and 0.10 mg/mL during the first testing phase, and to a concentration of 0.25 mg/mL during the second phase. The 0.10 mg/mL solution was prepared from the 0.30 mg/mL solution via serial dilution. All injections were administered at 1 mL/kg. Dosages were based on reports that 0.10 (Grottick and Higgins, 2002), 0.30 (MacQueen et al., 2018), and 0.25 (Andrzejewski et al., 2014; Grilly et al., 1998) mg/kg d-AMPH increased rodent performance in attention/vigilance-related tasks; dosages higher than 0.30 mg/kg have been demonstrated to be detrimental to performance of such tasks (McGaughy and Sarter, 1995; Paterson et al., 2011), and were therefore not included in the study.

4.4. Study 2A Statistical Analyses

Primary (% accuracy, % premature responses, and % omissions) and secondary (reward and correct/incorrect response latencies) were analyzed via two-way ANOVA. Prior to initiation of test phases, two-way ANOVAs of accuracy were used to confirm stability of responding across days, using day as the within-subjects factor and group as the between-subjects factor. After completion of the test phases, two-way ANOVAs were used to reveal any effects of d-AMPH on primary and secondary outcome variables, with d-AMPH dose as a within-subjects factor and group as a between-subjects factor.

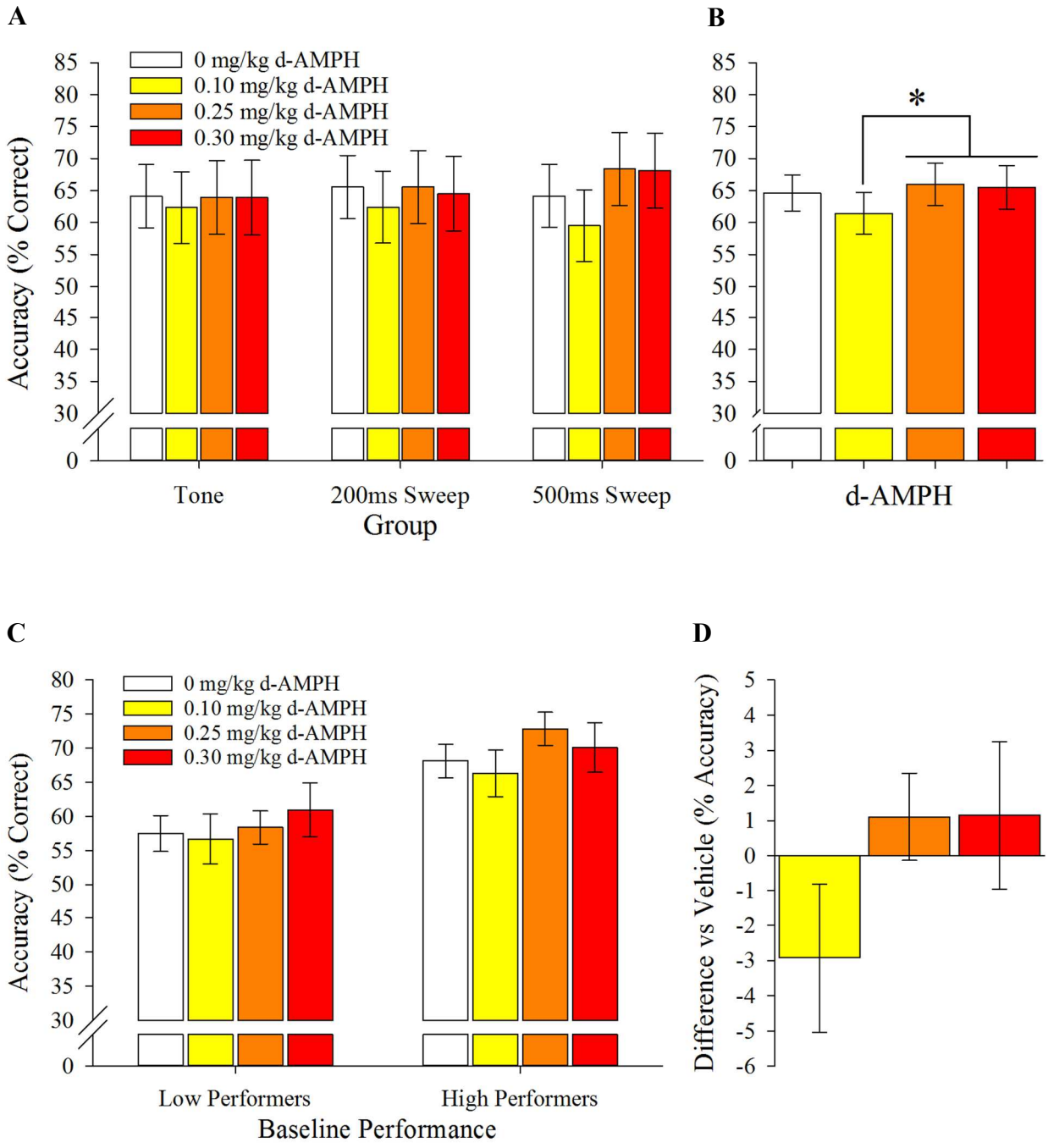
A paired samples T-Test was performed on baseline task accuracy across the two test phases in order to confirm that there was no effect of experimental time-point on task performance,

and to thereby allow data from both test phases to be analyzed together. Following the "median split" manipulation described in the Study 2A Progression section, a two-way ANOVA was used to determine whether d-AMPH had differentially affected high vs. low baseline performers. Drug dose was used as a within-subjects factor and baseline performance as a between-subjects factor; small sample sizes (n=4 rats per group before the median split manipulation) precluded the use of group as a between-subjects factor. "Difference scores" (see Study 2A progression) were analyzed via one-tailed one-sample T-Test (test value = 0) in order to determine whether d-AMPH increased accuracy versus vehicle. Side bias data were collected and monitored non-statistically during pre- and inter- testing phase intervals, but not during testing phases.

Any significant main or interactive effects revealed by above two-way ANOVAs were subjected to further analyses via Tukey *post hoc* comparisons. All data were analyzed using SPSS 24.0 (Chicago, IL) and were represented by mean and standard error of the mean. Alpha level was set to 0.05.

4.5. Study 2A Results

Graph 4.1. Study 2A Outcomes. No main or interactive effect of group (i.e. stimulus type) was observed on task accuracy. Data represented as mean ±S.E.M. (**A**). A main effect of d-AMPH was observed on task performance, with accuracy following administration of the two higher doses of d-AMPH (0.25 & 0.30 mg/kg) significantly higher than after administration of the low dose of d-AMPH (0.10 mg/kg). No difference was observed between any of the d-AMPH doses and vehicle. Data represented as mean ±S.E.M. (**B**). d-AMPH did not differentially affect the accuracy of low vs. high baseline performers. Data represented as mean ±S.E.M. (**C**). d-AMPH did not increase accuracy vs. vehicle. Data represented as mean difference scores ±S.E.M., where a score of "0" indicates no difference whatsoever vs. vehicle, positive scores indicate an increase in accuracy vs. vehicle, and negative scores indicate a decrease in accuracy vs. vehicle (**D**).


A
B
C
D
0 mg/kg d-AMPH
0.10 mg/kg d-AMPH
0.25 mg/kg d-AMPH
0.30 mg/kg d-AMPH
Accuracy (% Correct)
Tone
200ms Sweep
500ms Sweep
Group
*
d-AMPH
Low Performers
High Performers
Baseline Performance
Difference vs Vehicle (% Accuracy)

A two-way ANOVA of RSDT6 accuracy across the 10 days preceding initiation of the first phase of d-AMPH testing revealed no main effect of day [$F_{(9,108)}$=1.59, n.s.], indicating stability of response. A similar analysis conducted on accuracy across the five days preceding the second phase of testing returned the same results [$F_{(4,48)}$=1.56, n.s.]. Any variance of response observed during either testing phase could therefore be interpreted as having been driven by drug treatment, and not by latent task acquisition. Three rats (one from each group) were inactive during assessment on one or more test days, and were therefore excluded from analyses. As a result of these exclusions, sample size was 4 for each group. A paired samples T-Test was first used to verify that there was no effect of experimental time-point (test phase 1 vs 2) and/or task version (RSDT6A vs RSDT6B) on baseline task performance [t(11)=0.25, n.s.]; subsequent analyses could therefore be performed on pooled data collected across both phases.

A two-way ANOVA detected a main effect of drug on accuracy [$F_{(3,27)}$=4.60, p<0.05], although further analysis revealed that this effect was driven by a significant difference of the higher doses of d-AMPH (0.25 and 0.30 mg/kg) versus the low dose (0.10 mg/kg); no dose of d-AMPH was significantly different from vehicle (**Graph 4.1B**). No main or interactive effects of group were detected on accuracy [$F_{(3,27)}$=0.19, n.s.] (**Graph 4.2A**). Subsequent two-way ANOVAs revealed no main or interactive effects of drug or group on % premature responses or % omissions [Drug: F's$_{(3,27)}$<1, n.s.; Group: F's$_{(1,9)}$<1, n.s.](See **Supplementary Table 2** for Mean ±S.E.M.). There were also no main or interactive effects of drug or group on correct, incorrect, or reward latencies [Drug: F's$_{(3,27)}$<1, n.s.; Group: F's$_{(1,9)}$<1, n.s.](See **Supplementary Table 2** for Mean ±S.E.M.).

Following the "median split" grouping (see Study 2A Statistical Analyses), a two-way ANOVA detected the expected main effect of baseline performance [$F_{(1,10)}$=29.6, p<0.001], as well

as the main effect of drug detected by the first ANOVA [$F_{(3,30)}$-4.13, p<0.05], as described above; however, the absence of a baseline performance X drug interaction [$F_{(3,30)}$=0.61, n.s.] indicates that d-AMPH did not differentially affect low performers vs. high performers (**Graph 4.1C**), despite apparent differences. Analysis of difference scores via one-tailed one-sample T-Tests revealed that no dose of d-AMPH mediated any significant improvement over baseline accuracy [0.10 mg/kg: t(11)=-1.38, n.s.; 0.25 mg/kg: t(11)=0.89, n.s.; 0.30 mg/kg: t(11)=0.55, n.s.] (**Graph 4.1D**). Side preference scores were monitored non-statistically only during the pre- and inter-testing phase intervals, during which time no rat demonstrated strong, consistent side bias (cut-off value was 2; data not shown).

Chapter 5: Study 3

5.1. Study 3 Methods

5.1.2. Animals

Study 3 employed 8 male Long Evans rats (250-365g at training, average 310 g; Envigo, San Diego), none of which had been used in previous studies. Rats were housed and transported in exactly the same manner as those utilized by Studies 1 and 2, and were kept in the same AAALAC-approved facility. Rats were maintained at ~85% free-feeding body weight, and were provided with water *ad libitum*. Training began at 10 weeks of age, and rats were always trained during the dark period of their light/dark cycles. All procedures were approved by the University of California, San Diego (UCSD) Animal Care and Use Committee.

5.1.3. Preliminary Training

As in Studies 1 and 2, rats were initially trained in the basic HAB1 program until they had reliably associated the magazine light with food delivery. However, instead of proceeding to HAB2 as in previous studies, rats were moved to S_HAB2 – an alternative basic training module with similar specifications as the "guided" trial type utilized by the RSDT5&6. Like HAB2, S_HAB2 operated on a basic FR1 reward schedule, but used only one of two lateralized stimulus lights per trial. At the beginning of each trial, the rats were presented with a 500 ms auditory stimulus- either an upsweep (3 kHz-9.75 kHz) or a downsweep (9.75 kHz-3 kHz). As in the guided trials of the RSDT5&6, auditory stimuli were played from the same side of the chamber as the lit aperture (full speaker separation; **Figure 1.2B**); in each box, each sweep type (up or down) would only play from a single side of the chamber. The only difference between S_HAB2 trials and

RSDT5&6 guided trials was inter-trial interval – in order to ensure that the rats' heads would always be oriented in the same way at the beginning of auditory stimulus presentation, sweeps were programmed to play immediately following rats' withdrawal from the magazine.

The S_HAB2 was conceived as a means by which to circumvent the difficult transition from the HAB2, a simple FR1 training module in which any arbitrary nosepoke would be rewarded, to the RSDT, a comparatively stringent discrimination paradigm contingent upon a new sensory modality (audition). By instead training rats in the S_HAB2, it was hypothesized that the rats would a) recognize early on that auditory stimuli were salient, b) not have to suddenly learn to incorporate a new sensory modality into their response strategies upon being moved to the RSDT, and c) associate a given type of sweep (up or down) with its corresponding aperture (left or right) before being challenged by the RSDT. Once responding reliably in the S_HAB2, rats were moved to the **RSDT9** (**Table 5.1**). All training took place in the same apparatus used in Studies 1 and 2.

5.2. Study 3 Progression

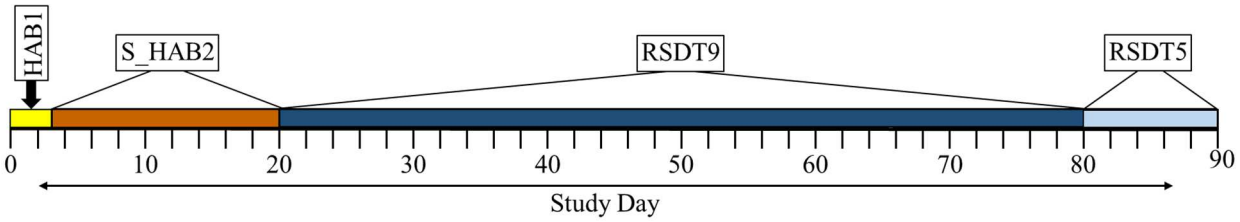


Figure 5.1. Study 3 Progression. Rats were trained for 90 days, including days spent training in the basic HAB1 program and the S_HAB2 program.. Rats were trained using 2 different permutations of the original RSDT1 program, the RSDT9 and the RSDT5; the latter of these programs was administered in Study 2, and the former was slightly modified from the RSDT8 used in Study 2. RSDT: Rat Sweep Discrimination Task.

Table 5.1. RSDT Program Specifications, Study 3 (Chronological Order).

Program	Program Specifications	Spatial Cues	Study Days
S_HAB2	FR1; 1 light coupled w/ auditory stim	Full Cues	D4-D20*
RSDT9	40 Standard, 40 Forced, 40 Guided Trials	Partial Cues	D21-D80
RSDT5	40 Standard, 40 Forced, 40 Guided Trials	Full Cues	D81-D90

RSDT: Rat Sweep Discrimination Task

Note: See Figure 2 & Table 2 respectively for speaker separation schematics (spatial cues) and trial type definition

** Individual rats spent 14-24 days in S_HAB2 before being moved to RSDT8; Average=17 days*

Auditory stimulus specifications for all Study 3 programs were based upon early training data from Study 2, in which rats trained with 500 ms sweeps learned the full spatial cue-assisted RSDT more quickly than rats trained with 200 ms sweeps or 500 ms pure tones (**Graph 3.1A**). Sweep frequencies were maintained at 3 and 9.75 kHz.

Visual representation of the progression of Study 3 is provided in **Figure 5.1**. The basic progression of Study 3 differed from earlier studies in that individual rats were moved from S_HAB2 to the RSDT as soon as they had reached criterion for behavior acquisition (>60 responses for 4 consecutive days), whereas Studies 1 and 2 did not progress to the RSDT until all rats had reached criterion in HAB2. This new strategy was implemented in response to the emergence of considerable inter-subject variability in acquisition of S_HAB2, by which point it had become clear that individual rats would take much longer than others to reach criterion. Waiting for all 8 rats to complete S_HAB2 before proceeding would have meant placing the study on hold for an indefinite amount of time, which was deemed unfeasible. This decision was later proven prudent, as 2 of the 8 rats never reached criterion in S_HAB2 within the entire 90-day study. Consequently, sample sizes for later stages of the study were reduced from 8 to 6.

Individual rats spent 14-24 days training in the S_HAB2 before reaching criterion (average = 17) (**Figure 5.1**, **Table 5.1)** and transitioning to the **RSDT9** (**Table 5.1**). Like the RSDT8 utilized in Study 2 (**Table 3.1**), the RSDT9 provided only partial spatial cues (partial speaker

separation, **Figure 1.2C**). Though rats had failed to eclipse 55% accuracy with partial spatial cues during Study 2 (**Graph 3.1E**), this failure was hypothesized to have been a consequence of Study 2 rats having been over-trained in the RSDT6 (**Table 3.1**), and thereby having become reliant upon full spatial cues. It was hypothesized that reliance upon spatial cues could be prevented by providing only partial cues at the beginning of RSDT training. The RSDT9 was identical to the RSDT8 utilized in Study 2 (**Table 3.1**), but with sample type distribution adjusted such that, of the 120 trials within each session, 40 were standard, 40 were forced, and 40 were guided (**Table 2.2**). It was intended that rats would be moved to the slightly more difficult RSDT8 (70 standard, 40 forced, and 10 guided trials; **Table 3.1**) once they began to perform reliably at or above 60% accuracy. The rats trained in the RSDT9 for 60 days, at which point they were moved to the **RSDT5** (**Table 5.1**), which provided full stimulus separation and utilized the same trial type distribution as the RSDT9. Rats' ability to perform the RSDT with full spatial cues was assessed over 10 days, after which the study was terminated.

Primary outcome variables were calculated for the RSDT9 in the same manner as for the RSDT5, following the formulae described in the Study 2 Progression section. Side preference values were calculated following the formula described in the Study 1 Progression section, and were monitored non-statistically across the study, as in Study 2.

5.3. Study 3 Statistical Analyses

Task performance (accuracy) was analyzed via repeated measures analyses of variance (ANOVA) and independent- and one-sample T-Tests. Since 2 of the 8 rats trained in Study 3 never progressed past S_HAB2, initial sample sizes for all analyses of Study 3 task performance

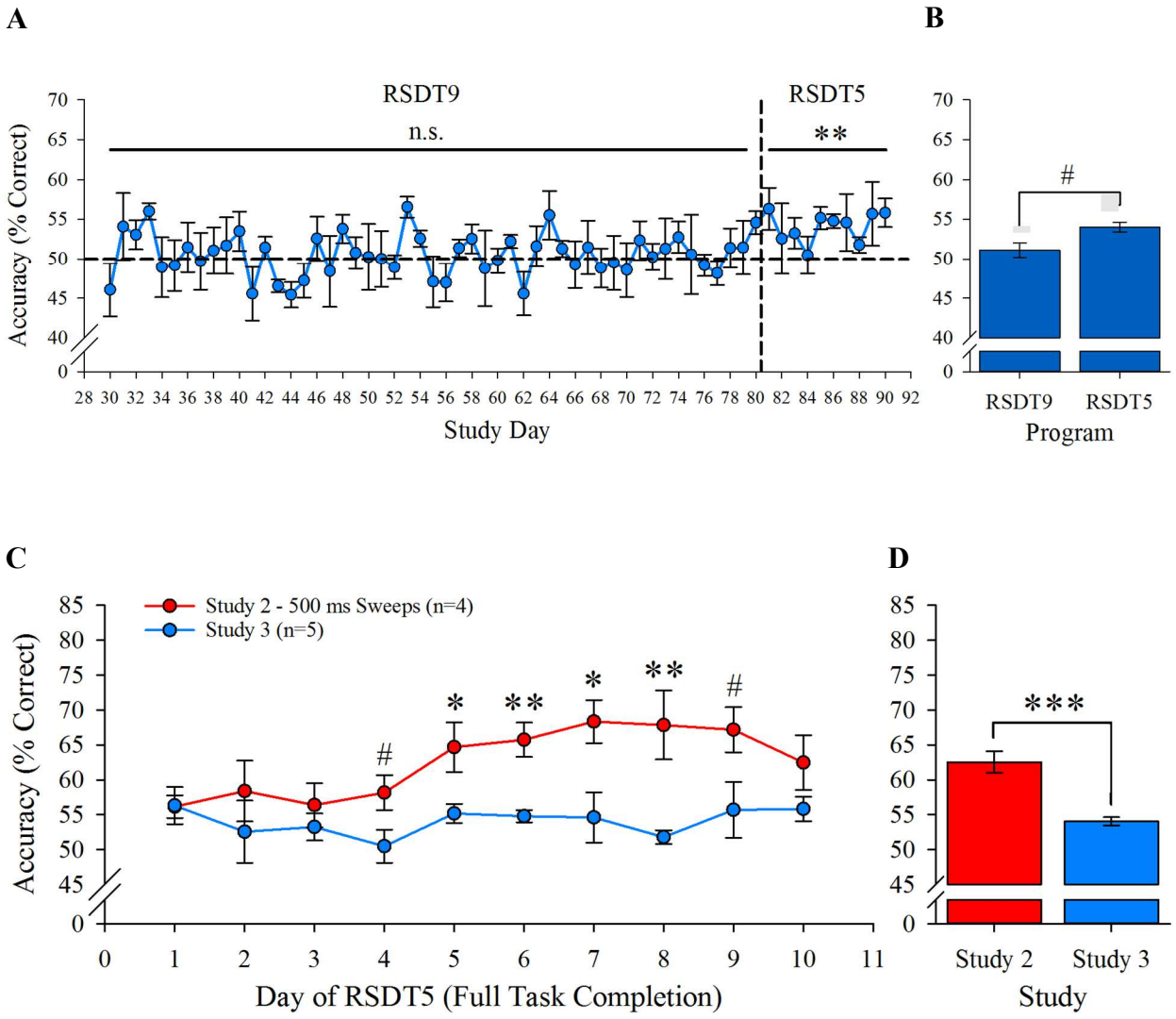
were 6. Development of strong, persistent side biases across entire phases of the study necessitated the exclusion of 1 further rat from analysis of the RSDT9 data, while 1 (different) rat was excluded from the RSDT5. Resultant sample sizes were 5 for the RSDT9 data, and 5 for the RSDT5 data. As in Study 2, certain rats developed strong side biases at various points in the study but later corrected themselves in subsequent days; these rats' data were included in the analyses.

Day-to-day progress within the RSDT9 and RSDT5 was assessed via repeated measures ANOVAs, using day as a within-subjects factor. As there was only one group of rats trained in Study 3, neither analysis examined any between-subjects factors. An independent samples T-Test was used to identify any significant difference between accuracy in the RSDT9 vs. RSDT5. Two-tailed one-sample T-Tests were used to compare accuracy to chance (test value = 50).

In order to determine whether training in the RSDT9 for 60 days had affected rats' ability to learn to complete the RSDT5 (i.e. whether overtraining with partial spatial cues had affected rats' ability to learn to use full spatial cues), an independent samples T-Test was used to compare Study 3 RSDT5 data to Study 2 RSDT5 data, the latter of which were generated directly after completion of HAB2. In the interest of maximizing construct validity, this analysis incorporated data only from those Study 2 rats trained using auditory stimuli of the same specifications as the Study 3 rats (i.e. 500 ms sweeps). This analysis was conducted on data generated across all 10 days of Study 3 RSDT5 (Study 3 D81-D90), and RSDT5 data from the first consecutive 10 days of Study 2 in which the 500 ms sweeps group of rats logged responses in all 120 trials of the session (Study 2 D9-D18; days 2-11 of RSDT5). One Study 2 rat was excluded from analysis due to inactivity across this 10-day window, leaving 4 rats in the Study 2 group and 5 rats in the Study 3 group.

5.4. Study 3 Results

Graph 5.1. Study 3 Outcomes. Rats, on average, did not perform significantly differently from chance in the RSDT9, but did perform significantly above chance in the RSDT5. Horizontal dotted line indicates chance (50%) performance. Vertical dotted line represents transition between RSDT versions, which are labeled at the top of the graph. **=p<0.01 vs 50, n.s.=p>0.10 vs 50. Data represented as mean ±S.E.M. (**A**). Rats tended to perform more accurately in the RSDT5 than in the RSDT9. #=p<0.10. Data represented as mean ±S.E.M. (**B**). Study 2 rats trained with 500 ms sweeps completed the RSDT5 with significantly higher accuracy than Study 3 rats on the 5th, 6th, 7th, and 8th consecutive days in which rats demonstrated complete task engagement (i.e. all 120 trials completed). This effect was seen as a non-significant trend on the 4th and 9th days. *=p<0.05, **=p<0.01, #=p<0.10 at a given timepoint. Data represented as mean ±S.E.M. (**C**). Across the first 10 days of the RSDT5 (with full task completion/engagement), Study 2 rats demostrated significantly higher accuracy than Study 3 rats. ***=p<0.001. Data represented as mean ±S.E.M.



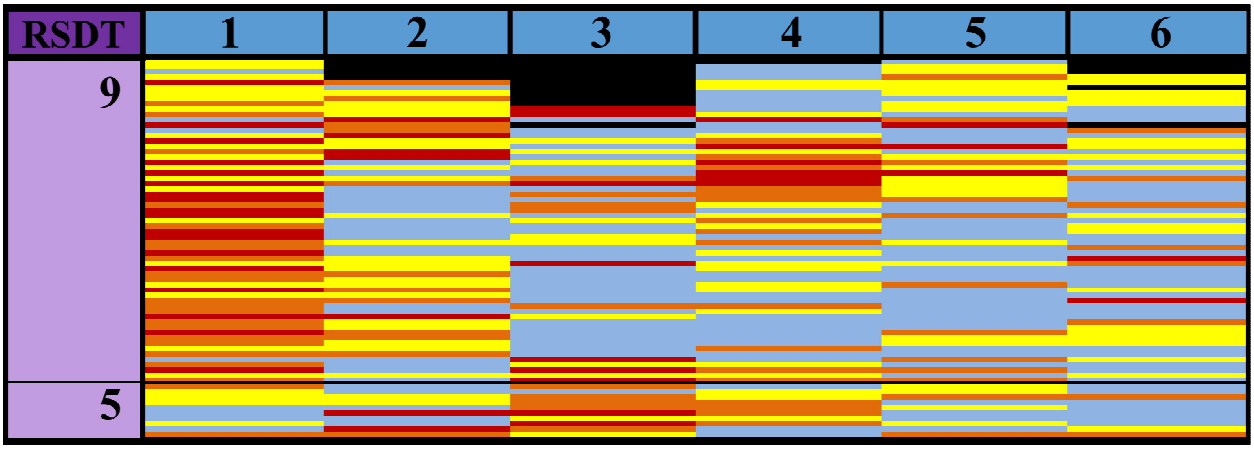


Figure 5.2. Illustration of side bias across entire study (RSDT9 & RSDT5). Side preference values were monitored non-statistically across the entire study, with a value of "2" or greater interpreted as genuine side bias. Daily preference values for each individual rat are represented as colored bars. Individual rats are indicated by number at the top of the chart, and daily preference values are represented in descending chronological order. RSDT version transition is delineated by a horizontal solid line. RSDT versions are indicated at left. Black bars represent days in which a given rat did not provide RSDT data, either because it was still training in the S_HAB2, or because of a system error. Rat #1 was excluded from RSDT9 data analysis due to a consistent side bias across the second half of that testing phase. Rat #3 was excluded from RSDT5 data analysis due to a strong and consistent bias across nearly all of that testing phase. Blue = preference value <1.5. Yellow = preference value >1.5 (acceptable). Orange = preference value >2. Red = preference value >3.

5.4.1. RSDT9 (Partial Spatial Cue) Performance

In order to determine rats' stability of performance in the RSDT9, a repeated measures ANOVA of accuracy was conducted across the last 10 days of the RSDT9, using day as the within-subjects factor. This analysis revealed no significant effect of day on accuracy [$F_{(9,36)}$=0.44, n.s.], indicating that rats' performance did not vary across the final 10 days of the 60-day RSDT9 training period. A one-sample T-Test of average accuracy across all 60 days of the RSDT9 (test value = 50) revealed that performance was no different from chance across the entire study [t(51)=1.57, n.s.] (**Graph 5.4A**). 7.16% (±0.36%) of the responses made in the RSDT9, on average, were premature; 1.24% (±0.17%) were omissions. Data from 5 of the 6 rats trained in the RSDT9 were included in these analyses – a strong side bias persisting across most of the second half of this phase of testing necessitated exclusion of one rat (rat #1, **Figure 5.2**). Following these

analyses, it was concluded that rats were not able to perform the RSDT with only partial spatial cues.

5.4.2. RSDT5 (Full Spatial Cue) Performance

The same rats were then retrained for 10 days of the RSDT5 in order to determine whether or not these rats were capable of utilizing full spatial cues to complete the task. A one-sample T-Test across these 10 days revealed that the rats were able to complete the task at above-chance levels [$t(9)=6.59$, $p<0.001$] (**Graph 5.4A**). However, though the results of this analysis were significant, the addition of spatial cues only marginally improved rats' performance over the partial-cue condition – a paired samples T-Test between RSDT9 and RSDT5 accuracy revealed only a non-significant trend towards improvement [$t(9)=-2.09$, $p=0.067$] (**Graph 5.4B**). Indeed, rats completed the RSDT5 at only ~54% accuracy on average (**Graph 5.4B**), which, though statistically higher than chance, cannot be considered competent performance. A repeated measures ANOVA conducted on all 10 days of RSDT5 data confirmed that rats' performance was stable across days [$F_{(9,36)}=0.55$, n.s.], indicating that no learning was taking place within this time period. 5.40% (±0.63%) of the responses made in the RSDT5, on average, were premature; 0.27% (±0.08%) were omissions. One rat was excluded from RSDT5 data analysis due to a persistent side bias across the majority of this phase of the study (rat #3, **Figure 5.2**).

5.4.3. Cross-Study RSDT5 Analysis

The low accuracy in the RSDT5 demonstrated by Study 3 rats suggested that training with only partial spatial cues for 60 days may have somehow negatively affected rats' ability to

incorporate full spatial cues into their response patterns. In order to determine whether training history affected task performance, Study 3 RSDT5 data were analyzed against Study 2 RSDT5 data, which were generated directly after completion of the entirely non-auditory HAB2. Only data from those Study 2 rats that had been trained using the same auditory stimulus specifications (500 ms sweeps) were included in this analysis. In order to minimize the confound of general operant training experience, only data from days on which rats demonstrated full task engagement (i.e. completion of all 120 trials in a session) were included. All 10 days of Study 3 RSDT5 data met this criterion and were included (D81-D90), as did data from Study 2 D9-D18 (i.e. the 2^{nd}-11^{th} days of RSDT5, **Figure 3.1**). One Study 2 rat did not meet the task engagement requirement for this analysis within this 10-day period and was excluded, making the sample size for Study 2 RSD5 data 4.

RSDT5 data from the two studies were compared via independent samples T-Tests. There was revealed a major difference between performance in the two studies, with Study 2 rats significantly more accurate than Study 3 rats [$t(18)=-5.12$, $p<0.001$,**Graph 5.1D**]. This difference was largely driven by data generated on the 5^{th} [$t(7)=-2.71$, $p<0.05$], 6^{th} [$t(7)=-4.62$, $p<0.01$], 7^{th} [$t(7)=-2.81$, $p<0.05$], and 8^{th} [$t(7)=-3.59$, $p<0.01$] days of full RSDT5 completion, and to a lesser extent, data generated on the 4^{th} [$t(7)=-2.22$, $p=0.062$] and 9^{th} [$t(7)=-2.15$, $p=0.069$] days (**Graph 5.1C**). Further analysis of the Study 2 data via repeated measures ANOVA revealed a main effect of day on accuracy across the first 9 days [$F_{(8,24)}=2.36$, $p=0.050$], and a similar trend across all 10 days [$F_{(9,27)}=2.16$, $p=0.059$]. The results of these ANOVAs indicate that Study 2 rats were not only considerably more accurate during their first 10 days of RSDT5 training (with full task engagement) than the Study 3 rats, but their performance also improved across days whereas that of the Study 3 rats remained stable. The implications of the results of Study 3 are that a) partial

spatial cues are not sufficient to drive acquisition of the RSDT, and b) over-training in such a paradigm can impair the incorporation of full spatial cues into response strategies when such cues are made available. Based on this interpretation, it was determined that the Study 3 rats were not likely receptive to further training and the study was terminated.

Chapter 6. Discussion

Herein, I have detailed attempts to develop a rat version of the Auditory Sweep Discrimination module of the Posit Science Corporation's Targeted Cognitive Training (TCT) suite, which spanned across three studies. In total, three different cohorts of Long Evans (LE) rats were trained in nine distinct permutations (**Sup. Table 1**) of the Rat Sweep Discrimination Task (RSDT; **Figure 1.1B**), each of which had been modified from previous versions in response to rat performance. Each new version of the RSDT differed from previous versions in terms of stimulus duration, speaker spacing (i.e. presence and degree of spatial cues; **Figure 1.2A-C**), and/or trial type distribution (**Table 2.2**), with each successive modification intended to facilitate association of auditory stimuli with their corresponding response apertures (**Sup. Table 1**). Despite rats' inability to perform any version at high levels (>85% accuracy), spatial localization of stimuli (RSDT5/6) enabled rats to attain ~65% accuracy – well above chance levels (**Graph 3.1C**). D-amphetamine (d-AMPH; 0.10, 0.25, and 0.30 mg/kg) did not affect performance of this version of the RSDT (**Graph 4.1A**), thereby failing to replicate a previous report of d-AMPH facilitating the effects of sweep discrimination training in schizophrenia patients (Swerdlow et al., 2017). Modest improvements were observed, however, though small sample sizes likely prevented their detection by statistical analyses. Overall, these studies indicate that the RSDT requires further refinement before it can be used to elucidate the mechanisms underlying human TCT.

In the original study (Study 1), it was hypothesized that male and female rats would acquire sweep discrimination with little to no assistance (RSDT1; **Table 2.1**). This original task was quickly adapted in response to rats' initial failures, with alternate trial types introduced to shape rats' responses (RSDT2-4; **Table 2.1**). Unfortunately, rats never reliably achieved above-chance task performance (>50% accuracy) within the 40 day study (**Graph 2.1A**). Learning was likely

limited by the development of side biases, which were considered sufficiently severe as to warrant termination of the study (**Figure 2.1C**). Rats' lack of task acquisition and side-bias development was observed irrespective of sex at all time-points.

Study 2 introduced spatial cues (RSDT5, RSDT6; **Table 3.1**) while still incorporating acquisition-promoting trial type distributions. Given that no effect of sex was observed on any measure in Study 1, only male rats were used in Studies 2 and 3 so as to limit factors and thereby allow for larger sample size (we intended to re-introduce the variable of sex in future studies). Nevertheless, sample sizes were limited in Study 2 due to the comparison of different sweep durations (200 vs. 500 ms) against tone discrimination training. Spatial cues were largely successful in promoting task acquisition, and all groups rapidly achieved above-chance performance (the 500 ms sweep group especially) (>65%; **Figure 7A**). Removal of these spatial cues resulted in chance-level performance, however (RSDT7; **Table 3.1**;**Graph 3.1F**). A brief re-assessment in the RSDT6 confirmed that rats could still perform the task at above 60% accuracy when fully defined spatial cues were provided (**Figure 7C**). Reduced spatial cues were then introduced (RSDT8; **Table 3.1**), but did not facilitate learning (**Graph 3.1F**). Although performance was statistically above chance levles, rats' ~55% accuracy (**Graph 3.1F**) was deemed insufficient to validate the task as a rat version of the human TCT. The study was terminated at this point. Though rats trained with 500 ms sweeps learned the spatial cue-augmented RSDT more quickly than the other two groups (**Graph 3.1A**), no significant difference between groups was observed within any RSDT program or across the entire study (**Graph 3.1C**). Nevertheless, this early effect of stimulus type on initial task acquisition recommended a sweep duration of 500 ms for subsequent studies.

Given that HAB2, the preliminary FR1 training program, trained rats to respond in either aperture for a reward, it was recognized that this early arbitrariness of response could interfere with the learning of specific auditory stimulus/aperture association in the RSDT; thus, the third study incorporated auditory stimuli into HAB2. The resultant new program, S_HAB2, was intended to a) begin association of a given auditory stimulus (a 500 ms upsweep or downsweep) with its corresponding aperture early, and to b) ease the transition from basic operant training to the RSDT (see Study 3 Primary Training). After a prolonged acquisition period (S_HAB2 acquisition having taken substantially longer than had HAB2 in previous studies; **Figure 5.1**), rats were moved to a version of the RSDT providing partial spatial cues (RSDT9, **Table 5.1**). Rats were trained in this paradigm for 60 days, during which time they never reliably performed at above-chance levels (**Graph 5.1A**). Rats were then trained for 10 days in a full-spatial cue version of the RSDT that had proven successful in promoting above-chance performance in Study 2 (RSDT5, **Table 5.1**). Rats performed statistically above chance with full spatial cues (**Graph 5.1A**), but still remained below 55% accuracy. Study 3 rats completed the RSDT5 with significantly lower accuracy than had the rats that had been initially trained with full spatial cues in the prior study (Study 2; **Graph 5.1D**). The 60 days of training with partial spatial cues had likely negatively affected Study 3 rats' ability to perform the RSDT with full spatial cues, which suggested that rats' receptivity to subsequent training modifications had been reduced. The study was ended following this conclusion.

The ultimate goal of the described training paradigms was to move rats from basic sweep discrimination tasks to more complicated versions with progressively greater face validity as models for human TCT. To wit, as rats became competent in these tasks (~90% accuracy), we intended to gradually incorporate more features of the human task until a training program

identical to the human version had been generated (i.e. two sweeps presented per trial instead of one, within-session modulation of task difficulty, etc.). Unfortunately, rats' inability to learn these early tasks prevented the attainment of this level of face validity; the only version of the RSDT that rats could complete with any semblance of reliability (~65%) was the version that provided heavy assistance in the form of spatial cues (RSDT6). Incorporating within-session task modulation on performance at ~65% accuracy was deemed unnecessary.

Having attained a limited level of face validity (e.g., frequency-modulated auditory stimuli, lateralized response outputs, etc.), the pharmacological predictive validity of the task was determined. D-AMPH has been recently demonstrated to facilitate the effects of sweep discrimination training in schizophrenia patients, which enabled us to perform a preliminary test of pharmacological predictive validity (albeit in normal, healthy rats) (Swerdlow et al., 2017). We failed to detect any significant main or interactive effects of d-AMPH on any measure of the RSDT6 vs vehicle (**Graph 4.1A,B**). Since d-AMPH had been previously reported to exert differential effects in rats exhibiting high vs low baseline task performance (Amitai et al., 2013), the data were subjected to a "median split" manipulation, so that data from rats demonstrating low baseline accuracy could be analyzed against those from rats demonstrating higher baseline accuracy. Although this analysis did not reveal any drug X baseline performance interaction, sample sizes of 4 rats per group prevented within-group interactive analyses. Closer inspection of group accuracy data, however, revealed that if sample sizes were artificially doubled, a significant enhancement of accuracy would emerge in 0.30 mg/kg-treated rats within the 500 ms sweeps group. The results of this manipulation imply that d-AMPH may, in fact, exert an effect on task performance that would become observable with larger sample sizes. A second study in pursuit

of this effect is currently underway, though time constraints prevented its inclusion in the current thesis.

Rats' unilaterally poor performance in most iterations of the RSDT was naturally disappointing. Expectations of better performance arose in-part from a previous study demonstrating that LE rats can reliably discriminate between 5 kHz tones of different durations (0.5 vs. 2 s) with at least 70% accuracy without the aid of spatial cues (full or partial)(Der-Avakian et al., 2013). The difficulties encountered by the present studies were therefore unlikely due to a basic inability of LE rats to learn an auditory discrimination task, but could instead reflect a limitation in study design and/or equipment. For example, overtraining was evident in Studies 2 and 3, which may have negatively affected learning of subsequent task versions. Given the absence of extant studies of auditory sweep discrimination in rodents, it was difficult to estimate acquisition time, and to therefore definitively identify performance plateaus. In the study cited above, LE rats required ~45 days to reach criterion (70% accuracy [33]), during which time rats logged long stretches of ostensibly stable, near-chance performance before spontaneously improving (Der-Avakian et al., 2013). This report had made interpretation of the progress of our own rats highly ambiguous, as the present studies, too, were largely characterized by long periods of low, stable performance (i.e. prolonged overtraining) (**Graph 3.4C, Graph 5.4A**). Overtraining in a given task can hinder rats' ability to modify their behavior when transferred to a new task with fundamentally different rules (Mackintosh, 1964) – a phenomenon that may explain rats' difficulty in transitioning from partial spatial cue conditions (RSDT8/9) to full spatial cue conditions (RSDT5/6).

Immediately after being (inadvertently) overtrained in the partial-cue RSDT8 in the final phase of Study 2 (46 days total; **Graph 3.1**), rats were retrained in the full-cue RSDT6 in Study

2A. Rats required 23 days of retraining to relearn the RSDT6, having apparently lost the ability to utilize full spatial cues (**Graph 4.1**). By way of comparison, when these same rats had been reassessed in the RSDT6 after 7 days of no-cue training earlier in Study 2 (RSDT7; **Graph 3.1**), it had been revealed that they had retained the ability to use spatial cues to complete the RSDT [no significant difference between D41 and D21-D35; $F_{(1,12)}<1$; **Graph 3.1C**]. This analysis shows that while 7 days of training in a task without spatial cues did not affect rats' ability to make use of spatial cues when they were made available, 46 days of training with only partial cues were sufficient to severely impair re-incorporation of full spatial cues into response strategies. Overtraining in a partial-cue program (RSDT9) exerted a similar effect in Study 3, though in this case it was the initial acquisition of full-cue use (RSDT5) that was affected, as opposed to re-acquisition. After training in the RSDT9 for 60 days, rats were trained in the RSDT5 for 10 days (**Figure 5.1**). Analysis of data from these 10 days against data gathered in Study 2 revealed that Study 2 rats were significantly more accurate during their first 10 days of full cue-assisted RSDT than Study 3 rats (**Graph 5.1D**). This result indicates that rats could more easily learn a spatially guided auditory task following completion of a basic non-auditory operant training program than following overtraining in an auditory task with only partial spatial cues.

While reward contingencies were the same between task versions, the full and partial spatial cue conditions exerted sufficiently differential effects upon performance that the two versions may be regarded as different tasks entirely. The sudden and severe drop in task performance observed when rats were moved from the full-cue RSDT6 to the no-cue RSDT7 in Study 2 (**Graph 3.1C**) suggests that the rats were not actually using the cues to associate auditory stimuli with their corresponding apertures. It would seem that instead, the rats were simply following stimuli to the correct aperture without noting stimulus quality (high or low, up or down),

and were therefore not building the associations necessary for the unassisted task – essentially, the full-cue task versions (RSDT5,6) were not auditory discrimination tasks, but auditory tracking tasks. Under the assumption that overtraining impairs rats' ability to modify their response strategies when transferred to a fundamentally different task (Mackintosh, 1964), it would follow that rats, when overtrained in a task in which auditory tracking was unfeasible (i.e. a genuine discrimination task), would have considerably more difficulty adjusting to the tracking task than would rats that had not been overtrained. Therefore, repeated extensions of study timeline and consequent overtraining likely directly hindered rats' receptiveness to subsequent training program modifications.

Equipment limitations also likely hindered learning in the RSDT. While Study 2 found sizeable differences in accuracy between full and partial cue conditions, rats' performance using partial cues was statistically and qualitatively indiscriminable from their performance with no spatial cues at all. Taken together, these results would imply that the partial spatial cues were not sufficiently defined to provide guidance. The dimensions of the sound-attenuating cabinets in which the training chambers were housed were such that the only possible way to position the speakers to provide an intermediate stimulus separation was to place them on top of the chamber (**Figure 1.2C**), rather than at the level of the response apertures. Although a hole in the ceiling enabled auditory stimuli to enter the chamber at sufficient volume (**Figure 1.1A**), it is likely that the acoustics of the chamber dissipated any lateralization of the stimuli. It is therefore probable that the "partial spatial cue" RSDT versions (RSDT8, 9) were in reality indistinguishable from the no-cue versions (RSDT1-4,7) in terms of rat experience.

This equipment limitation serendipitously validated the results from Study 1 by extending the window of observation of rats in a no spatial cue training regimen from 27 days to 60, and

thereby confirmed rats' inability to perform the unassisted task at above-chance levels. This equipment limitation also necessitated reinterpretation of results, however. Assuming that delivery of partial cues was ineffectual, Study 2 found that 28 days of training using full spatial cues (RSDT5+RSDT6) did not facilitate acquisition of sweep discrimination across 52 subsequent days of unassisted training (RSDT7+RSDT8). Meanwhile, Study 3 revealed that incorporating an auditory component into basic operant training (S_HAB2) was similarly ineffective on learning across 60 subsequent days of unguided training. Importantly, it remains possible that partial spatial cues may facilitate acquisition of sweep discrimination; however, operant chambers would need to be designed in such a way as to enable delivery.

Of all of the task iterations implemented across the three studies, rat performance was highest in the RSDT5 and RSDT6, which incorporated full auditory stimulus separation. Findings from these study phases raised an important issue in terms of stimulus audibility, however. Prior to initiation of testing, stimuli were verified to reach the interior of each training chamber at a volume of 48-50 dB, which, though lower than other rat auditory tasks (usually 60 dB, as in (Der-Avakian et al., 2013)), should theoretically have been sufficient given rats' auditory thresholds for the frequencies utilized (Kelly and Masterton, 1977). However, while the RSDT5 and RSDT6 were ultimately simple auditory tracking tasks (see above), rats' performance plateaued at only 55-70% accuracy (average across all rats = ~60%; **Graph 3.1C**). Given the minimal cognitive load of such tasks, performance should theoretically have been substantially higher; indeed, Burlile *et. al.* demonstrated that rats can readily learn an auditory tracking task to near perfection within a single session (4.5-50 kHz stimuli at 77dB)(Burlile et al., 1985). The incongruity between these results and those of the present studies is not likely due to behavioral aberrations of Study 2&3 rats, as measures of impulsivity and apathy (% premature responses and % omissions, respectively)

were not meaningfully outside normal parameters for tasks of vigilance and impulse control (Bari et al., 2008). The mediocre task performance observed in the present studies is therefore likely due to sub-optimal stimulus specifications.

Frequency was likely a more limiting factor than volume in the context of the present studies. While we did use a lower stimulus volume than is commonly employed by similar studies (see above), the difference was only ~10 dB (~17% lower than the lowest standard volume). The frequencies used by the present studies, however, deviated more meaningfully from optimal levels than did volume. Although estimates of rat audibility vary widely within extant literature, there is agreement that rats' hearing thresholds are lowest at frequencies between 8 kHz and 40 kHz (Borg, 1982; Cowles, 1943; Gourevitch and Hack, 1966; Harrison and Turnock, 1975; Jamison, 1951; Kelly and Masterton, 1977), indicating that rats are most sensitive to frequencies within that range. These "optimal" frequencies (the findings of Kelly & Masterson excepted (Kelly and Masterton, 1977)) lie entirely outside of the sweep ranges utilized by our studies. Although other studies have succeeded in training rats to respond to auditory stimuli of frequencies similar to ours (Der-Avakian et al., 2013; Der-Avakian et al., 2017; Floresco et al., 2018), these studies did not have the additional limitation of low volume. Rat auditory threshold curves clearly illustrate that audibility of lower frequencies requires higher tone volume than does audibility of higher frequencies (Borg, 1982; Cowles, 1943; Gourevitch and Hack, 1966; Harrison and Turnock, 1975; Jamison, 1951; Kelly and Masterton, 1977); this relationship implies that concomitant reduction of both frequency and volume would likely have a far greater impact on rats' ability to detect auditory stimuli than would reduction of one factor alone. Critically, any such reduction in stimulus detectability would likely translate to impairment in stimulus response, and may therefore explain the behavior observed in the present studies' tracking (full cue) paradigms.

Tonotopic mapping of the auditory cortex reveals a further limitation of the frequencies utilized by the present studies. Consistent with the audiograms referenced above, only 20% of the available cortical area is allocated to the lower four octaves of rat audibility range (~0.5-8 kHz), whereas the upper three octaves (~8-64 kHz) are represented by the remaining 80% (Sally and Kelly, 1988). Given that approximately 75% of the frequency window spanned by the Study 2&3 sweep stimuli fell within the bottom four octaves of rat audibility, it is possible that low cortical representation accounted for at least some of the sweeps' directional ambiguity. This putative link between cortical representation and discriminability follows from the hypothesis that tone discrimination may partly rely upon the spatial distance between frequency representations on the tonotopic map, the resolution of which increases with area of representation (Recanzone et al., 1993). Since sweep directionality is determined by subtle changes in component frequencies across stimulus presentation it is likely that sweep discrimination is even more heavily reliant upon cortical area (and therefore frequency range) than is simple tone discrimination.

Tonotopic mapping reveals a further relationship between stimulus frequency and perceptual resolution – individual neurons' sharpness of tuning is strongly and directly correlated with their characteristic frequencies. "Sharply" tuned neurons respond to a smaller range of frequencies (i.e. have greater specificity of response) than less sharply tuned neurons; in rats, neurons with higher characteristic frequencies (i.e. neurons that preferentially fire in response to higher frequencies) exhibit sharper tuning than neurons with lower characteristic frequencies (Sally and Kelly, 1988). Auditory sweeps, therefore, would vary not only in frequency across their presentation, but in perceptual resolution as well – to wit, as a sweep progresses, there should ostensibly occur some fold increase or decrease in sharpness of rats' auditory neuronal tuning. Following the characteristic frequency x Q_{10} (tuning sharpness) curve generated by Sally and Kelly

(Sally and Kelly, 1988), tuning to the high frequency used in Studies 2 and 3 (9.75 kHz) would have been approximately twice as sharp as to the low frequency (3 kHz); therefore, each sweep delivered in these studies would have either doubled or halved in resolution across its presentation.

This within-stimulus variability of resolution is fundamentally unavoidable in studies of sweep discrimination, though it does inform the design of future studies. Sweep directionality may be ambiguous if its component frequencies can only be perceived with low to middling resolution, as may have been the case in the present studies. This problem could potentially be mitigated by broadening sweeps' ranges to include ultrasonic frequencies, to which rats' cortical neurons are most sharply tuned. Theoretically, rats may be able to determine the directionality of sweeps with ambiguous lower frequencies based on the temporal position of the high-resolution upper frequency range (i.e. if a high resolution frequency range emerges from a low resolution frequency range, then the stimulus must be an upsweep). If this hypothesis is true, then rats' accuracy should increase with the difference in resolution between the high and low sweep components – given that neuron tuning sharpness increases as a function of characteristic frequency (Sally and Kelly, 1988), raising sweeps' upper frequency limit would increase this difference in resolution and thereby enhance discriminability.

Taking into consideration tonotopic mapping data together with rat auditory thresholds, an initial sweep frequency range of ~8-40 kHz may be recommended for future studies, in addition to a stimulus volume of at least 60 dB. Behavioral audiograms generated by Kelly and Masterson report that rats are most sensitive to tones of 8 and (to a lesser extent) 38 kHz (Kelly and Masterton, 1977), within which bounds other studies report alternative optimal frequencies (Borg, 1982; Cowles, 1943; Gourevitch and Hack, 1966; Harrison and Turnock, 1975; Jamison, 1951). In addition to providing good coverage of rats' auditory range, using these frequencies should also

enhance stimulus resolution by a) maximizing cortical spatial representation and b) engaging large populations of sharply tuned neurons. This proposed 32 kHz frequency range is of course considerably larger than that used in the human task (initial range = 1.5 kHz) (Swerdlow et al., 2017); however, this range would only be used during the early phases of training, and would be gradually narrowed thereafter. In a manner similar to the present studies' introduction of spatial cues and alternate trial types to facilitate task acquisition, future studies may be able to use frequency window to shape rats' behavior. Rats can readily learn an auditory discrimination task when the frequencies are sufficiently distinct – recent findings show that LE rats can learn to discriminate between complex auditory stimuli of low (5-10 kHz) and high (20-40 kHz) average frequencies at >85% accuracy within 10 days of training (Xiong et al., 2015). While these auditory stimuli were not sweeps, they were similar to sweeps in terms of salience (both being complex, "interesting" sounds), and were therefore likely to elicit a similar level of engagement from rats; conceivably, learning a discrimination task using these two stimulus types should be fairly similar. Determination of sweep directionality is dependent upon the ability to discriminate between high and low frequency ranges – an ability which, following the results of the above study, rats can readily acquire. It is therefore highly possible that if the frequencies used in the present studies were more distinct from each other (as they were in the above study), rats would have been able to acquire the behavior with minimal assistance.

In summary, attempts to develop a rat model of the Auditory Sweep Discrimination component of the Posit Science TCT regimen (the Rat Sweep Discrimination Task, RSDT) did not yield a task that was sufficiently refined for immediate application; however, we did succeed in generating a spatial cue-assisted version of the rat task that, pending an adequately powered drug study, may yet be demonstrated to have pharmacological predictive validity as a model of the

human task. Rats' acquisition and performance of the various RSDT versions developed may have been hindered by sub-optimal stimulus specifications, as well as by prolonged periods of overtraining. Future attempts to develop more reliable and face-valid versions of the RSDT may therefore be considered optimistically, given that much of the difficulty encountered by the described studies may be prevented by simply increasing stimulus frequency range and volume, and by avoiding repeated extensions of study timeline. Considerable upgrades to existing equipment are necessary before we can implement the former solution, however, and a more reliable method of detecting genuine performance plateaus is required for the latter.

Appendix

Supplemental Table 1. RSDT Program Specifications, Studies1-3.

Program	Trial Type Distribution	Frequencies	Stimulus Duration	Timeout Duration	Spatial Cues	Study
RSDT1	120S	4,7 kHz	1000 ms	4s	None	1
RSDT2	120F	4,7 kHz	1000 ms	4s	None	1
RSDT3	80S, 20G, 20FCα	4,7 kHz	1000 ms	4s	None	1
RSDT4	80S, 20G, 20FCα	4,7 kHz	1000 ms	7s	None	1
RSDT5	40S, 40F, 40G	4,7 kHz / 3,9.75 kHz*	200/500 ms**†	4s	Full	2, 3
RSDT6	70S, 40F, 10G	4,7 kHz / 3,9.75 kHz*	200/500 ms**	4s	Full	2, 2A
RSDT6A	120S	3,9.75 kHz	200/500 ms**	4s	Full	2A
RSDT6B	210S	3,9.75 kHz	200/500 ms**	4s	Full	2A
RSDT7	70S, 40F, 10G	3,9.75 kHz	200/500 ms**	4s	None	2
RSDT8	70S, 40F, 10G	4,7 kHz/3,9.75 kHz*	200/500 ms**	4s	Partial	2
RSDT9	40S, 40F, 40G	3,9.75 kHz	500 ms	4s	Partial	3

*: Changed during Study 2 (RSDT8), 2A (RSDT6), or 3 (RSDT5)
**: Stimulus duration was either 200 or 500 ms, depending upon group
†: Only 500 ms used during Study 3
RSDT: Rat Sweep Discrimination Task
S: Standard Trial; F: Forced Choice Trial; G: Guided Trial; FCα: Alternative Forced Choice Trial
Note: See Figure 2 and Table 2 for speaker separation (spatial cues) schematics and trial type definitions.

Supplemental Table 2. Primary & Secondary Outcome Variables; Study 2A; Group x d-AMPH

	500 ms Tone				200 ms Sweep				500 ms Sweep			
	VEH	0.10 mg/kg	0.25 mg/kg	0.30 mg/kg	VEH	0.10 mg/kg	0.25 mg/kg	0.30 mg/kg	VEH	0.10 mg/kg	0.25 mg/kg	0.30 mg/kg
% Accuracy	64.1±4.9	62.3±5.6	63.9±5.7	63.9±5.8	65.5±4.9	62.4±5.6	65.6±5.7	64.5±5.8	64.1±4.9	59.5±5.6	68.4±5.7	68.1±5.8
% Prem. Resp.	4.82±3.32	6.88±3.41	4.57±3.60	9.38±3.57	8.88±3.32	10.83±3.41	6.63±3.60	9.19±3.56	10.28±3.32	10.21±3.41	11.16±3.60	7.08±3.56
% Omissions	0.18±0.12	0.00±0.12	0.00±0.16	0.63±0.98	0.00±0.12	0.21±0.12	0.38±0.16	1.64±0.98	0.18±0.12	0.00±0.12	0.25±0.16	0.21±0.98
Correct Lat.	70.9±13.8	70.9±13.6	80.2±16.3	65.5±23.8	62.7±13.8	64.8±13.6	72.8±16.3	82.8±23.8	73.3±13.8	73.8±13.6	67.9±16.3	72.3±23.8
Incorrect Lat.	78.7±16.8	95.3±16.3	88.8±17.2	75.0±21.5	78.4±16.8	71.1±16.3	82.1±17.2	92.9±21.5	81.5±16.8	88.5±16.3	67.9±17.2	69.1±21.5
Reward Lat.	98.8±6.9	97.5±7.6	103.3±7.5	97.9±5.7	117.7±6.9	111.0±7.6	118.95±7.5	114.7±5.7	102.4±6.9	101.4±7.6	104.0±7.5	98.3±5.7

Bold indicates primary outcome variables.

% Accuracy = % correct responses; % Prem. Resp. = % of trials ended by a premature response; % Omissions = % of trials in which no response to auditory stimulus was made; Lat. = Latency, the time (centi-seconds) elapsed before the indicated event: a) a correct response, b) an incorrect response, or c) collection of a reward.

Data represented as means ±S.E.M.

Bibliography

Adcock, R. A., Dale, C., Fisher, M., Aldebot, S., Genevsky, A., Simpson, G. V., Nagarajan, S., Vinogradov, S., 2009. When top-down meets bottom-up: auditory training enhances verbal memory in schizophrenia. Schizophr Bull 35, 1132-1141.

Amitai, N., Weber, M., Swerdlow, N. R., Sharp, R. F., Breier, M. R., Halberstadt, A. L., Young, J. W., 2013. A novel visuospatial priming task for rats with relevance to Tourette syndrome and modulation of dopamine levels. Neurosci Biobehav Rev 37, 1139-1149.

Andrzejewski, M. E., Spencer, R. C., Harris, R. L., Feit, E. C., McKee, B. L., Berridge, C. W., 2014. The effects of clinically relevant doses of amphetamine and methylphenidate on signal detection and DRL in rats. Neuropharmacology 79, 634-641.

Bari, A., Dalley, J. W., Robbins, T. W., 2008. The application of the 5-choice serial reaction time task for the assessment of visual attentional processes and impulse control in rats. Nat Protoc 3, 759-767.

Biagianti, B., Fisher, M., Neilands, T. B., Loewy, R., Vinogradov, S., 2016. Engagement with the auditory processing system during targeted auditory cognitive training mediates changes in cognitive outcomes in individuals with schizophrenia. Neuropsychology 30, 998-1008.

Borg, E., 1982. Auditory thresholds in rats of different age and strain. A behavioral and electrophysiological study. Hear Res 8, 101-115.

Burlile, C. J., Feldman, M. L., Craig, C., Harrison, J. M., 1985. Control of responding by the location of sound: role of binaural cues. J Exp Anal Behav 43, 315-319.

Cope, Z. A., Powell, S. B., Young, J. W., 2016. Modeling neurodevelopmental cognitive deficits in tasks with cross-species translational validity. Genes Brain Behav 15, 27-44.

Cowles, J. T., Pennington, L.A., 1943. An Improved Conditioning Technique for Determining Auditory Acuity of the Rat. J. Psychol. 15, 41-47.

Dale, C. L., Brown, E. G., Fisher, M., Herman, A. B., Dowling, A. F., Hinkley, L. B., Subramaniam, K., Nagarajan, S. S., Vinogradov, S., 2016. Auditory Cortical Plasticity Drives Training-Induced Cognitive Changes in Schizophrenia. Schizophr Bull 42, 220-228.

Der-Avakian, A., D'Souza, M. S., Pizzagalli, D. A., Markou, A., 2013. Assessment of reward responsiveness in the response bias probabilistic reward task in rats: implications for cross-species translational research. Transl Psychiatry 3, e297.

Der-Avakian, A., D'Souza, M. S., Potter, D. N., Chartoff, E. H., Carlezon, W. A., Jr., Pizzagalli, D. A., Markou, A., 2017. Social defeat disrupts reward learning and potentiates striatal nociceptin/orphanin FQ mRNA in rats. Psychopharmacology (Berl) 234, 1603-1614.

Fisher, M., Herman, A., Stephens, D. B., Vinogradov, S., 2016. Neuroscience-informed computer-assisted cognitive training in schizophrenia. Ann N Y Acad Sci 1366, 90-114.

Fisher, M., Holland, C., Merzenich, M. M., Vinogradov, S., 2009. Using neuroplasticity-based auditory training to improve verbal memory in schizophrenia. Am J Psychiatry 166, 805-811.

Fisher, M., Holland, C., Subramaniam, K., Vinogradov, S., 2010. Neuroplasticity-based cognitive training in schizophrenia: an interim report on the effects 6 months later. Schizophr Bull 36, 869-879.

Fisher, M., Loewy, R., Carter, C., Lee, A., Ragland, J. D., Niendam, T., Schlosser, D., Pham, L., Miskovich, T., Vinogradov, S., 2015. Neuroplasticity-based auditory training via laptop computer improves cognition in young individuals with recent onset schizophrenia. Schizophr Bull 41, 250-258.

Floresco, S. B., Montes, D. R., Tse, M. M. T., van Holstein, M., 2018. Differential Contributions of Nucleus Accumbens Subregions to Cue-Guided Risk/Reward Decision Making and Implementation of Conditional Rules. J Neurosci 38, 1901-1914.

Gourevitch, G., Hack, M. H., 1966. Audibility in the rat. J Comp Physiol Psychol 62, 289-291.

Green, M. F., Kern, R. S., Heaton, R. K., 2004. Longitudinal studies of cognition and functional outcome in schizophrenia: implications for MATRICS. Schizophr Res 72, 41-51.

Grilly, D. M., Pistell, P. J., Simon, B. B., 1998. Facilitation of stimulus detection performance of rats with d-amphetamine: a function of dose and level of training. Psychopharmacology (Berl) 140, 272-278.

Grottick, A. J., Higgins, G. A., 2002. Assessing a vigilance decrement in aged rats: effects of pre-feeding, task manipulation, and psychostimulants. Psychopharmacology (Berl) 164, 33-41.

Harrison, J. M., Turnock, M. T., 1975. Animal psychophysics: improvements in the tracking method. J Exp Anal Behav 23, 141-147.

Jamison, J. H., 1951. Measurement of auditory intensity thresholds in the rat by conditioning of an autonomic response. J Comp Physiol Psychol 44, 118-125.

Kelly, J. B., Masterton, B., 1977. Auditory sensitivity of the albino rat. J Comp Physiol Psychol 91, 930-936.

Mackintosh, N. J., 1964. Overtraining and transfer within and between dimensions in the rat. Quarterly Journal of Experimental Psychology 16, 250-256.

MacQueen, D. A., Minassian, A., Kenton, J. A., Geyer, M. A., Perry, W., Brigman, J. L., Young, J. W., 2018. Amphetamine improves mouse and human attention in the 5-choice continuous performance test. Neuropharmacology 138, 87-96.

McGaughy, J., Sarter, M., 1995. Behavioral vigilance in rats: task validation and effects of age, amphetamine, and benzodiazepine receptor ligands. Psychopharmacology (Berl) 117, 340-357.

Murthy, N. V., Mahncke, H., Wexler, B. E., Maruff, P., Inamdar, A., Zucchetto, M., Lund, J., Shabbir, S., Shergill, S., Keshavan, M., Kapur, S., Laruelle, M., Alexander, R., 2012. Computerized cognitive remediation training for schizophrenia: an open label, multi-site, multinational methodology study. Schizophr Res 139, 87-91.

Paterson, N. E., Ricciardi, J., Wetzler, C., Hanania, T., 2011. Sub-optimal performance in the 5-choice serial reaction time task in rats was sensitive to methylphenidate, atomoxetine and d-amphetamine, but unaffected by the COMT inhibitor tolcapone. Neurosci Res 69, 41-50.

Popov, T., Jordanov, T., Rockstroh, B., Elbert, T., Merzenich, M. M., Miller, G. A., 2011. Specific cognitive training normalizes auditory sensory gating in schizophrenia: a randomized trial. Biol Psychiatry 69, 465-471.

Portfors, C. V., 2007. Types and functions of ultrasonic vocalizations in laboratory rats and mice. J Am Assoc Lab Anim Sci 46, 28-34.

Ramsay, I. S., Fryer, S., Boos, A., Roach, B. J., Fisher, M., Loewy, R., Vinogradov, S., Mathalon, D. H., 2018. Response to Targeted Cognitive Training Correlates with Change in Thalamic Volume in a Randomized Trial for Early Schizophrenia. Neuropsychopharmacology 43, 590-597.

Rapoport, J. L., Buchsbaum, M. S., Weingartner, H., Zahn, T. P., Ludlow, C., Mikkelsen, E. J., 1980. Dextroamphetamine. Its cognitive and behavioral effects in normal and hyperactive boys and normal men. Arch Gen Psychiatry 37, 933-943.

Recanzone, G. H., Schreiner, C. E., Merzenich, M. M., 1993. Plasticity in the frequency representation of primary auditory cortex following discrimination training in adult owl monkeys. J Neurosci 13, 87-103.

Sally, S. L., Kelly, J. B., 1988. Organization of auditory cortex in the albino rat: sound frequency. J Neurophysiol 59, 1627-1638.

Swerdlow, N. R., 2011. Are we studying and treating schizophrenia correctly? Schizophr Res 130, 1-10.

Swerdlow, N. R., 2012. Beyond antipsychotics: pharmacologically-augmented cognitive therapies (PACTs) for schizophrenia. Neuropsychopharmacology 37, 310-311.
Swerdlow, N. R., Bhakta, S. G., Light, G. A., 2018. Room to move: Plasticity in early auditory information processing and auditory learning in schizophrenia revealed by acute pharmacological challenge. Schizophr Res.

Swerdlow, N. R., Tarasenko, M., Bhakta, S. G., Talledo, J., Alvarez, A. I., Hughes, E. L., Rana, B., Vinogradov, S., Light, G. A., 2017. Amphetamine Enhances Gains in Auditory Discrimination Training in Adult Schizophrenia Patients. Schizophr Bull 43, 872-880.

Thomas, M. L., Bismark, A. W., Joshi, Y. B., Tarasenko, M., Treichler, E. B. H., Hochberger, W. C., Zhang, W., Nungaray, J., Sprock, J., Cardoso, L., Tiernan, K., Attarha, M., Braff, D. L., Vinogradov, S., Swerdlow, N., Light, G. A., 2018. Targeted cognitive training improves auditory and verbal outcomes among treatment refractory schizophrenia patients mandated to residential care. Schizophr Res.

Vinogradov, S., Fisher, M., de Villers-Sidani, E., 2012. Cognitive training for impaired neural systems in neuropsychiatric illness. Neuropsychopharmacology 37, 43-76.

Xiong, Q., Znamenskiy, P., Zador, A. M., 2015. Selective corticostriatal plasticity during acquisition of an auditory discrimination task. Nature 521, 348-351.

Young, J. W., Geyer, M. A., 2015. Developing treatments for cognitive deficits in schizophrenia: the challenge of translation. J Psychopharmacol 29, 178-196.

Young, J. W., Geyer, M. A., Rissling, A. J., Sharp, R. F., Eyler, L. T., Asgaard, G. L., Light, G. A., 2013. Reverse translation of the rodent 5C-CPT reveals that the impaired attention of people with schizophrenia is similar to scopolamine-induced deficits in mice. Transl Psychiatry 3, e324.