Effects of Nicotine on Response Inhibition and Fos Activation in Spontaneously Hypertensive and Wistar Kyoto Rats

by

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ABSTRACT

Smoking remains the leading cause of preventable death in the United States, and early initiation is associated with greater difficulty quitting. Among adolescent smokers, those with attention-deficit hyperactivity disorder (ADHD), characterized by difficulties associated with impulsivity, hyperactivity, and inattention, smoke at nearly twice the rate of their peers. Although cigarette smoking is highly addictive, nicotine is a relatively weak primary reinforcer, spurring research on other potential targets that may maintain smoking, including the potential benefits of nicotine on attention, inhibition, and reinforcer efficacy. The present study employs the most prevalent rodent model of ADHD, the spontaneously hypertensive rat (SHR) and its control comparison Wistar Kyoto (WKY) to examine the effects of acute and chronic subcutaneous nicotine injections on performance in three operant response inhibition paradigms. Functional activation in select regions of the prefrontal cortex and striatum was also explored. Acute (0.1, 0.3, 0.6 mg/kg) and chronic (0.3 mg/kg) nicotine increased impulsive responding regardless of strain, dose, or operant schedule. Dose-dependent decreases in latency to initiate the task were also observed. SHR receiving daily nicotine injections showed less activation in the nucleus accumbens shell compared to saline controls. Despite close similarities, one of the three operant tasks did not detect response inhibition deficits in SHR relative to WKY. A closer examination of these tasks may highlight critical components involved in the amelioration of response inhibition deficits.

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CHAPTER 1

GENERAL INTRODUCTION

Response inhibition is broadly defined as the ability to withhold a response (Bardo, Cain, & Bylica, 2006). Response inhibition deficits are a central feature of several psychiatric disorders, including attention-deficit hyperactivity disorder (ADHD), bipolar disorder, and substance use disorders (Grant, Levine, Kim, & Potenza, 2005). Accurate assessment of these deficits is critical to accomplishing three goals: (A) identifying people in need of treatment, (B) identifying environmental arrangements or drugs that alleviate the deficits, and (C) uncovering the neural underpinnings of the behavioral deficit.

Although disorders of inhibitory control are typically diagnosed using clinical interviews and behavior rating scales (Costello, Edelbrock, Kalas, Kessler, & Klaric, 1982; Shaffer, Fisher, Lucas, Dulcan, & Schwab-Stone, 2000), laboratory measures offer several advantages. Laboratory tasks can be administered relatively quickly compared to clinical diagnoses, thereby reducing costs and allowing for more participants or more frequent observations. Increased ease of administration also facilitates data collection at multiple time points, or in conjunction with the manipulation of variables, e.g., reinforcement contingencies, drugs, or distractors. Laboratory tasks rely on quantitative dependent measures that are more amenable to replication than qualitative ones, increasing overall consistency. Lastly, laboratory tasks used on human subjects often translate well to experiments with non-human animals, whereas clinical reports such as interviews or parent/teacher ratings offer no animal analogue (Nichols & Waschbusch, 2004).

Behavioral tasks designed to assess response inhibition typically require subjects to withhold a previously reinforced response. Beyond that similarity, however, response inhibition tasks vary widely in their design and implementation, and the inferences that can be drawn from their dependent measures. We begin by discussing several standard response inhibition tasks used with humans and animals. After evaluating the contribution of each task to the three aforementioned goals, we offer a potential alternative task, and introduce a quantitative model to further enhance the resolution of response inhibition data.

Go/no-go and stop signal task

Response inhibition is often assessed in humans using variations of the go/no-go task (Newman, Widom, & Nathan, 1985; Wodka et al., 2007), or the stop signal task (SST; Aron, Fletcher, Bullmore, Sahakian, & Robbins, 2003; Logan, Schachar, & Tannock, 1997). Both tasks are designed to measure 'action inhibition,' or the ability to inhibit a pre-planned physical response (Eagle, Bari, & Robbins, 2008). Participants are required to perform hurried responses on "go" trials and withhold responses on 'no-go' or 'stop' trials (Aron, Fletcher, et al., 2003). In go/no-go tasks, subjects must respond quickly to a "go" stimulus on the majority of trials, and withhold responding in the presence of a "stop" stimulus on other trials. Estimates of action inhibition from go/no-go performance are calculated from the proportion of errors of commission, i.e., the proportion of incorrect responses during "stop" trials. In the SST, a "go" stimulus is presented on *every* trial; on select trials a "stop" signal is presented shortly after the "go" signal, thus participants must inhibit responding *after* they have already been presented

with a "go" stimulus. The dependent measure, the stop-signal reaction time, is an estimate of the minimum time needed to execute the stop response, which is derived from Logan and Cowan's (1984) race model.

The SST estimates the speed at which an ongoing response is inhibited, whereas the go/no-go task is an estimate of the ability to inhibit the initiation of a response. Although the distinction may seem slight, the go/no-go task involves response choice selection in addition to inhibition, whereas the SST only requires the cancellation of a response that has already been selected (Dalley, Everitt, & Robbins, 2011; Eagle et al., 2008; Eagle & Baunez, 2010). As a result, the SST is regarded as more dynamic and "cognitively pure" than the go/no-go task (Aron & Poldrack, 2005).

Discriminant validity, or the ability to accurately discriminate psychiatric populations from normal controls, is an important feature of a response inhibition task. A meta-analysis of seven studies reported that SST accurately distinguishes children with ADHD from normal controls (Oosterlaan, Logan, & Sergeant, 1998). Poor performance on the SST has been correlated with off task and hyperactive behavior in classroom situations (r = .65), and with teacher ratings of inattention and hyperactivity (r = .37) (Pliszka, Borcherding, Spratley, Leon, & Irick, 1997). Similarly, results from go/no-go tasks show that children with ADHD produce more errors than controls (Van der Meere, Marzocchi, & De Meo, 2005; Wodka et al., 2007).

SST performance is also sensitive to pharmacological effects. Stop signal performance improves under the administration of various stimulants, including those used to treat ADHD (Aron, Dowson, Sahakian, & Robbins, 2003; Boonstra, Kooij, Oosterlaan, Sergeant, & Buitelaar, 2005; de Wit, Enggasser, & Richards, 2002; Eagle et

al., 2008), as well as modafinil (R. E. Morgan, Crowley, Smith, LaRoche, & Dopheide, 2007). Interestingly, these drugs improve the stop signal reaction time without affecting the "go" reaction time, or the time required to initiate a response. This suggests that these stimulants (MPH, d-amphetamine, modafinil) are affecting processes related to inhibition, not simply improving overall reaction time. Alternatively, cocaine *increases* stop signal reaction time (Fillmore, Rush, & Hays, 2002), supporting existing evidence that cocaine is detrimental to response inhibition in humans (C. R. Li, Milivojevic, Kemp, Hong, & Sinha, 2006; Moeller et al., 2002) and non-human primates (Jentsch & Taylor, 1999; Liu, Heitz, & Bradberry, 2009).

Animal analogues to the go/no-go task and SST have been used in lesion studies and with knockout mice, to explore the neurobiology underlying response inhibition (for review, see Eagle, Bari, & Robbins, 2008). Go/no-go tasks have also provided evidence that a variety of drugs are detrimental to action inhibition, including nicotine in rodents (Kolokotroni, Rodgers, & Harrison, 2011), selective α-2 antagonists in primates (Arnsten & Li, 2004), and alcohol and ketamine in humans (Finn, Justus, Mazas, & Steinmetz, 1999; C. J. Morgan, Mofeez, Brandner, Bromley, & Curran, 2004).

The SST and go/no-go tasks are generally regarded as useful tools for studying response inhibition, but they are not without their limitations. An inability to withhold responding in either task might be interpreted as deficient motor processing, not inhibition (Van der Meere et al., 2005). To account for this possibility, the "go" reaction time is used to measure the relative duration required to execute the response, independent of inhibitory processing. Manipulations that affect inhibitory performance, but not "go" reaction time are presumed to operate on inhibitory processes, whereas

manipulations that affect both become difficult to interpret. This limits the utility of these tasks for studying the effects of drugs that directly affect reaction time, such as alcohol. The SST utilizes visual go signals, but auditory stop signals, drawing criticism because children with ADHD may be particularly poor at auditory processing (Nigg, 2000; Van der Meere et al., 2005).

Differential reinforcement of low rates schedule

Another method for assessing response inhibition is the differential reinforcement of low rates (DRL) schedule (Skinner, 1938). DRL requires subjects to wait a specified interval between consecutive responses; only responses made after the interval elapses are considered 'correct,' and are reinforced. Responses are often made on a mechanical lever or button, but DRL schedules can be administered manually as well. Their simple design and implementation makes them highly attractive to laboratory researchers. Unlike the SST and go/no-go tasks, which require the inhibition of responses to discrete stimuli, DRL is considered a measure of 'waiting impulsivity,' (Eagle et al., 2008; Robinson et al., 2009) because subjects must inhibit responding for a specific period of time, i.e., successfully 'wait' in between responses. Performance can be summarized by the proportion of correct responses, or by the mean waiting interval (i.e., mean duration between consecutive responses).

Although DRL schedules originated from early operant animal work and remain more prevalent there, they have been shown to discriminate between children with and without ADHD (Gordon, 1979; McClure & Gordon, 1984; Solanto, 1990) and children

with conduct disorder (Shapiro, Quay, Hogan, & Schwartz, 1988), while others have found no differences (Daugherty & Quay, 1991).

Despite a relatively small body of supporting clinical literature, DRL is still widely used in animal research as a response inhibition task. DRL schedules have been used with animals to examine the effects of a variety of drugs on response inhibition, including nicotine (Kirshenbaum et al., 2011; Kirshenbaum, Brown, Hughes, & Doughty, 2008; Kirshenbaum, Johnson, Schwarz, & Jackson, 2009; C. Morrison & Armitage, 1967; C. F. Morrison, 1968; Popke, Fogle, & Paule, 2000; Popke, Mayorga, Fogle, & Paule, 2000), caffeine (Webb & Levine, 1978), and cocaine (Wenger & Wright, 1990; Woolverton, Kandel, & Schuster, 1978). In general, these stimulants reduce the time between consecutive responses in humans and non-human animals, suggesting that stimulants increase impulsive responding. However, stimulant medications used to treat ADHD, such as MPH or d-amphetamine, also appear to impair DRL responding (Emmett-Oglesby, Taylor, & Dafter, 1980; Ferguson et al., 2007; Seiden, Andresen, & MacPhail, 1979) or have no effect (Andrzejewski et al., 2014). These results are inconsistent with evidence from human literature suggesting that ADHD medications *improve* inhibitory control.

The failure of DRL schedules to corroborate human literature on the beneficial effects of ADHD medications on inhibitory control highlights a serious limitation of the task. One potential explanation is that DRL performance relies on time estimation, which may be altered by stimulants. If stimulant drugs cause time to be overestimated (i.e., time seems to pass more quickly), this could increase premature responding under DRL schedules.

Alternatively, the sensitivity of DRL to changes in motivation may underlie deficient performance under MPH and d-AMPH. In DRL schedules, the ability to withhold a response is confounded with reinforcer efficacy (Doughty & Richards, 2002; Hill, Covarrubias, Terry, & Sanabria, 2012). Hence DRL performance worsens as the quality of the reinforcer or deprivation level increases; under circumstances where the reinforcer is devalued, animals tend to respond less frequently. In DRL schedules, where reinforcement is contingent on waiting, infrequent responding may actually improve the proportion of correct responses, and increase the duration of the mean waiting interval. If MPH or d-AMPH increase the reinforcing efficacy of food reward, the result could be deficient DRL performance, regardless of the drugs' effects on inhibitory control.

A potential alternative: the Fixed Minimum Interval (FMI) schedule

The Fixed Minimum Interval (FMI) schedule of reinforcement (Mechner & Guevrekian, 1962), like the DRL schedule, is a form of 'waiting' task that purports to measure response inhibition (Hill et al., 2012). It is implemented similarly to DRL schedules, but in FMI schedules the response that initiates the waiting interval differs from the response that terminates it.

In FMI schedules, reinforcement is contingent upon successfully withholding a target response for a programmed time interval. Intervals are initiated with a lever press and terminated with a head entry into the food hopper (the target response). Head entries made *after* the programmed interval elapses are reinforced. Although this arrangement is similar to the DRL schedule, a key difference is that in FMI schedules the topography and location of the initial response (lever press) differs from the terminal response (head

entry). In this way, FMI schedules dissociate the capacity to withhold the target response (inferred from the intervals between initial and terminal responses) from the efficacy of the reinforcer in maintaining initial responses (inferred from the intervals between trial onset and initial response) (Hill et al., 2012; Mechner & Guevrekian, 1962).

FMI schedules have been used rather sparingly in comparison to DRL. More recently, however, our laboratory has evaluated FMI schedules as an alternative to DRL. Using FMI schedules we have shown that the mean waiting interval, an index of inhibitory performance, decreases under chronic stress (Mika et al., 2012), and increases under acute MPH (Hill et al., 2012). The latter finding is particularly important when considering the effects of MPH on DRL performance. If FMI schedules are sensitive to ameliorative effects of MPH, they may offer added utility over DRL schedules in the evaluation of drugs that alleviate response inhibition deficits.

The purpose of Chapters 2 and 3 was to evaluate FMI performance in response to acute (Ch. 2) and chronic (Ch. 3) nicotine, a drug with potentially therapeutic effects on ADHD symptoms (see Ch. 2 introduction for details). Chapter 4 aims at identifying causes for the differences in performance observed between FMI and DRL schedules.

The temporal regulation model

In each experiment presented here, the temporal regulation (TR) model (Hill et al., 2012; Mika et al., 2012; Sanabria & Killeen, 2008) was applied to estimate parameters of the distribution of waiting intervals (see Appendix for detailed model description). The distribution of waiting intervals produced in response-withholding tasks is often contaminated with short "burst" waiting intervals that do not appear to be

sensitive to the criterial waiting requirement, but instead occur at random and follow an exponential distribution (Sanabria & Killeen, 2008). The TR model uses a mixture of two distributions—one exponential, and one gamma—to model the distribution of waiting intervals, and allows for the analysis of response withholding performance independently of "burst" responses (Sanabria & Killeen, 2008). The TR model assumes that waiting intervals can be categorized as 'timed' (i.e., the subject is engaged in the timing task), or 'non-timed' (i.e., the subject is not engaged in the task). 'Timed' waiting intervals are characterized by a gamma distribution, and 'non-timed' responses, such as rapid burst responses or extended intervals of chamber exploration, are characterized by an exponential distribution.

There are two estimates derived from the TR model that serve as dependent variables throughout each experiment. Response inhibition performance is indexed by θ, the mean timed waiting interval (i.e., 'non-timed' intervals are excluded). The parameter *P* represents the proportion of 'timed' responses.

CHAPTER 2

DETRIMENTAL EFFECTS OF ACUTE NICOTINE ON THE RESPONSE-WITHHOLDING PERFORMANCE OF SPONANEOUSLY HYPERTENSIVE AND WISTAR KYOTO RATS

Attention-deficit hyperactivity disorder (ADHD) is among the most common childhood psychiatric disorders, affecting between 2% and 10% of children worldwide (Froehlich et al. 2007; Skounti et al. 2007), with symptoms often persisting into adulthood (Kessler et al. 2006). Barkley (1997) suggested that the core features of ADHD are deficits in response inhibition capacity and sustained attention. Response inhibition capacity refers to the ability to withhold a reinforced response; it is typically assessed in humans using Go/No-Go tasks (e.g., Wodka et al. 2007). Sustained attention refers to the effortful maintenance of responding to a stimulus; it is typically assessed using vigilance tasks (e.g., Stins et al. 2005).

ADHD is associated with early initiation of tobacco smoking, and with habitual smoking during adolescence (Lambert and Hartsough 1998; Milberger et al. 1997). The higher prevalence of smoking among individuals with ADHD may be due to tobacco smoking ameliorating some ADHD-related deficits (Blume et al. 2000; Gehricke et al. 2007; Khantzian 1997; Pomerleau et al. 2000). Because nicotine is the main psychoactive ingredient of tobacco, it is possible that nicotine enhances response inhibition and/or sustained attention.

Effects of nicotine on response inhibition capacity

The effects of nicotine on response inhibition capacity in humans are somewhat disparate. Although acute nicotine has been shown to enhance the capacity to withhold an ongoing response in the STT regardless of smoking or ADHD status (Potter et al. 2012; Potter and Newhouse 2008; Potter and Newhouse 2004), these findings are inconsistent with other data from the SST (Wignall & de Wit, 2011) and the continuous performance task (Bekker, Böcker, Van Hunsel, van den Berg, & Kenemans, 2005). The effects of acute nicotine on estimates of response inhibition capacity obtained from errors of commission in vigilance tasks are also inconsistent: whereas some studies report substantial nicotine-induced enhancement in vigilance-related response inhibition capacity (Myers et al. 2013; Myers et al. 2008), other studies report very mild effects (Levin et al. 1998), effects related only to irrelevant stimuli (Dawkins et al. 2007), and dose-dependent effects (Bekker et al. 2005). Inconsistent effects of acute nicotine have also been observed in biased visual discrimination tasks (Barr et al. 2008; Wignall and de Wit 2011) that are discriminative of ADHD status (Tripp and Alsop 1999).

Unlike humans, acute nicotine administration in rodents consistently increases impulsive responding in a wide range of tasks, including the five-choice serial reaction time task (5-CSRTT; Bizarro et al. 2004; Blondel et al. 2000; Hahn et al. 2002; Semenova et al. 2007), the differential reinforcement of low rates (DRL) schedule of reinforcement (Kirshenbaum & Brown, 2008; Kirshenbaum et al., 2011, 2009; Mayorga, Popke, Fogle, & Paule, 2000; Popke, Fogle, et al., 2000), the stop-signal task (Kirshenbaum et al. 2011), and the go/no-go discrimination (Kolokotroni et al. 2011). Only the temporal response differentiation task, which involves holding down a lever for

a target interval, appears to be insensitive to nicotine-induced premature responding (Popke et al. 2000b).

Effects of nicotine on sustained attention

Acute nicotine appears to reliably enhance sustained attention in individuals with and without ADHD (Conners et al. 1996; Gehricke et al. 2006; Levin et al. 1998; Levin et al. 2000, 2001; Warburton 1992; for review see Heishman et al. 2010). Measures obtained from rats in the 5-CSRTT suggest that acute nicotine improves sustained attention in outbred rodent models as well (Bizarro et al. 2004; Blondel et al. 2000; Day et al. 2007; Hahn et al. 2002; Mirza and Stolerman 1998; Semenova et al. 2007). Results from a visual signal detection task, another test of vigilance, also support the notion that nicotine improves attention in rodents (Rezvani et al. 2002; Rezvani and Levin 2004).

Human and animal studies are consistent in supporting the ameliorating effects of nicotine on sustained attention, but *not* on response inhibition capacity. In this study, we consider an explanation to the latter negative findings. Nicotine may not appear to enhance response inhibition capacity in past studies due to (a) the animal models used, and/or (b) the method by which response inhibition capacity was assessed in these models.

An animal model of ADHD

The negative effects of nicotine on response inhibition capacity may be due to the choice of animal model in which these effects were tested. Subjects in these studies typically are outbred rats that may not have inhibitory deficits to rescue. The effect of

nicotine on response inhibition capacity may be different in animals that model ADHD-related inhibitory deficits. The spontaneously hypertensive rat (SHR) is the most widely studied animal model of ADHD (Sagvolden 2000; Sagvolden et al. 2009). Despite the prevalent use of this strain, the majority of evidence suggests that sustained attention is not compromised in SHR (Van den Bergh et al. 2006; Thanos et al. 2010; but see Sagvolden and Xu 2008). In contrast, performance in response-withholding paradigms, such as the DRL schedule, consistently shows a reduced response inhibition capacity in SHR compared to its normoactive control, the Wistar Kyoto rat (WKY; Evenden and Meyerson 1999, Ferguson et al. 2007, Orduña et al. 2009; Sagvolden and Berger 1996, Sanabria and Killeen 2008; van den Bergh et al. 2006). Therefore, despite its limitations as an animal model of ADHD, SHR appears to be an ideal model to test whether or not nicotine enhances response inhibition capacity.

Assessing response inhibition capacity

In DRL schedules, the ability to withhold a response for an incentive is confounded with the reinforcing efficacy of the incentive (Doughty and Richards 2002; Hill et al. 2012). Nicotine may appear to reduce response inhibition capacity when in reality it is enhancing the efficacy of a reinforcer. The present study implements a novel response inhibition paradigm, the fixed minimum interval (FMI) schedule of reinforcement (see general introduction for review), that empirically isolates response inhibition capacity from reinforcer efficacy (Hill et al. 2012). Such dissociation is not possible with DRL schedules, because initial and terminal responses are identical. Put simply, levels of motivation significantly affect presumed measures of response inhibition capacity in the

DRL, but not in the FMI. This difference between FMI and DRL may explain why methylphenidate (MPH), which enhances response inhibition capacity in individuals with ADHD (Aron et al. 2003; Boonstra et al. 2005; DeVito et al. 2009), also enhances response-withholding performance of rats in the FMI (Hill et al. 2012), but not in the DRL (Van den Bergh et al. 2006; Ferguson et al. 2007; Hill et al. 2012; Orduña et al. 2009). In FMI schedules, acute MPH selectively reduces the interval between trial onset and initial response (Hill et al., 2012); because this interval and the interval between initial and terminal response are confounded in DRL schedules, MPH often induces short intervals between consecutive responses (Emmett-Oglesby et al. 1980; Ferguson et al. 2007; Orduña et al. 2009; Pearl and Seiden 1976; Seiden et al. 1979; but see Hill et al. 2012). We hypothesize that a similar confound explains nicotine-induced response-withholding deficits inferred from DRL performance. Thus, it was expected that the FMI schedule would reveal a lower baseline response inhibition capacity in SHR relative to WKY. Also, in agreement with the symptom amelioration hypothesis of ADHD-related smoking, it was expected that the FMI would reveal a nicotine-induced enhancement of reinforcer efficacy and inhibitory capacity.

Methods

Subjects

Twenty-three male rats, 12 Spontaneously Hypertensive Rats (SHR/NCrl) and 11 Wistar Kyoto (WKY/NHsd), arrived on post-natal day (PND) 25 and were pair-housed according to strain. Initially, 10 rats of each strain were received, but 3 WKY died within 3 days of arrival. One rat, left without a cagemate, was added to another pair to make one

cage of 3 WKY. Before examining any performance data, a second cohort (4 WKY, 2 SHR) was added to compensate for the attrition. Both cohorts experienced identical procedures throughout the experiment. Shortly upon arrival, the duration of access to food was reduced daily from 24 h to 18 h, 12 h, and finally 1 h. For both SHR and WKY, this feeding regimen yielded weights at the beginning of each session that were, on average, 75.5% of mean ad libitum weights estimated from growth charts provided by the breeders. The average difference in weight between cagemates under food restriction was approximately 9% of their estimated mean ad libitum weights, which was similar to the 10% difference observed when food was freely available. Water was available in the home cages ad libitum throughout the duration of the experiment.

Apparatus

Experiments were conducted in 10 MED Associates (St. Albans, VT) modular test chambers (three chambers were 305 mm long, 241 mm wide, and 210 mm high; seven chambers were 305 mm long, 241 mm wide, and 292 mm high), each enclosed in a sound- and light-attenuating box equipped with a ventilating fan. The front and back walls and the ceiling of the test chambers were made of Plexiglas; the front wall was hinged and served as a door to the chamber. One of the two aluminum side panels served as a test panel. The floor consisted of thin metal bars positioned above a catch pan. A square opening (51 mm sides) located 15 mm above the floor and centered on the test panel provided access to the hopper (MED Associates, ENV-200-R2M) and was furnished with a head entry detector (ENV-254-CB). Each activation of a dispenser delivered a single 45-mg sucrose pellet (TestDiet, Richmond, IN) to the hopper. A

multiple tone generator (MED Associates, ENV-223) was used to produce 3 kHz tones at approximately 75 dB through a speaker (MED Associates, ENV-224AM) centered on the top of the wall opposite to the test panel, 240 mm above the floor of the chamber. Two retractable levers (ENV-112CM) flanked the food hopper, and three-color light stimuli (ENV-222M) were mounted above each lever and could be illuminated yellow, green, and red. Lever presses were recorded when a force of approximately 0.2 N was applied to the end of the lever. The ventilation fan mounted on the rear wall of the sound-attenuating chamber provided masked noise of approximately 60 dB. The test chambers could be dimly illuminated by a houselight located behind the wall opposite to the test panel. Experimental events were arranged via a Med-PC® interface connected to a PC controlled by Med-PC IV® software.

Procedure

Figure 1 describes the sequence of events. Sessions were conducted once daily, 7 days a week. Training initiated with autoshaping, consisting of pairing lever insertion with the delivery of a sucrose pellet. Once all rats were responding reliably to the lever, FMI training began. During FMI sessions, reinforcement was contingent upon the rat successfully waiting a given interval of time. The waiting interval was initiated by a lever press and terminated with a head entry into the food hopper. All terminal responses resulted in the retraction of the lever and a 5.5-s blackout period, after which the lever was reinserted and the next trial began. Each session ended after 60 min or after 150 sucrose pellets were delivered, whichever happened first. For a detailed description of training and the arrangement of sessions, see Mika et al. (2012).

At the onset of FMI training, the criterial waiting time (*t*) was set to 0.5 s. Each correct response increased *t* by 1.25%; *t* was carried over from one session to the next until *t* = 6 s, and remained constant thereafter. Once the 6-s criterion had been established, a conjunctive variable interval (VI) 9-s schedule was introduced and gradually increased across sessions, to a VI 30-s schedule. The conjunctive VI 30-s schedule was implemented to reduce the between-subject variability in rate of reinforcement that would otherwise result from unequal performance (for details, see footnote 1). When there were no noticeable upward or downward trends in the proportion of correct responses in four consecutive days, as determined by visual inspection of the data, the treatment phase began.

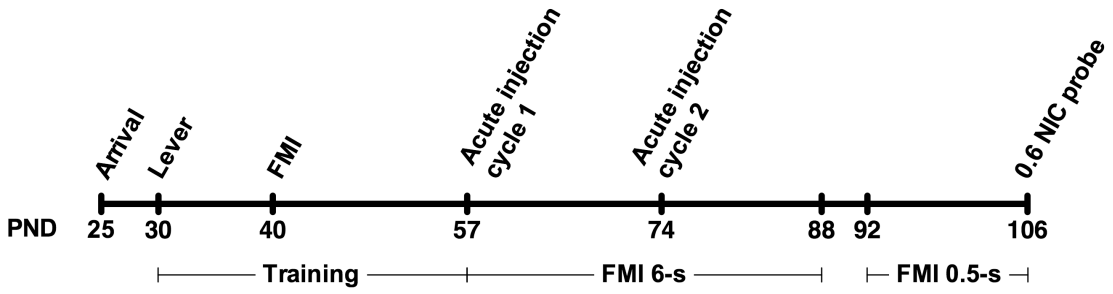


Figure 1. Timeline of events. Rats arrived at the facility on PND 25; after an acclimation period, they began lever training on PND 30. FMI training started on PND 40, and once performance stabilized on the FMI 6-s schedule, the treatment phase began on PND 57. During treatment, rats were injected with saline, three different doses of nicotine (0.1, 0.3, 0.6 mg/kg), and pre-fed in counterbalanced order. Rats were subjected to each treatment cycle twice, with the second cycle beginning on PND 74. On PND 92, the criterial waiting time for the FMI schedule was reduced from 6 s to 0.5 s. After 14 days of experience with FMI 0.5-s, all animals received 0.6 mg/kg nicotine immediately prior to session (PND 106).

Nicotine and pre-feeding treatments

Prior to PND 52, when treatment commenced, all rats were drug naïve. Treatment consisted of *either* a subcutaneous (s.c.) injection of saline, 0.1, 0.3, or 0.6 mg/kg of nicotine, 10 min prior to session, or ad libitum access to food in the home cage during the hour leading up to session start. Treatment was implemented twice per week, with at least two rest days between treatments. This drug regimen was chosen because neither tolerance nor sensitization effects were evidenced in a similar regimen implemented by Kirshenbaum et al. (2008) using DRL 4.5-s and Sprague Dawley rats. Within this range of doses, acute s.c. nicotine disrupts DRL performance (Kirshenbaum et al., 2008), timing (Hinton and Meck, 1996), improves performance in 5-CSRTT (Bizarro and Stolerman 2003; Hahn et al. 2003), induces place preference, and enhances social rewards (Thiel et al. 2009).

FMI 6-s sessions were conducted daily. (-)Nicotine hydrogen tartrate (Sigma-Aldrich, St. Louis, MO) was dissolved in saline, and sodium hydroxide was added until the pH of the solution was approximately 7.2. Nicotine dose was calculated as the base, and injection volume was based on body weight at the time of injection. All animals experienced two determinations of each dose and two pre-feeding (PF) sessions. Each animal received each treatment (dose or PF) once (cycle 1) and then again in the same order (cycle 2). Treatment order within each cycle was counterbalanced across animals.

FMI with minimal delay

Once the two cycles of treatment were completed, two daily FMI 6-s sessions were conducted in the absence of nicotine. Immediately following, 15 daily sessions were

conducted in which the criterial time was reduced to 0.5 s (FMI 0.5-s). No injections were administered until the 15th day, when all rats received 0.6 mg/kg nicotine immediately prior to the FMI session. The purpose of this condition was to determine whether selected effects of nicotine on FMI 6-s performance were dependent on the 6-s waiting period.

Dependent measures

The primary dependent measures were median latency to initial lever press, and selected parameters of the distribution of inter-response times (IRTs), computed for each rat over individual sessions. Latencies are the intervals between lever presentation (start of the trial) and initial lever press. Latencies were classified into two categories based on the outcome of the previous trial: (1) post-R latencies: those following correct reinforced trials, and (2) post-N latencies: those following non-reinforced trials, including those following trials in which a correct response went unreinforced because the conjunctive VI had not elapsed, as well as those following incorrect responses (i.e., IRT < t).

IRT refers to the time elapsed between the initial lever press and the terminal head entry. The Temporal Regulation model was applied to estimate parameters of the distribution of IRTs (Mika et al. 2012; Sanabria and Killeen 2008). The model assumes that, at the beginning of every trial, rats sometimes enter a *timing state* with probability P. When in a timing state, rats produce IRTs that are gamma-distributed, centered close to the criterial FMI interval (here, 6 s). When rats are not in a timing state, they produce IRTs at a constant average rate, and as such, non-timing IRTs are exponentially

distributed. Thus, according to the Temporal Regulation model, a mixture of two distributions, one gamma and one exponential, underlie the distribution of IRTs:

$$\Pr(\text{IRT} = t \mid t < \delta) = 0$$

$$\Pr(\text{IRT} = t \mid t \geq \delta) = P\Gamma(t - \delta; n, c) + (1 - P)(1 / K) \exp(-(t - \delta) / K). \quad (1)$$

In Equation 1, the probability of entering a timing state, P, is the mixture weight of a gamma distribution with shape parameter n and scale parameter c. Both distributions, gamma and exponential, are shifted rightwards to account for the minimum time required to complete an IRT, δ. Thus, the mean duration of timed IRTs is $nc + \delta$ and the mean duration of non-timed IRTs is $K + \delta$. Our analysis was primarily concerned with estimates of P and of the rescaled mean of the gamma distribution, $\theta = (nc + \delta) / 6$ s. If the mean timed IRT is shorter or longer than the 6-s criterial time, estimates of θ are, respectively, less than or greater than 1. Estimates of θ served as indices of response inhibition capacity (Sanabria and Killeen, 2008).

Data Analysis

IRTs and latencies were collected on every treatment day, on the day preceding each treatment (*no-treatment days*), and on the last 2 experimental days (FMI 0.5-s on no-treatment and on 0.6 mg/kg nicotine). Median latencies and estimates of θ were log-transformed. The proportion of correct IRTs (i.e., IRT > 6 s), the proportion of correct IRTs reinforced (i.e., reinforced IRTs / total IRTs), and estimates of P were log-odds transformed. These transformations follow suggestions on the estimation of population parameters in a similar model by Cheung, Neisewander and Sanabria (2012). All

dependent measures are reported as back-transformed mean ± SEM. *P* and θ were estimated for each rat using the method of maximum likelihood (Myung 2003).

Analysis of variance (ANOVA). ANOVA was implemented to establish the statistical significance of the effects of strain (SHR vs. WKY) and nicotine dose (vehicle vs. 0.1 vs. 0.3 vs. 0.6 mg/kg) on proportions of correct IRTs and correct IRTs reinforced. The effects of strain, nicotine dose, pre-feeding, schedule (FMI 6-s vs. 0.5-s) and treatment cycle (first vs. second) were examined on rate of reinforcement, median latencies and estimates of *P* and θ, using a significance threshold of $\alpha = .05$. When sphericity was violated according to Mauchly's test, a Huynh-Feldt correction was implemented. Only significant main effects or interaction effects were followed by post hoc 2-tailed t-tests. Significant cycle effects were followed by a separate ANOVA in each cycle. Only significant effects are reported.

Nicotine effects. Separate 2 × 2 × 4 (strain × cycle × dose) mixed-design ANOVAs were conducted on median latencies and estimates of *P* and θ to establish the dose effects of nicotine on these dependent measures, and whether those effects were modulated by strain and treatment cycle.

Pre-feeding effects. Separate 2 × 2 × 2 (strain × cycle × feeding status: not pre-fed at vehicle vs. pre-fed) mixed-design ANOVAs were conducted on median latencies and estimates of *P* and θ to establish the effects of pre-feeding on these dependent measures, and whether such effects were modulated by strain and treatment cycle. This analysis was intended to identify effects related to a potential nicotine-induced reduction in appetite (Dandekar et al. 2011; Wellman et al. 2005). Nicotine effects that matched

pre-feeding effects were discounted as potentially related to appetite reduction and not to performance enhancement.

Schedule effects on latencies. Separate 2 × 2 × 2 (strain × schedule: FMI 0.5-s vs. 6-s on 0.6 mg/kg in second cycle; treatment: no-treatment vs. treatment day) mixed-design ANOVAs were conducted on post-R and post-N latencies to establish whether strain and nicotine effects on latency were modulated by the length of the criterial waiting time. Only the second cycle of 0.6 mg/kg FMI 6-s treatment was used in this comparison to minimize the confound between schedule and order effects (FMI 6-s was implemented before FMI 0.5-s, see Figure 1).

Stability of rate of reinforcement. Rate of reinforcement was measured as the number of reinforcers obtained per 60-min session. Although the maximum number of reinforcers obtainable in a session was 150 pellets, no rat reached this limit in any experimental session. The conjunctive VI schedule was expected to keep rate of reinforcement relatively constant across experimental manipulations. Stability of rate of reinforcement was tested by conducting a 2 × 2 × 4 (strain × cycle × dose) and a 2 × 2 × 2 (strain × cycle × feeding status) mixed-design ANOVAs, with reinforcers per session as the dependent measure.

Coefficient of variation. The estimation of the coefficient of variation (CV) of timed IRTs is often included as part of the analysis of FMI performance (Sanabria and Killeen 2008). Higher CV is indicative of less precise timing (Sanabria and Killeen 2008). In this study we monitored CV across manipulations, but do not report it because baseline timing is not less precise in SHR than in WKY (Orduña et al. 2009; Orduña et al. 2008; Sanabria and Killeen 2008). This null finding was replicated here in the vehicle

condition, CV_{SHR} = 0.18 +/- 0.01, CV_{WKY} = 0.20 +/- 0.01. Thus, SHR does not appear to be an adequate model of timing deficits in ADHD (Toplak et al. 2006); effects of nicotine in this domain are, therefore, uninterpretable.

Results

Temporal Regulation Parameters

In all conditions the distribution of IRTs were well described by a mixture of two underlying distributions, one gamma (timed IRTs) and one exponential (non-timed IRTs) (Eq. 1). Figure 2 illustrates the goodness-of-fit of this model to performance under vehicle and under the highest dose of nicotine, 0.6 mg/kg.

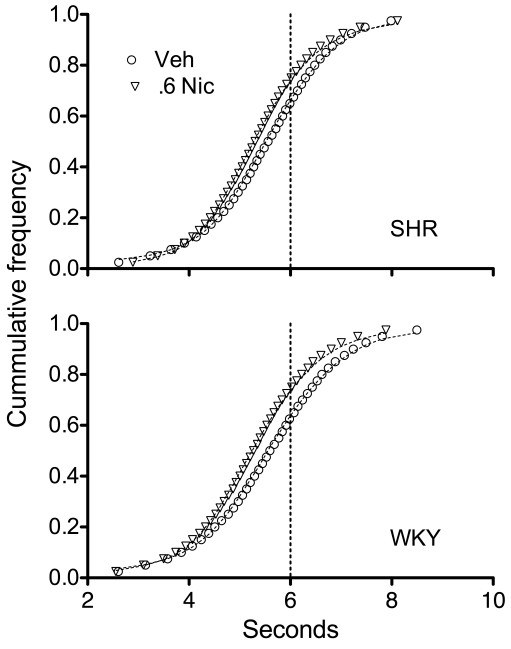


Figure 2. Mean cumulative frequency distributions of IRTs produced by SHR (top) and WKY (bottom) after s.c. injections of vehicle (circles) and 0.6 mg/kg nicotine (downward triangles). Data are organized in 39 bins, each containing approximately equal number of IRTs. Dotted lines are fits of the Temporal Regulation (TR) model of response inhibition capacity (Eq. 1).

Nicotine effects. A significant main effect of nicotine dose was observed on the proportion of correct IRTs, $F(3,63) = 4.23$, $p = .009$. Post hoc paired-sample t-tests revealed that all doses of nicotine reduced the proportion of correct IRTs, from an average of 51% at baseline to 33-36% under nicotine; $t(22)$ ranged between 2.25, $p = .035$, and 3.00, $p = .007$. No significant effect of nicotine dose or strain was observed on the proportion of correct IRTs reinforced, which was on average 54%.

Figure 3 shows the effects of strain and dose on estimates of θ and *P*. A significant main effect of dose on θ estimates was observed, $F(3, 63) = 4.64$, $p = .005$. Post hoc paired-samples t-tests revealed that, relative to vehicle, all doses of nicotine significantly reduced θ; $t(22)$ ranged between 2.58, $p = .017$, and 3.31, $p = .003$. Significant strain × dose interaction effects on estimates of *P* were observed, $F(3, 63) = 5.05$, $p = .003$. Post hoc t-tests on *P* revealed that it was (a) significantly higher for SHR than WKY on 0.1 mg/kg nicotine, $t(21) = 2.33$, $p = .030$, (b) significantly higher for SHR on 0.1 and 0.3 mg/kg nicotine than on vehicle, $t(11) = 4.41$, $p = .001$, and $t(11) = 4.05$, $p = .002$, respectively, and (c) significantly higher for WKY on 0.3 and 0.6 than on vehicle, $t(10) = 2.79$, $p = .019$, and $t(10) = 2.64$, $p = .024$. Overall, these results suggest that nicotine reduced response inhibition capacity, but dose-dependently increased the sensitivity to the temporal contingencies of reinforcement, requiring a lower dose for the latter improvement in SHR.

Pre-feeding effects. Figure 3 shows the effects of pre-feeding (PF) on estimates of *θ* and *P*. A significant strain × cycle × feeding status interaction effect was observed on θ estimates, $F(1, 21) = 5.61$, $p = .028$. A separate analysis was conducted on θ estimates at each cycle. No significant effect of strain or feeding status was observed on θ

in either cycle, suggesting that the 3-way interaction effect on θ was an effect of cycle modulated by strain and feeding status. No significant effects of strain, cycle, or feeding status were observed on *P*.

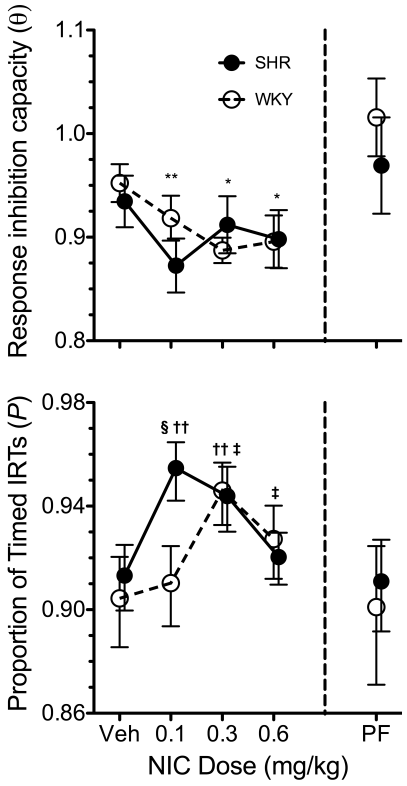


Figure 3. Mean estimates of θ (top) and *P* (bottom) for SHR (solid circles) and WKY (open circles) rats across vehicle (Veh), 0.1, 0.3, and 0.6 mg/kg s.c. nicotine (left of vertical dashed line) and pre-feeding (PF; right of vertical dashed line). *Significant difference between dose and Veh. §Significant simple main effect of strain at a dose level. †Significant difference between dose and Veh in SHR. ‡Significant difference between dose and Veh in WKY. Symbols are repeated with lower p-values (i.e., *p < .050, **p < .010, ***p < .001). A significant strain × cycle × feeding status interaction effect was observed on θ, but no significant main or interaction effect of strain or feeding status on θ were observed in either cycle. All doses of nicotine reduced estimates of θ relative to Veh. Nicotine increased estimates of *P* in a dose-dependent manner and differentially across strains. The nicotine dose-response curve of *P* was shifted leftwards in SHR relative to WKY.

Latencies

Nicotine effects. Figure 4 (left) shows the effects of strain and dose on post-R and post-N latencies. Significant effects of dose on post-R and post-N latencies were observed [post-R: $F(3, 63) = 19.83$, $p < .001$; post-N: $F(3, 63) = 28.16$, $p < .001$]. Post hoc paired-sample t-tests revealed that all doses of nicotine significantly shortened post-R and post-N latencies relative to vehicle; $t(22)$ ranged between 2.36, $p = .027$, and 8.21, $p < .001$. No significant effect of strain was observed. Thus, regardless of strain or of the outcome in the preceding trial, even the lowest dose of nicotine was effective in reducing the time between trial onset and first lever press.

Pre-feeding effects. Figure 4 (PF) shows the effects of strain and feeding status on post-R and post-N latencies. Significant main effects of feeding status on post-R and post-N latencies were observed [post-R: $F(3, 63) = 20.08$, $p < .001$; post-N: $F(3, 63) = 62.20$, $p < .001$]. All latencies were roughly 2 s longer when rats were pre-fed. No significant strain × feeding status interaction effect was observed. Thus, regardless of strain or of the outcome of the preceding trial, pre-feeding increased the time between trial onset and first lever press.

Schedule effects. Figure 4 (right) shows the effects of an s.c. injection of 0.6 mg/kg on FMI 0.5-s and FMI 6-s performance. Significant main effects of schedule and nicotine were observed in both post-R [respectively: $F(1, 21) = 61.56$, $p < .001$; $F(1,21) = 27.92$, $p < .001$] and post-N latencies [respectively: $F(1,21) = 4.58$, $p = .044$; $F(1,21) = 51.06$, $p < .001$]. No significant effect of strain or interaction effect was observed. These results suggest that the nicotine-induced shortening of latencies was not schedule-dependent, and was observable even when reinforcement was minimally delayed.

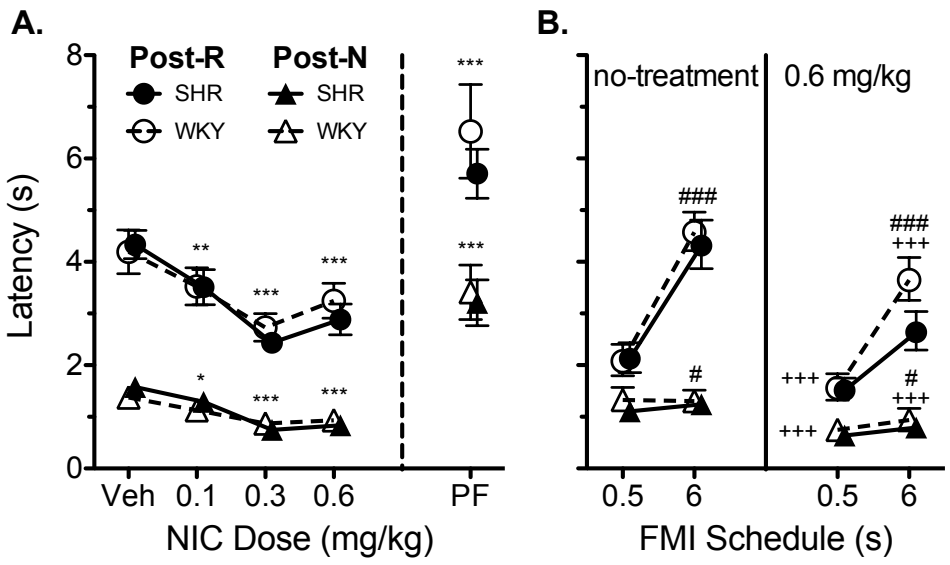


Figure 4. Mean median latencies to the initial response across nicotine and pre-feeding (PF) treatment conditions (left panel) and across FMI schedules (right panel; FMI 6-s data is from cycle 2) for SHR and WKY rats. "Post-R" denotes the latencies following reinforced trials; "Post-N" denotes latencies following non-reinforced trials. *Significant difference between dose and Veh. #Significant main effect of FMI schedule, +Significant main effect of nicotine relative to no-treatment (day preceding injection). Symbols are repeated with lower p-values (i.e., $^{*}p < .050$, $^{**}p < .010$, $^{***}p < .001$). Nicotine and shorter FMI target time reduced median post-R and post-N latencies; pre-feeding increased median post-R and post-N latencies.

Stability of Rate of Reinforcement

A significant strain × dose effect on rate of reinforcement was observed, $F(3,63) = 2.84$, $p = .045$. Post hoc paired-sample t-tests revealed that WKY obtained fewer reinforcers under 0.6 mg/kg nicotine than under vehicle, $t(10) = 3.29$, $p = .008$. This means that the effect of nicotine on WKY performance was confounded with the effects of reduced rate of reinforcement only at the highest dose. At other doses in WKY and all doses in SHR, these effects were not confounded.

A significant strain × cycle × feeding status effect on rate of reinforcement was observed, $F(1,21) = 310.42$, $p = .004$. Post hoc t-tests were conducted separately in each

cycle. Post hoc comparisons revealed that, in the second cycle, pre-feeding substantially reduced the rate of reinforcement of WKY relative to no pre-feeding, $t(10) = 2.98$, $p = .014$, and relative to pre-fed SHR, $t(21) = 2.97$, $p = .007$.

Discussion

Nicotine and Response Inhibition

The acute administration of nicotine reduced estimates of θ similarly for SHR and WKY (Figure 3). This result suggests that acute nicotine reduces response inhibition capacity in both strains. Although this finding is inconsistent with our expectations, it is consistent with nicotine-induced reductions in IRTs observed in DRL studies using Sprague Dawley rats (Kirshenbaum et al. 2008, 2009, 2011). Performance in DRL schedules cannot be readily interpreted in terms of response inhibition capacity, because it is also sensitive to reinforcer-efficacy manipulations (e.g., reinforcer magnitude; Doughty and Richards 2002). In contrast, estimates of θ are not significantly sensitive to changes in reinforcer efficacy via pre-feeding (Figure 3). Yet, nicotine also appears to reduce θ, suggesting that the effect of nicotine on DRL performance may not be solely explained on the basis of enhanced reinforcer efficacy.

An alternative account of our results would suggest that reduced estimates of θ resulted from a faster internal clock under acute nicotine (Hinton and Meck 1996). Intervals trained without nicotine may be perceived as being longer under nicotine, thus yielding shorter timed IRTs when nicotine was acutely administered. The accelerative effects of nicotine on timing, however, have only been demonstrated using the peak interval method (Hinton and Meck 1996; Meck 2007), which is vulnerable to

confounding motivational effects (Galtress and Kirkpatrick 2009; Ludvig et al. 2011; Plowright et al. 2000; Sanabria and Thrailkill 2009). Timing estimates that are more robust to motivational manipulations, such as those obtained from the temporal bisection procedure (Galtress and Kirkpatrick, 2010), do not suggest an accelerative effect of nicotine on the internal clock (Ward et al. 2009). These findings do not support an explanation of nicotine-induced effects on θ based on timing mechanisms.

Neither changes in reinforcer efficacy nor changes in rate of reinforcement can explain nicotine-induced reductions in estimates of θ. Pre-feeding did not have a significant effect on estimates of θ (Figure 3), but it increased latencies, an effect opposite that of nicotine. The robustness of θ to the pre-feeding manipulation suggests that, consistent with prior findings (Mechner and Guevrekian 1962), mean timed IRTs are robust against changes in reinforcer efficacy. Relative to baseline, rate of reinforcement was significantly lower only for WKY at the highest dose of nicotine. The stability of rate of reinforcement in both strains across most conditions was primarily due to the conjunctive VI schedule of reinforcement that imposed a minimum (but variable) amount of time between reinforcers. The limited effect of nicotine on rate of reinforcement cannot account for reductions in estimates of θ in both strains at every dose of nicotine. Thus, it appears that nicotine-induced reductions in estimates of θ reflect a nicotine-induced reduction in the capacity of both SHR and WKY to withhold a reinforced response.

Given that nicotine reduces response inhibition capacity so consistently in rodent models, it is unclear why the effects of nicotine on response inhibition capacity appear to vary so much among human studies. The key to these inconsistencies may be the

underlying processes assessed by divergent methodologies. For instance, nicotine-induced improvements in human response-inhibition capacity are primarily observed in the stop-signal task and in the Stroop task (Potter et al. 2012; Potter and Newhouse 2008; Potter and Newhouse 2004; Wignall and de Wit 2011). In these tasks, the behavior to be withheld is prepotent because it is either already initiated (stop signal task) or because it is strongly associated with a present stimulus (Stroop task). In contrast, most rodent paradigms, including DRL and FMI schedules, are based on behavior that is potentiated by its consequences. Nicotine has detrimental effects on performance in analogous tasks in humans, such as the biased visual discrimination task used by Barr et al. (2008).

The absence of strain effects on estimates of θ at baseline tempers our interpretation of the measure of response inhibition capacity obtained from FMI performance. To the extent that θ reflects response inhibition capacity, and SHR models inhibitory deficits associated with ADHD, lower estimates of θ would be expected in SHR relative to WKY. This difference between strains has been observed systematically in the DRL (Ferguson et al. 2007, Orduña et al. 2009; Sagvolden and Berger 1996, Sanabria and Killeen 2008; van den Bergh et al. 2006), but was not observed in the present study using the FMI. It is thus likely that procedural differences between FMI and DRL schedules are responsible for these divergent results. Various features of the FMI—the longer resting period between withholding trials, the response-initiated nature of these trials, the separation of initial and terminal responses—may facilitate the response-withholding performance of SHR.

Nicotine and reinforcer efficacy

Mechner and Guevrekian (1962) demonstrated that FMI latencies, but not IRTs, are sensitive to reinforcer deprivation. Our results are consistent with those findings, showing that pre-feeding increased latencies regardless of strain and of the outcome of the preceding trial (i.e., post-R vs. post-N; Figure 4, left). These results support the interpretation of changes in latencies as reflecting changes in reinforcer efficacy. The reduction in latencies following nicotine administration (Figure 4, left) thus suggests that nicotine enhanced the reinforcing efficacy of sucrose pellets. These data are consistent with reports of nicotine-induced enhancement of reinforcer efficacy for appetitive reinforcers or food-related cues (see Donny et al. 2011) in both rats (Grimm et al. 2012; Raiff and Dallery 2006; Wing and Shoaib 2010) and humans (Epstein et al. 1992; Perkins 1992).

In principle, however, it is possible that the reduction in latencies following nicotine administration reflects a nicotine-induced reduction in the sensitivity to the delay of reinforcement. Prior research (Morgan 1972) and the positive relationship between latencies and FMI target time (0.5 s vs. 6 s; Figure 4), support the notion that latencies are sensitive to delay of reinforcement. If nicotine effects on latencies were mediated by changes in sensitivity to delay of reinforcement, it would be expected that the elimination of the delay of reinforcement would also eliminate the effect of nicotine on latencies. Contrary to that expectation, however, nicotine produced shorter latencies even in FMI 0.5 s, when delay to reinforcement was minimal (Figure 4, right panels). Such an effect supports the hypothesis that changes in latencies induced by nicotine reflect changes in reinforcer efficacy and not in sensitivity to the delay of reinforcement.

Nicotine and sensitivity to timing contingencies

In all conditions the distribution of IRTs were well described by a mixture of two underlying distributions, one gamma (timed IRTs) and one exponential (non-timed IRTs) (Eq. 1, Figure 2). Generally, more than 90% of the IRTs were timed, signified by parameter *P*. The remaining 10% of intervals (1-*P*) appear to be produced absent of the control of the timing contingencies, as they are produced at a constant rate. Although it has not been explicitly evaluated in the FMI schedule, this loss of control by the contingencies of reinforcement may be attributed to lapses in attention (Killeen et al. 2013; Sagvolden et al. 1998).

In the present study, the proportion of timed intervals did not differ significantly at baseline (vehicle) between SHR and WKY (Figure 3, bottom). This lack of significant baseline differences has also been observed in paradigms designed to assess sustained attention in rodents, such as the 5-CSRTT (van den Bergh et al. 2006) and the visual stimulus position discrimination task (Thanos et al. 2010). The evidence available suggests that the SHR is not an adequate model of ADHD-related deficits in sustained attention.

Nevertheless, the effects of nicotine on *P* may be informative. It was observed that *P* increased following nicotine administration, tracing an inverted-U dose response function that peaked at a lower dose for SHR (0.1 mg/kg) than for WKY (0.3 mg/kg; Figure 3). This effect suggests that, relative to WKY, SHR required a lower dose of nicotine to increase the proportion of timed IRTs. If *P* were to be interpreted in terms of attentional processes, these results would be the opposite of what would be expected. This is because the SHR, compared to WKY, has fewer α4β2 nicotinic receptors

(Wigestrand et al. 2011), which appear to mediate the enhancement of sustained attention in rodents induced by nicotine (Rezvani et al. 2011; Young et al. 2013). Furthermore, estimates of *P* do not appear to increase with MPH treatment in rats (Hill et al. 2012), even though past research has shown that MPH enhances sustained attention in both rats (Paine et al. 2007) and humans (Epstein et al. 2006; Riccio et al. 2001). A potential explanation to these seemingly contradictory findings requires that nicotine and MPH enhance sustained attention through different mechanisms (Levy and Hobbes 1996; McGaughy et al. 1999*), and P* indexes a nicotine-sensitive attention-like process that is not necessarily mediated by α4β2 nicotinic receptors. Although it is yet unclear what neural processes are indexed by *P*, these processes are likely to have implications in the research and treatment of attentional deficits.

Conclusion

This investigation provides the first evidence, in an animal model of ADHD, for nicotine-induced reduction in response inhibition capacity that is not confounded with reinforcer-efficacy effects. In particular, our data suggest that nicotine hinders the capacity of rats to withhold a response that has been instrumentally reinforced. This result undermines the hypothesis that smoking among individuals with ADHD is facilitated by an ameliorating effect of nicotine on response inhibition deficits.

Acute nicotine increased the degree to which rats responded to reinforcement contingencies. This effect peaked at a lower dose for the animal model of ADHD (0.1 mg/kg) than for its control (0.3 mg/kg). Along with observations from past research and pre-feeding manipulations, it suggests that the mechanism by which nicotine enhances the sensitivity to reinforcement contingencies involve neither α4β2 nicotinic receptors—

which are often involved in sustained attention—nor motivational mechanisms on which MPH appear to operate (Levy and Hobbes 1996). Future research may unveil the specific mechanisms supporting this effect.

Footnote 1

The VI schedule was implemented as follows: A timer ran throughout the session. Reinforcement became available when the timer completed a specified interval, with one exception: if the interval elapsed after the initial response, reinforcement was not available until the subsequent trial. After each reinforcer, the timer was reset and a new interval was specified. If a correct response was made before reinforcement became available, the rat was exposed to the 3kHz tone, but sucrose reinforcement was withheld. Intervals were specified by sampling without replacement from a 12-item Fleschler-Hoffman distribution (Fleshler & Hoffman, 1962). The VI-schedule requirement progressed in daily succession (9, 15, 20, 30 s), until all rats were performing at VI 30-s and t = 6 s. The VI schedule was implemented to reduce the between-subject variability in rate of reinforcement that would otherwise result from unequal performance. With this control in place, differences in performance could be reliably attributed to the experimental manipulation and not to differences in rate of reinforcement.

CHAPTER 3

EFFECTS OF CHRONIC NICOTINE ON RESPONSE INHIBITION AND FOS ACTIVATION IN SPONTANEOUSLY HYPERTENSIVE AND WISTAR KYOTO RATS

Smoking remains the leading cause of preventable death in the United States, and early initiation is associated with greater difficulty quitting. Among adolescent smokers, those with attention-deficit hyperactivity disorder (ADHD), characterized by difficulties with impulsivity and inattention, smoke at nearly twice the rate of their peers (Lambert and Hartsough 1998; Milberger et al. 1997). Although cigarette smoking is highly addictive, nicotine is relatively weak primary reinforcer (Chaudhri et al., 2006; Palmatier et al., 2006, 2007), prompting research on other potential targets that maintain smoking, including the potential benefits of nicotine on attention (Barr et al., 2008; M Ernst, Heishman, Spurgeon, & London, 2001; Warburton & Mancuso, 1998), inhibition (Potter & Newhouse, 2004, 2008), and reinforcer efficacy (Chaudhri et al., 2006; Palmatier et al., 2006, 2007; Raiff & Dallery, 2006). The present study examines the effects of a chronic subcutaneous (s.c.) nicotine injections (0.3 mg/kg) on response inhibition and brain activation in an animal model of ADHD, the spontaneously hypertensive rat (SHR).

Compared to acute effects, the effects of chronic nicotine administration on behavioral measures of response inhibition have received less attention. However, the majority of existing data seems to suggest that chronic nicotine hinders performance in response inhibition tasks. Levin et al. (2001) administered transdermal nicotine patches to non-smokers with and without ADHD for four weeks, but failed to find significant effects on measures of response inhibition obtained from the continuous performance

task. In rodents, chronic nicotine has been shown to increase premature responding in the 5-CSRTT (Amitai & Markou, 2009; Blondel, Sanger, & Moser, 2000; Blondel, Simon, Sanger, & Moser, 1999; Hahn, Shoaib, & Stolerman, 2002; Semenova, Stolerman, & Markou, 2007), well as in the differential reinforcement of low rates (DRL) schedule (Kirshenbaum et al., 2008). In a rodent adaptation of the Go/No-go task, chronic nicotine decreased the proportion of correct "No-go" trials (Kolokotroni, Rodgers, & Harrison, 2012). In a delay discounting paradigm, chronic nicotine increases impulsive choice (Dallery & Locey, 2005; Kayir, Semenova, & Markou, 2014), with deleterious effects observed 30 days post-nicotine treatment (Dallery & Locey, 2005). Although delay discounting is a measure of impulsive choice, not response inhibition, both measures are tied to impulsivity and appear to be sensitive to the effects of nicotine.

Previously, we demonstrated that acute nicotine administration was detrimental to response-withholding performance in the fixed minimum interval (FMI) schedule of reinforcement (Mazur, Wood-Isenberg, Watterson, & Sanabria, 2014). FMI schedules require subjects to wait a specified duration between responses in order for reinforcement to be delivered. Waiting intervals are initiated with a lever press, and terminated with a head entry into the food hopper. Terminal responses that occur prior to the criterial waiting time are deemed premature, i.e., impulsive. The FMI schedule is similar to other response-withholding tasks that invoke a waiting requirement, such as DRL. However, waiting intervals generated in FMI are less susceptible to motivational confounds (Hill et al., 2012; Mazur et al., 2014), and unlike other rodent response-withholding tasks, FMI schedules are capable of detecting benefits of methylphenidate on response inhibition (Hill et al., 2012).

The present study aimed to expand the findings of Mazur et al. (2014) to the effects of chronic nicotine administration—which may differ from effects of acute dosing—on FMI performance. The unique action of acetylcholine nicotinic receptors (nAChRs) may lead to differences in performance between an acute or chronic dosing regimen. Whereas exposure to agonists typically results in receptor downregulation, chronic nicotine exposure leads to an upregulation of nAChRs and increase in nicotine binding in the brain at a wide range of doses (0.45-5 mg/kg; Brennan, Lea, Fitzmaurice, & Truman, 2010; Wonnacott, 1990), an effect that may contribute to nicotine dependence (Picciotto, Zoli, Rimondini, & Léna, 1998). Upregulation of nAChRs is only observed after repeated exposure to nicotine dosing, suggesting that chronic dosing may be advantageous for comparing the effects of nicotine in rodents to those of regular smokers.

In humans, nicotine has a half-life of around 2 h, compared to 45 min in rats. This means that in rats receiving a daily injection schedule, nicotine is cleared entirely before the next injection. These conditions make repeated daily injections an undesirable design for studying the effects of nicotine withdrawal, which typically requires more constant delivery of nicotine via osmotic mini-pumps (Matta et al., 2007). Nonetheless, it has been shown that five daily subcutaneous (s.c.) injections of nicotine (0.1 and 0.4 mg/kg) increased extracellular dopamine in the nucleus accumbens and increased spontaneous locomotor activity in rats, compared to a single injection of the same dose (Benwell & Balfour, 1992). Cedex (1992) found that 12 repeated injections of nicotine (0.4 and 0.8 mg/kg) produced an increase in DA utilization in the mPFC, and enhanced locomotor effects in rats compared to a single dose. Dallery and Locey (2005) administered daily s.c. injections of nicotine (0.3 mg/kg) and observed nicotine-induced

increases in impulsive choice that persisted 30 days after nicotine treatment was terminated. Although repeated nicotine injections may not sustain blood concentrations high enough to induce dependence or withdrawal, neurochemical and long lasting behavioral effects can occur that would not be observable under and acute dosing regimen.

Prolonged nicotine exposure may result in a reduction of some aversive effects, while enhancing rewarding effects. Non-smokers who are administered nicotine report fewer positive effects compared to smokers, and more aversive effects such as decreased alertness, stronger symptoms of nicotine toxicity, and tremors (Foulds et al., 1997; Perkins et al., 1990; Soria et al., 1996). Non-smokers report diminished aversive effects of nicotine with continued exposure (Brennan et al., 2010). Thus, the atypical action of nicotine on nAChrs, combined with the initial aversive effects of nicotine exposure, support the notion that potentially beneficial effects of nicotine may only be visible after repeated dosing.

In order to determine if nicotine ameliorates response inhibition deficits, the present study employed an animal model of ADHD. The SHR is the most extensively studied animal model of ADHD (Sagvolden, 2000; Sagvolden et al., 2009). Compared to their normotensive control strain, the Wistar Kyoto (WKY), the behavior of the SHR appears to parallel core characteristics of ADHD, most consistently impulsivity (Evenden and Meyerson 1999, Ferguson et al. 2007, Orduña et al. 2009; Sagvolden and Berger 1996, Sanabria and Killeen 2008; van den Bergh et al. 2006).

Similarities between individuals with ADHD and SHRs are not limited to their behavioral profiles. SHRs show disregulation of mesocortical and mesolimbic DA

pathways, marked by reduced dopamine release and D2 receptor hypofunctioning (Q. Li et al., 2009; Linthorst, van Giersbergen, Gras, Versteeg, & de Jong, 1994; V. A. Russell, 2002; V. a Russell, 2000; Viggiano, Vallone, Ruocco, & Sadile, 2003; Viggiano, Vallone, & Sadile, 2004). Similarly, imaging data from patients with ADHD suggest a hypofunctioning dopamine system in the prefrontal cortex (PFC) and nucleus accumbens, evidenced by reduced availability of D2/D3 dopamine receptors and dopamine transporters (DAT; Volkow et al., 2007, 2009), reduced activation of the PFC (Monique Ernst et al., 2003), and reduced frontal cortex volume (Krain & Castellanos, 2006). Genetic studies, although inconclusive, also point to links between ADHD and polymorphisms of dopamine receptor genes resulting under expression dopamine receptors (for review, see Faraone et al., 2005).

Functional activation in several relevant brain regions was examined via Fos-like immunoreactive (Fos-IR) labeling to determine differences in activation between SHR and WKY during the task, and to determine the effects of nicotine on activation in SHR. Sub-regions of the prefrontal cortex (PFC) and striatum were selected for their role in response inhibition, addiction, and influence on DA release in response to psychoactive drugs via the corticolimbic and mesolimbic pathways. Specifically, the nucleus accumbens shell (AcbSh) was targeted because of its importance in modulating motivational salience, and establishing learned associations between motivational events, (Kalivas & Volkow, 2005). The nucleus accumbens core (AcbC) was of interest due to its mediation of the expression of learned behaviors, and its anatomical association with the orbitofrontal cortex (OFC), a region implicated in inhibition and addiction. (Eagle & Baunez, 2010; Rubia et al., 2009; Winstanley, Eagle, & Robbins, 2006) The OFC is also

critically involved in compulsive drug taking and in motivation by stimuli predicting drug availability (Kalivas & Volkow, 2005). Lesions to the infralimbic cortex (ILC) are associated with increases in impulsive responding in the 5-CSRTT (Winstanley et al., 2006), and nicotinic receptors in the ILC are suggested to play a critical role in mediating the effects of nicotine on impulsivity (Tsutsui-Kimura et al., 2010).

The present study examined the effects of a chronically administered systemic injection of a moderate dose of nicotine (0.3 mg/kg) on the response withholding performance of the SHR in a FMI schedule of reinforcement. The selected dose is within the range of previous studies using repeated subcutaneous injections to examine effects of nicotine on operant behavior (Dallery & Locey, 2005; Kirshenbaum et al., 2008), and falls well within the range of typical systemic doses used in behavioral research (approx. 0.05-0.8 mg/kg; Matta et al., 2007). Also, 0.3 mg/kg acute nicotine produced the most pronounced behavioral effect in a previous study using an FMI schedule (Mazur et al., 2014).

Although the majority rodent research suggests that nicotine induces response inhibition deficits, not alleviates them, the use of a rodent task that is capable of detecting beneficial effects of stimulants on response inhibition (Hill et al., 2012) may be useful for studying the effects of chronic nicotine. To the extent that SHR performance in FMI schedules models inhibitory deficits related to ADHD, timed waiting intervals were expected to be shorter in SHR than in WKY. To the extent that a chronic nicotine regimen alleviates inhibitory deficits, the duration of timed waiting intervals was expected to increase in SHR after extended exposure to nicotine. In terms of functional activation, it was be expected that compared to WKY, SHR would show a

hypofunctioning prefrontal system, similar to what is observed in humans with ADHD, and that repeated exposure to nicotine would facilitate activation in the nucleus accumbens. Thus, the experiment was designed to make two important comparisons: baseline differences between SHR and WKY, and the effects of nicotine on SHR.

Method

Subjects

Thirty male rats, 20 Spontaneously Hypertensive Rats (SHR/NCrl) and 10 Wistar Kyoto (WKY/NHsd), arrived on post-natal day (PND) 25 and were pair-housed according to strain. Subjects were maintained on a reverse light cycle (lights out 0700 to 1900 hours), with daily sessions beginning at 0800 hours. Once healthy weights were established, the duration of access to food was reduced daily from 24 h to 18 h, 12 h, and finally 1 h post-session. Water was available in the home cages ad libitum throughout the duration of the experiment.

Apparatus

Experiments were conducted in 10 MED Associates (St. Albans, VT) modular test chambers (three chambers were 305 mm long, 241 mm wide, and 210 mm high; seven chambers were 305 mm long, 241 mm wide, and 292 mm high), each enclosed in a sound- and light-attenuating box equipped with a ventilating fan. The front and back walls and the ceiling of the test chambers were made of Plexiglas; the front wall was hinged and served as a door to the chamber. One of the two aluminum side panels served as a test panel. The floor consisted of thin metal bars positioned above a catch pan. A

square opening (51 mm sides) located 15 mm above the floor and centered on the test panel provided access to the hopper (MED Associates, ENV-200-R2M) and was furnished with a head entry detector (ENV-254-CB). Each activation of a dispenser delivered a single 45-mg sucrose pellet (TestDiet, Richmond, IN) to the hopper. A multiple tone generator (MED Associates, ENV-223) was used to produce 3 kHz tones at approximately 75 dB through a speaker (MED Associates, ENV-224AM) centered on the top of the wall opposite to the test panel, 52 mm from the ceiling of the chamber. Two retractable levers (ENV-112CM) flanked the food hopper, and three-color light stimuli (ENV-222M) were mounted above each lever and could be illuminated yellow, green, and red. Lever presses were recorded when a force of approximately 0.2 N was applied to the end of the lever. The ventilation fan mounted on the rear wall of the sound-attenuating chamber provided masked noise of approximately 60 dB. The test chambers could be dimly illuminated by a houselight located behind the wall opposite to the test panel. Experimental events were arranged via a Med-PC® interface connected to a PC controlled by Med-PC IV® software.

Procedure

Sessions were conducted once daily, 7 days a week. Training initiated with autoshaping, consisting of pairing lever insertion with the delivery of a sucrose pellet. Once all rats were responding reliably to the lever, FMI training began. During FMI sessions, reinforcement was contingent upon the rat successfully waiting a given interval of time. The waiting interval was initiated by a lever press, and terminated with a head entry into the food hopper.

Sessions began with a 300-s acclimation period, during which the chamber remained inoperative and dark. After the acclimation period, the start of each subsequent trial was signaled by the insertion of the lever and illumination of the house light. The first response on the lever resulted in the house light being turned off and the illumination of the 3-color stimulus lights, signaling the start of the waiting interval. Criterial waiting time *t* is the amount of time that had to pass between the *initial* lever press and the *terminal* head entry response in order for reinforcement to be delivered. "Correct" responses were defined as terminal responses made after *t* had elapsed, and were reinforced with sucrose paired with a 3 kHz tone. Premature "incorrect" responses (i.e., responses prior to *t*) were not reinforced. All terminal responses resulted in the retraction of the lever and a 10-s blackout period, after which the lever was reinserted and the next trial began. Each session ended after 45 min or after a rat obtained 100 food pellets, whichever happened first.

At the onset of training, the criterial waiting time (*t*) was set to 0.5 s, and increased by 1.25% for each correct response. The value of *t* was carried over from one session to the next until $t = 6$ s, and remained constant thereafter. Once the 6-s criterion had been established, a conjunctive variable interval (VI) schedule was introduced. The VI schedule was implemented as follows: A timer ran throughout the session. Reinforcement became available when the timer completed a specified interval, with one exception. If the interval elapsed after the initial response, reinforcement was not available until the subsequent trial. After each reinforcer, the timer was reset and a new interval was specified. If a correct response was made before reinforcement became available, the rat was exposed to the 3 kHz tone, but sucrose reinforcement was withheld.

Intervals were specified by sampling from a 12-item Fleschler-Hoffman distribution (Fleshler & Hoffman, 1962). The VI-schedule requirement progressed in daily succession (9, 15, 20, 30 s), until all rats were performing at VI 30-s and t = 6 s. The VI 30-s and 6-s criterial time were fixed across sessions for the remainder of the experiment. As soon as the VI and criterial waiting time were fixed, a 12-s limited-hold was implemented upon lever extension. Waiting intervals longer than 12-s were recorded as ineffective responses and did not result in reinforcer delivery.

The VI schedule was implemented to reduce the between-subject variability in rate of reinforcement that would otherwise result from unequal performance. With this control in place, differences in performance could be reliably attributed to the experimental manipulation and not to differences in rate of reinforcement. A VI 30-s schedule meant that, regardless of performance, on average reinforcement was set up (i.e., was delivered with the next correct response) every 30 s.

Throughout FMI training, two variables were tracked daily: Median latency to the initial response and mean waiting interval. Performance was evaluated for stability after a minimum of 10 sessions with t = 6 s and VI = 30 s. Performance was deemed stable when, within 5 consecutive days, the mean waiting interval and the mean median latency of each strain (a) did not change in the same direction for more than 2 consecutive days (b) did not vary by more than 1.5 s. Once stability was achieved by both strains, each rat received three daily injections of saline to acclimate them to the injection process before chronic nicotine treatment was initiated. SHR were assigned into two treatment groups of 10 (SHR-NIC, SHR-Veh) such that baseline performance was roughly equivalent between groups. WKY rats constituted a single group (WKY-Veh).

Nicotine Regimen

During the treatment phase, each rat received daily injections of either 0.3 mg/kg nicotine (SHR-NIC), or a saline solution (SHR-Veh, WKY-Veh), approximately 10 min prior to the start each session. Injections were delivered subcutaneously, and the volume of each injection was matched to body weight such that each rat received 0.1 ml per 100 g of body weight. Daily FMI sessions were identical to those just prior to the start of treatment (i.e., t = 6 s, and VI = 30 s). Treatment continued until stability criteria (identical to FMI training) were established.

Dependent Measures

The primary dependent measures were mean waiting interval and the latency to the first lever press in each trial. Latencies refer to the time elapsed between the lever presentation (start of the trial) and the first lever press. For analysis, latencies were classified into two categories based on the outcome the previous trial: (1) latencies following correct reinforced trials, (2) latencies following unreinforced trials. The unreinforced category includes trials during which a correct response went unreinforced because the VI had yet to elapse.

Temporal Regulation Model

The Temporal Regulation (TR) model (Sanabria & Killeen, 2008) was applied to estimate parameters of the distribution of waiting intervals. The TR model assumes that a mixture of two distributions—one gamma and one exponential—underlie the

distribution of waiting intervals. Our primary index of response inhibition was θ, the mean of the gamma-distributed, timed waiting intervals. Parameter *P* is the proportion of timed waiting intervals. Increases in *P* reflect and increase in gamma distributed waiting intervals relative to exponentially distributed waiting intervals. For a detailed explanation of the Temporal Regulation model and its parameters, see Appendix A.

Tissue preparation and Fos immunohistochemistry

To capture peak Fos protein expression (Nestler, Barrot, & Self, 2001; Sonnenberg, Macgregor-Leon, Curran, & Morgan, 1989), all rats were overdosed with sodium pentobarbital (100 mg/kg, i.p.) 115 min following placement in the chambers on the last day of FMI testing. Rats were transcardially perfused with phosphate buffered saline (pH 7.4) and 4% paraformaldehyde (pH 7.4), and brains were removed and post-fixed in 4% paraformaldehyde and stored at 4°C overnight. Brains were then cryoprotected in 15% and 30% sucrose over 2d, and stored at 4°C until sectioning. Brains were sectioned on a cryostat at 20 μm. Multiple series of slides were taken at each level of section for separate cresyl violet and immunohistochemistry staining procedures. Sections mounted on slides were then stored at -80°C until tissue processing. One series of slides was stained with cresyl violet to identify and confirm subregions of interest for Fos analysis. Another series of slides containing subregions of interest were processed for immunohistochemistry against Fos protein, which will be termed Fos-like immunoreactive (Fos-IR) labeling. Target sections were washed three times in 1x phosphate buffered saline (1xPBS, pH 7.4) and incubated in 5% normal goat serum/1xPBS/ 0.4% Triton X for 60 min at room temperature. Rabbit polyclonal

antibody (anti-Fos, Santa Cruz Biotechnology, sc-52) was utilized to recognize Fos in specific sections containing the nucleus accumbens core, nucleus accumbens shell, dorsolateral striatum, orbitofrontal cortex, and infralimbic cortex. This antibody was used at a dilution of 1:2500 in 5% normal goat serum/1xPBS/ 0.4% Triton X. Following incubation (48 h, 4°C), sections were incubated with avidin–biotin-peroxidase complex (Vectastain ABC kit) for 45 min, then washed again in 1xPBS and processed using DAB with nickel-intensification (DAB peroxidase substrate kit, Vector Laboratories). Brain sections from each experimental group were processed similarly throughout all stages of the procedure. This procedure was adapted from Nikulina et al. (2004) and used recently (Hoffman et al., 2013, 2014).

Data Analysis

Data from the final five days of the training and the subsequent 17 treatment days were included for analysis. Median latencies and estimates of θ were log-transformed, and estimates of *P* were log-odds transformed. These transformations follow suggestions on the estimation of population parameters in a similar model by Cheung, Neisewander and Sanabria (2012). All dependent measures are reported as back-transformed mean ± SEM. *P* and θ were estimated for each rat using the method of maximum likelihood (Myung 2003).

Dependent measures where evaluated under baseline, acute nicotine, and chronic nicotine conditions. The baseline condition was comprised from data collected over the final five days of FMI training. Data collected from the first day of the treatment phase

were used to evaluate the effects of acute nicotine, and data from the final three days of treatment were used to evaluate chronic-nicotine effects.

Model parameters for each condition were derived by pooling the waiting intervals across the sessions in that condition, and estimating a single set of parameters per condition for each rat. Latencies were pooled identically to waiting intervals; the median latency of each rat served as the dependent measure.

ANOVA was implemented to establish the statistical significance of the effects of strain (SHR-Veh vs. WKY-Veh) and nicotine (SHR-Veh vs. SHR-Nic) on latencies and model parameters. Two separate ANOVAs were performed for each dependent measure. Effects of strain were evaluated only for groups receiving vehicle using a 2 x 3 strain (SHR-Veh, WKY-Veh) x condition (Baseline, Acute, Chronic) mixed design ANOVA. Effects of nicotine were evaluated only for SHR groups using a 2 x 3 treatment (SHR-Veh, SHR-Nic) x condition mixed design ANOVA. Only significant main effects of condition and significant interaction effects were followed by post hoc 2-tailed t-tests; only significant effects are reported.

Differences in Fos-IR labeling were analyzed using independent samples t-tests to evaluate effects of strain (SHR-Veh vs. WKY-Vehi) and nicotine (SHR-Veh vs. SHR-Nic). The Benjamini-Hochberg procedure (Thissen, Steinberg, & Kuang, 2002) was applied to adjust t-critical values to control for false discovery when using multiple comparisons.

Results

In all conditions the distribution of waiting intervals were well described by a mixture of two underlying distributions, one gamma (timed waiting intervals) and one exponential (non-timed waiting intervals). Figure 5 illustrates the goodness-of-fit of this model to performance under baseline (BL) and under the first injection of nicotine, 0.3 mg/kg (Ac).

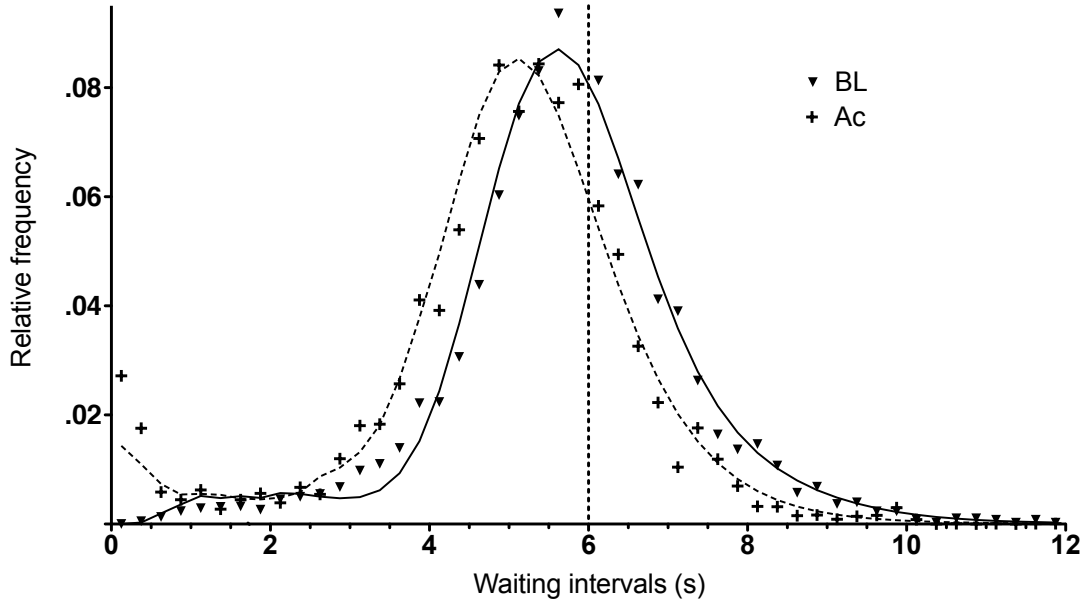


Figure 5. Mean relative frequency distributions of waiting intervals produced by the SHR-NIC group during baseline (BL; downward triangles) and under the first injection of nicotine (Ac; plus sign). Data are organized in 39 bins of 0.5 s, and lines represent mean fits of the Temporal Regulation (TR) model.

Temporal Regulation Parameters

Response inhibition performance, θ. Figure 6 depicts mean estimates of θ during baseline and drug treatment phases. A 2 x 3 (drug x condition) ANOVA for effects of nicotine on θ under baseline (BL), acute nicotine (Ac), and chronic nicotine (Ch) conditions was not significant. However, a post-hoc independent samples t-test for

effects of drug (SHR-NIC vs SHR-Veh) on mean values of θ over the entire course of the treatment period (17 injection days) revealed a significant decrease in θ in the SHR-NIC group ($t(32)=10.3$, $p<.001$).

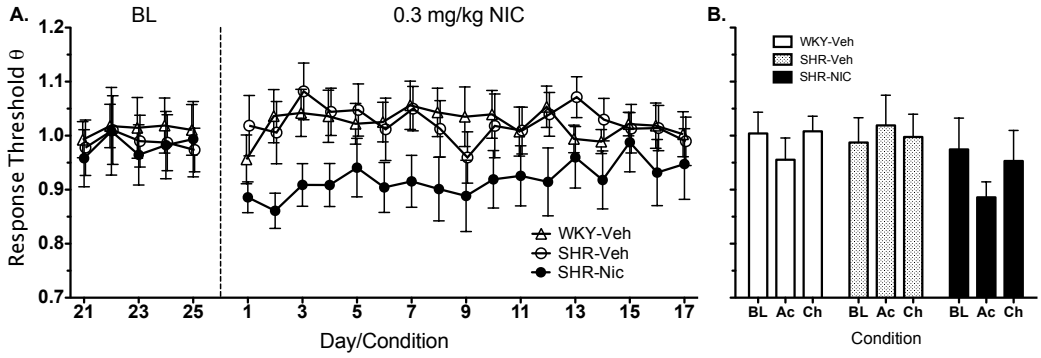


Figure 6. Mean estimates of θ, an index of response inhibition, depicted by day (A) and pooled according to condition (B). Baseline (BL) and chronic nicotine (Ch) estimates were derived from pooling data from the final five days of baseline, and from days 15-17 of chronic nicotine treatment, respectively. Acute nicotine (Ac) estimates were derived from data from day 1 of nicotine injections.

Proportion of timed waiting intervals, *P*. No significant effects were observed on estimates of *P*.

Latency to initiate the waiting interval

Figure 7 depicts the mean latency to initiate waiting intervals for each group. For analysis, latencies were subdivided into two categories: latencies that occurred following a trial in which reinforcement was delivered (Post-R), and latencies that occurred following a trial in which reinforcement was *not* delivered (Post-N).

Post-R latencies. A significant drug x condition interaction was observed on SHR Post-R latencies ($F(2)=4.390$, $p=.020$). Follow up post-hoc paired samples t-tests in the SHR-NIC group revealed that, compared to BL, Post-R latencies were significantly reduced in the Ac ($t(9)=2.344$, $p=.044$), and Ch ($t(9)= 2.407$, $p=.039$) conditions.

Post-N latencies. A significant main effect of drug on Post-N latencies was observed ($F(1)=5.581$, $p=.030$). Nicotine decreased Post-N latencies.

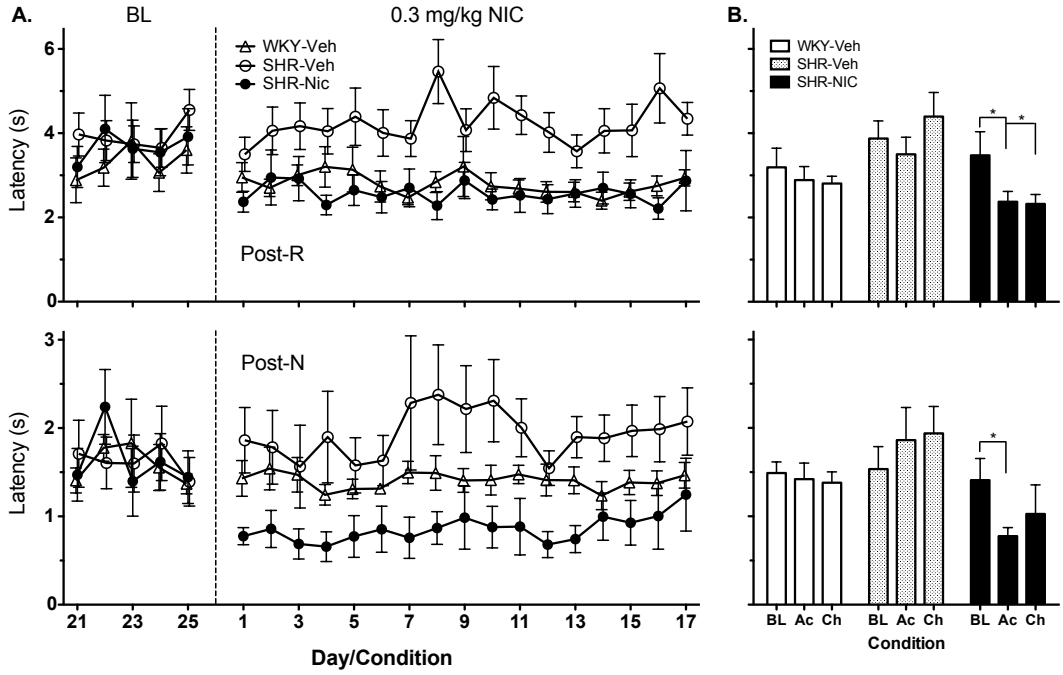


Figure 7. A. Mean of the median latency to the initial response, depicted by group. Post-R latencies (top) are latencies to initiate the waiting interval following reinforced trials, and Post-N latencies (bottom) are latencies following trials in which reinforcement was not delivered. B. Baseline (BL) and chronic nicotine (Ch) estimates were derived from pooling data from the final five days of baseline, and from days 15-17 of chronic nicotine treatment, respectively. Acute nicotine (Ac) estimates were derived from data from day 1 of nicotine injections.

Fos-IR Labeling

Figure 8 depicts Fos-IR labeling. Independent-samples t-tests revealed significantly greater functional activation within the nucleus accumbens shell in SHR-Veh than in SHR-Nic ($t(15)=2.627$, $p=.019$). Functional activation in the OFC was greater in WKY than SHR ($t(17)=2.244$, $p=.038$), however the Benjamini-Hochberg

correction for false discovery rate adjusted α to .025, so this difference failed to meet the criterion for significance.

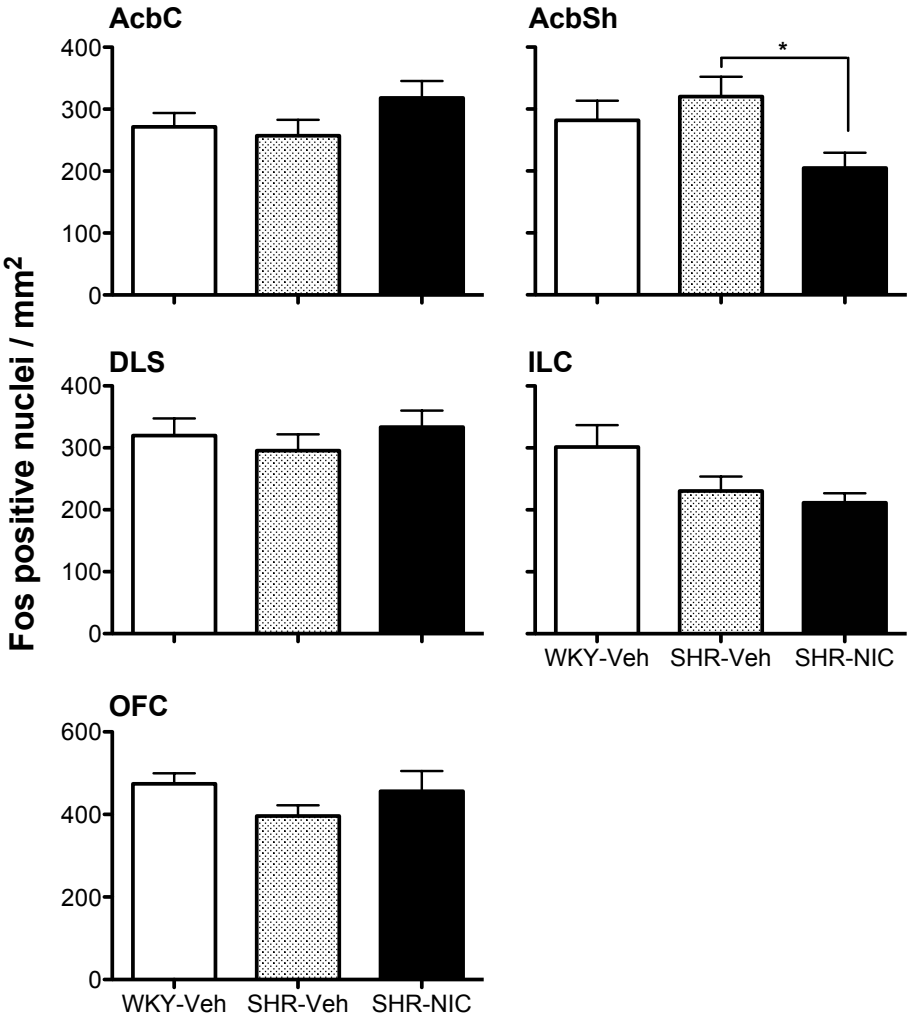


Figure 8. Fos-positive nuclei/mm^2 depicted by brain region. AcbC=nucleus accumbens core, AcbSh=nucleus accumbens shell, DLS=dorsolateral striatum, ILC=infralimbic cortex, OFC= orbitalfrontal cortex. *Denotes significant difference between groups; $p<.05$.

Discussion

The present study examined the effects of chronic nicotine exposure on the response withholding performance of SHR and WKY rats under a FMI schedule of

reinforcement. The hypothesis, that repeated, long-term exposure to nicotine would improve response inhibition performance in SHR, an animal model of ADHD, was not supported. An analysis of the effects of nicotine on θ over the course of the injection period (17 days) revealed a significant decrease in θ in the SHR-NIC group. While this is opposite our predictions, it is in agreement with the majority of existing rodent research (Amitai & Markou, 2009; Blondel et al., 2000, 1999; Hahn et al., 2002; Kirshenbaum et al., 2008; Kolokotroni et al., 2012; Semenova et al., 2007). The present findings also are inconsistent with the hypothesis that high prevalence of smoking among the ADHD population is due to nicotine's ability to ameliorate ADHD-related inhibitory deficits.

One potential interpretation of the present data is that reduced estimates of θ could result from a nicotine-induced speeding of the internal clock (Hinton and Meck 1996). However, such effect has only been observed in the peak interval procedure (Hinton and Meck 1996), and timing performance rapidly recovered to baseline after two days of nicotine administration. The present data do not suggest a rapid recovery of baseline performance following nicotine treatment. Furthermore timing estimates that are more robust to motivational manipulations, such as those obtained from the temporal bisection procedure (Galtress and Kirkpatrick, 2010), do not suggest an accelerative effect of nicotine on the internal clock (Ward et al. 2009). Combined with evidence that chronic nicotine can increase appetite for sucrose in rats (Jias & Ellison, 1990), an explanation of nicotine-induced effects on θ based on timing mechanisms is not supported.

It is important to note that the SHR did not display baseline response inhibition deficits relative to WKY. This lack of strain differences was also observed on FMI schedules in a previous study (Mazur et al., 2014), and acute nicotine (0.1, 0.3, 0.6

mg/kg) produced equivalent decrements in both strains. Testing the hypothesis that nicotine ameliorates response inhibition deficits hinges on the presence of deficits at baseline. Nonetheless, it is unlikely that the detrimental effects of nicotine observed here would be reversed if SHR had performed worse than WKY. Given the similarities between FMI and DRL schedules, it is interesting that SHR display robust response inhibition deficits in DRL procedures (Bull, Reavill, Hagan, Overend, & Jones, 2000; Ferguson et al., 2007; Orduña, Valencia-Torres, & Bouzas, 2009; Sagvolden & Berger, 1996; Sanabria & Killeen, 2008; van den Bergh et al., 2006), but not in FMI.

The first injection of nicotine reduced the latency to initiate waiting intervals in SHR, regardless of whether or not reinforcement was delivered on the previous trial (Figure 7). In the case of Post-R latencies, this effect was still observable at the end of the chronic condition. The finding that nicotine reduces response latencies is consistent with data from FMI schedules (Mazur et al., 2014), as well as response latencies obtained from the 5-CSRTT (Blondel et al., 2000; Mirza & Stolerman, 1998; Semenova et al., 2007; Stolerman, Mirza, Hahn, & Shoaib, 2000).

Evidence from Fos-IR labeling did not support our hypothesis that chronic nicotine would increase general activation in the nucleus accumbens. On the contrary, nicotine-treated SHR showed significantly less activation in the AcbSh than controls. Several studies targeting the AcbSh have provided evidence that chronic pretreatment with nicotine leads to an increase in DA availability compared to saline pretreatment (Benwell & Balfour, 1992; Cadoni & Di Chiara, 2000; Carboni, Bortone, Giua, & Di Chiara, 2000; Di Chiara, 2000; Nisell & Marcus, 1997). Chronic nicotine has also been observed to increase Fos-IR labeling in the AcbSh (Nisell, Nomikos, Chergui, Grillner, &

Svensson, 1997; Salminen, Seppa, & Ga, 1999; Shim et al., 2001). Although evidence suggests that chronic nicotine treatment leads to a reduced DA output compared to the initial nicotine exposure (Cadoni & Di Chiara, 2000; Carboni et al., 2000; Di Chiara, 2000; Nisell & Marcus, 1997), others have found that chronic nicotine exposure potentiates the DA response in the AcbSh (Benwell & Balfour, 1992). Nevertheless, we were unable to find published evidence of a nicotine-induced reduction in Fos-IR labeling. Papa et al. (2002) found that repeated methylphenidate injections decreased DA binding sites in the AcbSh of the SHR. Given the dysregulated dopamine system of the SHR (Q. Li et al., 2009; Linthorst et al., 1994; V. A. Russell, 2002; V. a Russell, 2000; Viggiano et al., 2003, 2004), the observed effect of nicotine on the AcbSh may be relatd to strain-specific characteristics of the SHR. It is also important to note that unlike the studies mentioned above, the present study timed tissue collection to coincide with peak Fos expression during the final experimental FMI session. Thus, in the nicotine-treated SHR group, Fos expression could result from exposure to nicotine or from exposure to the task, rendering results difficult to interpret.

Although the FMI schedule used did not detect response inhibition deficits in SHR, it seems clear that chronic nicotine administration does not provide a benefit for our marker of response inhibition performance (θ). Further research is warranted to determine why SHR do not display response inhibition deficits in FMI, despite demonstrating robust deficits in DRL schedules.

CHAPTER 4

PERFORMANCE OF SPONTANEOUSLY HYPERTENSIVE AND WISTAR KYOTO RATS ON EXTERNALLY- VS. SELF-PACED REPONSE WITHOLDING TASKS: EFFECTS OF ACUTE NICOTINE

Attention-deficit hyperactivity disorder (ADHD), marked by difficulties with inhibition, hyperactivity, and attention, is among the most common childhood psychiatric disorders, affecting between 2% and 10% of children worldwide (Froehlich et al. 2007; Skounti et al. 2007). Deficits in response inhibition are a core feature of ADHD, defined by an inability to withhold prepotent responses, including those that have been previously reinforced (Aron & Poldrack, 2005; Barkley, 1997; Grant et al., 2005). The spontaneously hypertensive rat (SHR) is the most common rodent model of ADHD, and is frequently employed in the study of response inhibition deficits. However, a variety of behavioral tasks are available to assess response inhibition in rodents, with varying results. Under certain conditions SHR show response inhibition deficits, and under others they do not. Research suggests that children with ADHD perform better on externally paced tasks compared to self-paced tasks (Koschack, Kunert, Derichs, Weniger, & Irle, 2003; Sonuga-Barke, Taylor, & Heptinstall, 1992). The present study reviews differences between several rodent response inhibition paradigms, and examines the contribution of self-pacing vs. experimenter-pacing to SHR performance on two variations of the differential reinforcement of low rates (DRL) schedule free-operant DRL (FO-DRL; Ferster & Skinner, 1957), and discrete-trials DRL (DT-DRL; F. Logan, 1961).

DRL schedules require animals to wait a specified duration between consecutive responses in order to receive reinforcement. Performance is measured by the ability to successfully wait between responses (i.e., respond at a sufficiently low rate), and is considered a measure of 'waiting' impulsivity (Eagle et al., 2008).

Across a wide range of response inhibition indices (e.g., mean inter-response time, responses per reinforcer, peak latency, mean timed waiting interval), SHR generally perform worse than WKY on FO-DRL schedules (Table 1). This effect is observed regardless of age, which ranged from approximately post-natal day (PND) 50 to PND 260, or the criterial waiting time, which ranged from 5 to 72 s. Of the seven FO-DRL experiments listed, only one failed to find response inhibition deficits in SHR relative to WKY (Ferguson et al., 2007). In this exceptional case, extended training (106 sessions) and breeder selection (see Sagvolden et al., 2009) may have contributed to this divergence from the general trend.

Table 1. Performance of SHR and WKY rats on free operant DRL schedules

Task	Approx age	Performace differences	# sessions	Author
DRL-5	PND 260	LE, WKY > SHR	NL	Sanabria & Killeen (2008)
DRL-10	PND 90	WIS, WKY > SHR	70	Orduña et al. (2009)
DRL-10	PND 90	WIS, WKY > SHR	30	Orduña et al. (2009)
DRL-10	PND 148	SD > SHR, WKY	106	Ferguson et al. (2007)
DRL-16	PND 80	WKY > SHR	42	Sagvolden & Berger (1996)
DRL-60	PND 260	LE, WKY > SHR	NL	Sanabria & Killeen (2008)
DRL-60	NL	WKY > SHR > SD	5	Bull et al. (2000)
DRL-72	PND 50	WKY > SHR	NL	van den Bergh et al. (2006)

Ages are best approximation of PND at the beginning of testing. NL=not listed

It seems that FO-DRL schedules are suitable for observing response inhibition deficits in SHR. This appears not to be the case for the fixed minimum interval (FMI) schedule of reinforcement (Mechner & Guevrekian, 1962). Similar to DRL, FMI schedules require subjects to wait a specified interval between responses. However, in FMI schedules the initial response differs from the terminal response (i.e., two different levers, or a lever press followed by a hopper beam break), whereas initial and terminal responses in DRL schedules are co-located (i.e. consecutive presses on a single lever, consecutive beam breaks). Recent evidence showed that SHR on FMI 6-s schedules do not display response inhibition deficits relative to WKY (Mazur et al., 2014; Mazur et al., submitted). This finding is surprising, considering that both schedules require animals to withhold responding for a given interval.

Two major differences between FMI and FO-DRL schedules are that (1) in FO-DRL schedules the target response for initial and terminal responses are the same, whereas in FMI they are not, and (2) FO-DRL is a free-operant procedure where multiple waiting intervals can be produced in rapid succession, whereas FMI is a discrete-trial procedure. This means that in FMI schedules each waiting interval produced is followed by an ITI such that only one waiting interval can be produced per trial.

The difference between the free-operant arrangement in FO-DRL schedules and discrete-trials arrangement in FMI schedules may account for the absence of evidence of strain differences under FMI schedules. In a free-operant design, subjects control the pace at which waiting intervals are generated. Although subjects do not control the start of each waiting interval, their rate of responding dictates the rate at which waiting

intervals are produced. Subjects are permitted to respond rapidly, producing a large number of short intervals. This is contrary to a discrete-trials design, where the program places external limits on the pace at which waiting interval can be generated, via the ITI and resetting of the trial (see Figure 9 for schedule diagram).

Research in humans with ADHD on self-paced vs. externally paced tasks suggests that when participants are permitted to control the pace of stimulus presentation, children with ADHD perform worse than controls, but when the pace of stimulus presentation is externally controlled by the experimenter, children with ADHD perform similarly to controls (Koschack, Kunert, Derichs, Weniger, & Irle, 2003; Sonuga-Barke, Taylor, & Heptinstall, 1992). The differences between self-paced and externally paced performance is attributed to a faster response style, where children with ADHD respond more rapidly and spent less time attending to stimuli (Denckla, 1996; Koschack et al., 2003).

A similar distinction may be made between FMI and FO-DRL. Two major differences between FMI and FO-DRL schedules are that (1) in FO-DRL schedules the target response for initial and terminal responses are the same, whereas in FMI they are not, and (2) FO-DRL is a free-operant procedure where multiple waiting intervals can be produced in rapid succession, whereas FMI is a discrete-trial procedure. This means that in FMI schedules each waiting interval produced is followed by an ITI such that only one waiting interval can be produced per trial.

To test the hypothesis that response inhibition deficits in SHR are ameliorated by an externally-paced task, we implemented a discrete-trials DRL (DT-DRL) task that was as similar as possible to the FMI task used in past research (Mazur et al., 2014, submitted). To our knowledge this is the first study to examine SHR vs. WKY

performance on a DT-DRL task. To the extent that externally controlling the pace at which waiting intervals are generated contributes to improved performance in impulsive/hyperactive subjects, we expected SHR to perform equally well as WKY on a DT-DRL task. For comparison, the same cohort of SHR and WKY were also exposed to a FO-DRL procedure. In the case of FO-DRL, we expected SHR to perform markedly worse than WKY, evidenced by shortened mean waiting intervals.

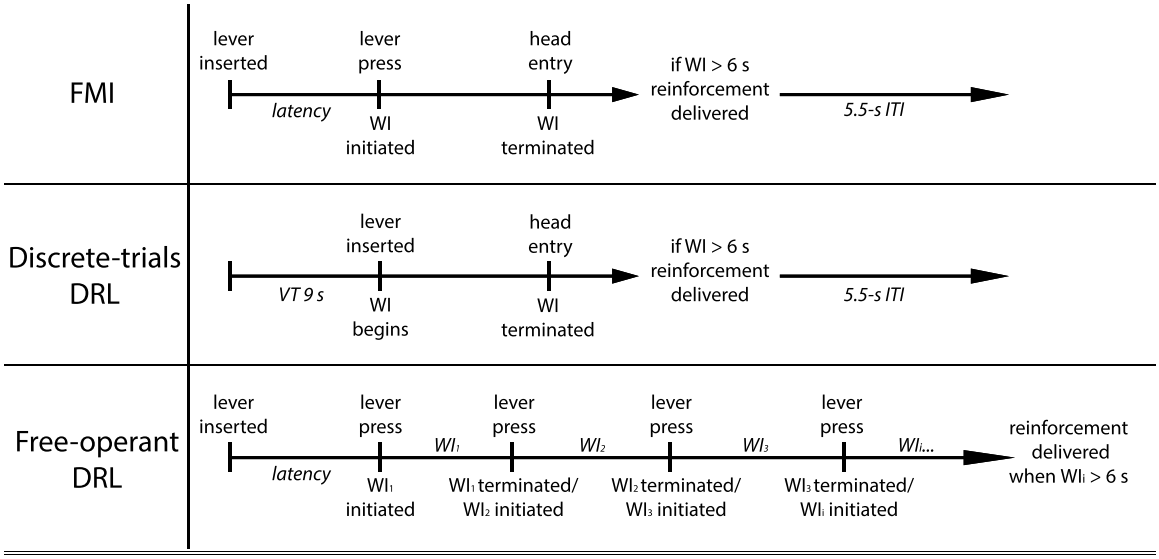


Figure 9. Depiction of events from one reinforcer to the next for three operant schedules: FMI, discrete-trials DRL, and free-operant DRL; (WI=waiting interval).

Methods

Subjects

Subjects were sixteen male rats, 8 Spontaneously Hypertensive Rats (SHR/NCrl) and 8 Wistar Kyoto (WKY/NHsd), post-natal day (PND) 50. Subjects were pair-housed according to strain and maintained on a reverse light cycle (lights out 0700 h to 1900 h), with daily sessions beginning at 10 am. Once healthy weights were established, the

duration of access to food was reduced daily from 24 h to 18 h, 12 h, and finally 1 h post-session (given 30 min after the end of the session). Water was available in the home cages ad libitum throughout the duration of the experiment.

Apparatus

Experiments were conducted in 10 MED Associates (St. Albans, VT) modular test chambers (three chambers were 305 mm long, 241 mm wide, and 210 mm high; seven chambers were 305 mm long, 241 mm wide, and 292 mm high), each enclosed in a sound- and light-attenuating box equipped with a ventilating fan. The front and back walls and the ceiling of the test chambers were made of Plexiglas; the front wall was hinged and served as a door to the chamber. One of the two aluminum side panels served as a test panel. The floor consisted of thin metal bars positioned above a catch pan. A square opening (51 mm sides) located 15 mm above the floor and centered on the test panel provided access to a dipper (MED Associates, ENV-202M-S) fitted with a cup (MED Associates, ENV-202C) that could hold 0.01 cc of a liquid reinforcer (33% sweetened condensed milk diluted in tap water; Great Value brand, Walmart, Bentonville, AK). The receptacle was furnished with a head entry detector (ENV-254-CB). A multiple tone generator (MED Associates, ENV-223) was used to produce 3 kHz tones at approximately 75 dB through a speaker (MED Associates, ENV-224AM) centered on the top of the wall opposite to the test panel, 240 mm above the floor of the chamber. Two retractable levers (ENV-112CM) flanked the food hopper, and three-color light stimuli (ENV-222M) were mounted above each lever and could be illuminated yellow, green, and red. Lever presses were recorded when a force of approximately 0.2 N was applied to the end of the lever. The ventilation fan mounted on the rear wall of the

sound-attenuating chamber provided masked noise of approximately 60 dB. The test chambers could be dimly illuminated by a houselight located behind the wall opposite to the test panel. Experimental events were arranged via a Med-PC® interface connected to a PC controlled by Med-PC IV® software.

Procedure

Sessions were conducted once daily, 7 days a week. During all DRL sessions, reinforcement was contingent upon the rat successfully waiting a given interval of time between consecutive responses. The criterial waiting time was 6 s; *correct* responses were reinforced with 5-s access to 0.01 ml of sweetened condensed milk. Premature *incorrect* responses were not reinforced. All sessions began with a 300-s acclimation period, during which the chamber remained inoperative and dark.

Discrete-trial DRL. After the acclimation period, each trial began with the illumination of the house light and initiation of VT 9-s timer, sampled from a Fleshler-Hoffman distribution (Fleshler & Hoffman, 1962). Once the VT interval elapsed, the houselight was turned off, the right lever was inserted, and the 3-color stimulus lights above the lever were illuminated, signaling the start of the waiting interval. The waiting interval was terminated with a head entry into the food hopper; lever presses were recorded but had no programmed effect. The criterial waiting time *t* was the minimum amount of time that had to pass between the lever insertion and the terminal head entry response in order for reinforcement to be delivered. *Correct* responses were defined as terminal responses made after *t* had elapsed since the initial response, and were reinforced with 5-s access to 0.01 ml of sweetened condensed milk. paired with a 0.5-s

3kHz tone. Premature *incorrect* responses (i.e., terminal responses prior to *t*) were not reinforced. All terminal responses resulted in the retraction of the lever and a 5.5-s blackout period, after which the lever was reinserted and the next trial began. Each session ended after 45 min or after 150 sucrose pellets were delivered, whichever happened first.

At the onset of DRL training, the criterial waiting time (*t*) was set to 0.5 s, and was increased by 1.25 percent after each correct response. The value of *t* was carried over from one session to the next until $t = 6$ s, and remained constant thereafter. Once the 6-s criterion had been established, the probability of the availability of reinforcement for correct responses was changed from 100 percent to 40 percent: At the start of each trial, there was a 40 percent chance that reinforcement would be set up (i.e., available) for a correct response. Once reinforcement was set up, its availability was carried over from trial to trial until a correct response was recorded. This probability was reduced to mitigate the between-subject variability in rate of reinforcement that would otherwise result from unequal performance. Thirty-two sessions were conducted.

Nicotine administration. After 32 sessions, a nicotine probe was conducted. Each rat was administered one subcutaneous injection of nicotine (0.3 mg/kg), and one subcutaneous injection of saline in a counterbalanced order, separated by four days. Both injections were administered 5 min prior to the start of the session.

Free Operant DRL. Four days after the last injections from the previous condition FO-DRL training began. In this procedure, initial and terminal responses were both made by a lever press. After the acclimation period, the left lever was inserted (note

that only the right lever was used in the previous condition). The first response on the lever initiated the waiting interval; subsequent responses on the lever simultaneously terminated the current waiting interval and began a new one.

At the onset of FO-DRL training, the criterial waiting time (*t*) was set to 0.5 s and was increased in the same manner described in the DT-DRL condition. Premature responses did not end the trial or result in a timeout. Instead, the waiting interval was reset and the lever remained extended until a waiting interval greater than 6 s was produced. Correct responses were reinforced with 5-s access to 0.01 ml of sweetened condensed milk paired with a 0.5-s 3kHz tone. Thirty sessions were conducted.

Nicotine administration. After 30 sessions, a nicotine probe was conducted. Each rat was administered one subcutaneous injection of nicotine (0.3 mg/kg), and one subcutaneous injection of saline in a counterbalanced order, separated by four days. Both injections were administered 5 min prior to the start of the session.

Temporal Regulation Model

The Temporal Regulation (TR) model (Sanabria & Killeen, 2008) was applied to estimate parameters of the distribution of waiting intervals. The TR model assumes that a mixture of two distributions—one gamma and one exponential—underlie the distribution of waiting intervals. Our primary index of response inhibition performance, θ, represents the mean of the timed waiting intervals. Parameter *P* is the proportion of timed waiting intervals. Increases in *P* reflect and increase in gamma distributed waiting intervals relative to exponentially distributed waiting intervals. For a detailed explanation of the Temporal Regulation model and its parameters, see Appendix A.

Data Analysis

Individual waiting intervals from the final five days of testing were pooled and used for the analysis of strain differences. *P* and θ were estimated for each rat using the method of maximum likelihood (Myung 2003). Latencies were pooled identically to waiting intervals, and the median latency for each rat was used to calculate the group mean. Strain differences were evaluated using an independent-samples t-test of the pooled values for each dependent measure. Non-significant effects ($p > .050$) were not reported.

Effects of nicotine were evaluated using a 2 x 2 mixed design ANOVA with strain (SHR vs. WKY) as the between-subjects factor and drug (Veh vs. NIC) as the within-subjects factor.

Results

In all conditions, the distribution of waiting intervals was well described by a mixture of two underlying distributions, one gamma (timed waiting intervals) and one exponential (non-timed waiting intervals). Figure 10 illustrates the goodness-of-fit of this model to performance in the last five days of training (pooled).

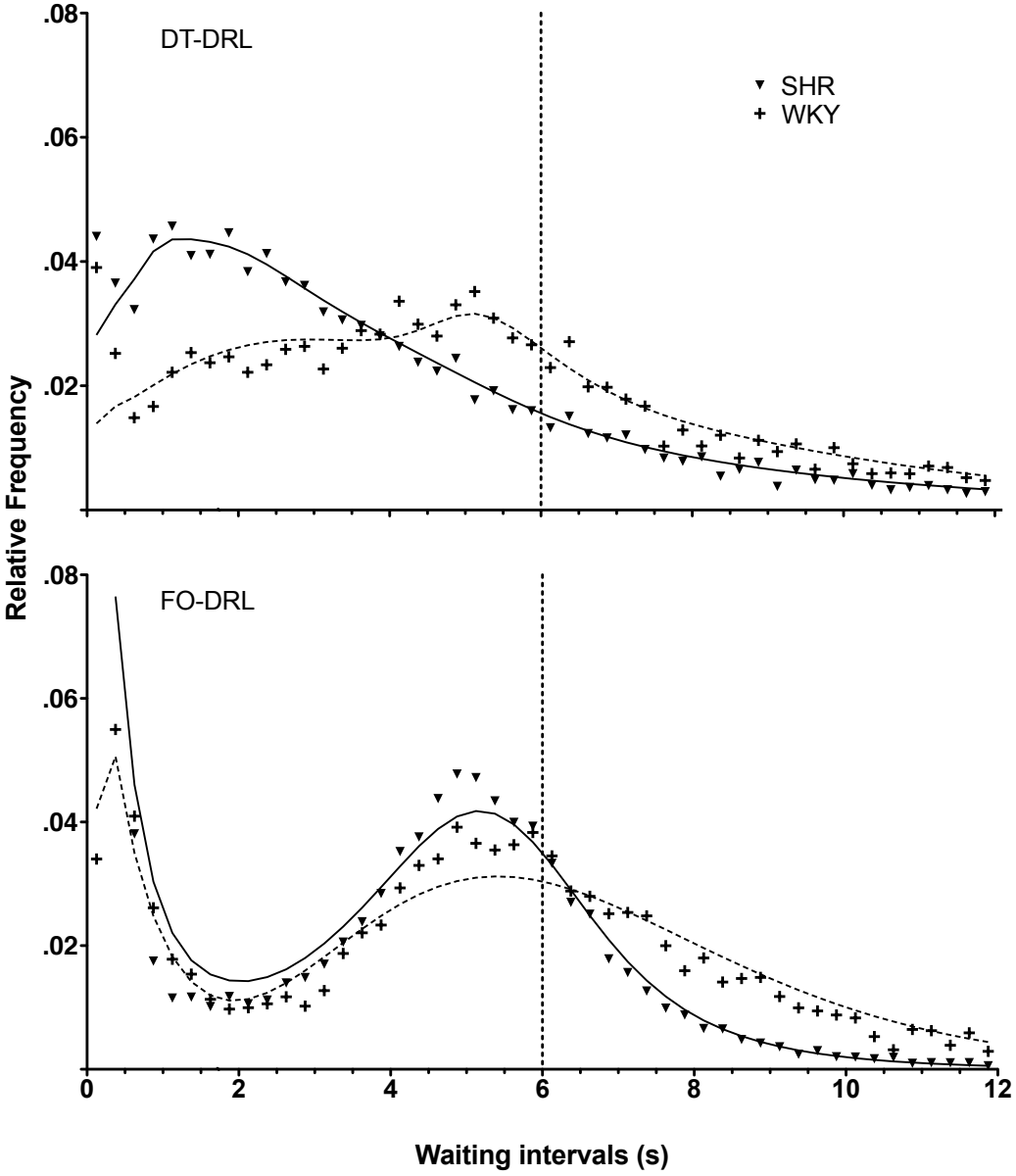


Figure 10. Mean relative frequency distributions of waiting intervals produced by the SHR (downward triangles) and WKY (plus sign) rats on DT-DRL (top) and FO-DRL schedules (bottom) schedules of reinforcement. Data are organized in 39 0.25-s bins, and lines represent fits of the Temporal Regulation (TR) model.

Discrete-trials DRL

Mean waiting interval. Mean waiting intervals for each strain are depicted in Figure 11 (top). Independent samples t-tests revealed a that mean waiting intervals were

significantly shorter for SHR than WKY over the last 5 days of non-injection sessions ($t(14)=4.808$, $p<.001$). A 2 x 2 (strain x drug) repeated measures ANOVA revealed that nicotine significantly decreased mean waiting intervals ($F(1,1)=16.729$, $p=.001$).

Response threshold, θ. Independent samples t-tests revealed that θ was significantly lower in SHR relative to WKY over the last 5 days of non-injection sessions ($t(14)=2.526$, $p= .024$). A 2 x 2 (strain x drug) repeated measures ANOVA revealed a significant main effect of drug—nicotine significantly decreased mean waiting intervals ($F(1,1)=8.177$, $p=.013$).

Proportion of timed waiting intervals, *P*. An independent samples t-test for effects of strain revealed that *P* was significantly higher for WKY than SHR ($t(14)=4.113$, $p=.001$). A 2 x 2 (strain x drug) repeated measures ANOVA only revealed a significant effect of strain ($F(1,1)=9.309$, $p=.009$) where WKY produced a larger proportion of gamma distributed waiting intervals.

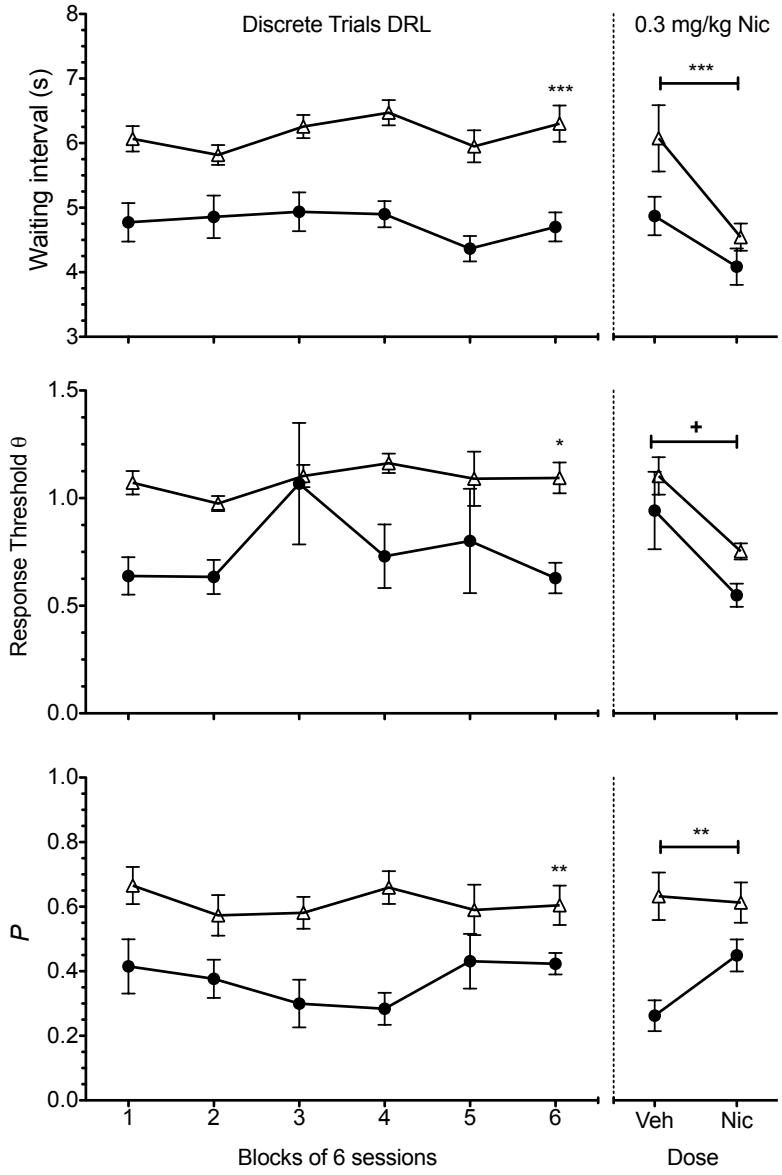


Figure 11. Left panels depict mean waiting intervals (top), mean estimates of θ (middle) and P (bottom) for discrete-trial DRL sessions. Right panels depict effects of veh and 0.3 mg/kg injections. *Denotes significant effect of group. +Denotes significant effect of drug. Symbols are repeated with lower p-values (i.e., $^{*}p < .050$, $^{**}p < .010$, $^{***}p < .001$)

Free operant DRL

Mean waiting interval. Mean waiting intervals for each strain are depicted in Figure 12 (top). Independent samples t-tests revealed that SHR produced significantly shorter mean waiting intervals compared to WKY over the last 5 days of non-injection

sessions ($t(14)=4.093$, $p= .001$). A 2 x 2 (strain x drug) repeated measures ANOVA revealed a significant main effect of drug ($F(1,1)=18.580$, $p=.001$), and a significant effect of strain ($F(1,1)=26.935$, $p< .001$). Nicotine significantly decreased mean waiting intervals and SHR had significantly shorter mean waiting intervals than WKY.

Response threshold, θ. Independent samples t-tests revealed that θ was significantly lower in SHR relative to WKY over the last 5 days of non-injection sessions ($t(14)=3.228$, $p= .006$). A 2 x 2 (strain x drug) repeated measures ANOVA revealed a significant main effect of drug ($F(1,1)=13.436$, $p=.003$), and a significant main effect of strain ($F(1,1)=9.810$, $p= .007$). Nicotine significantly decreased estimates of theta, and SHR had significantly shorter estimates of theta than WKY.

Proportion of timed waiting intervals, *P*. A 2 x 2 (strain x drug) repeated measures ANOVA revealed a significant main effect of drug ($F(1,1)=8.165$, $p=.013$). Nicotine decreased the proportion of timed waiting intervals.

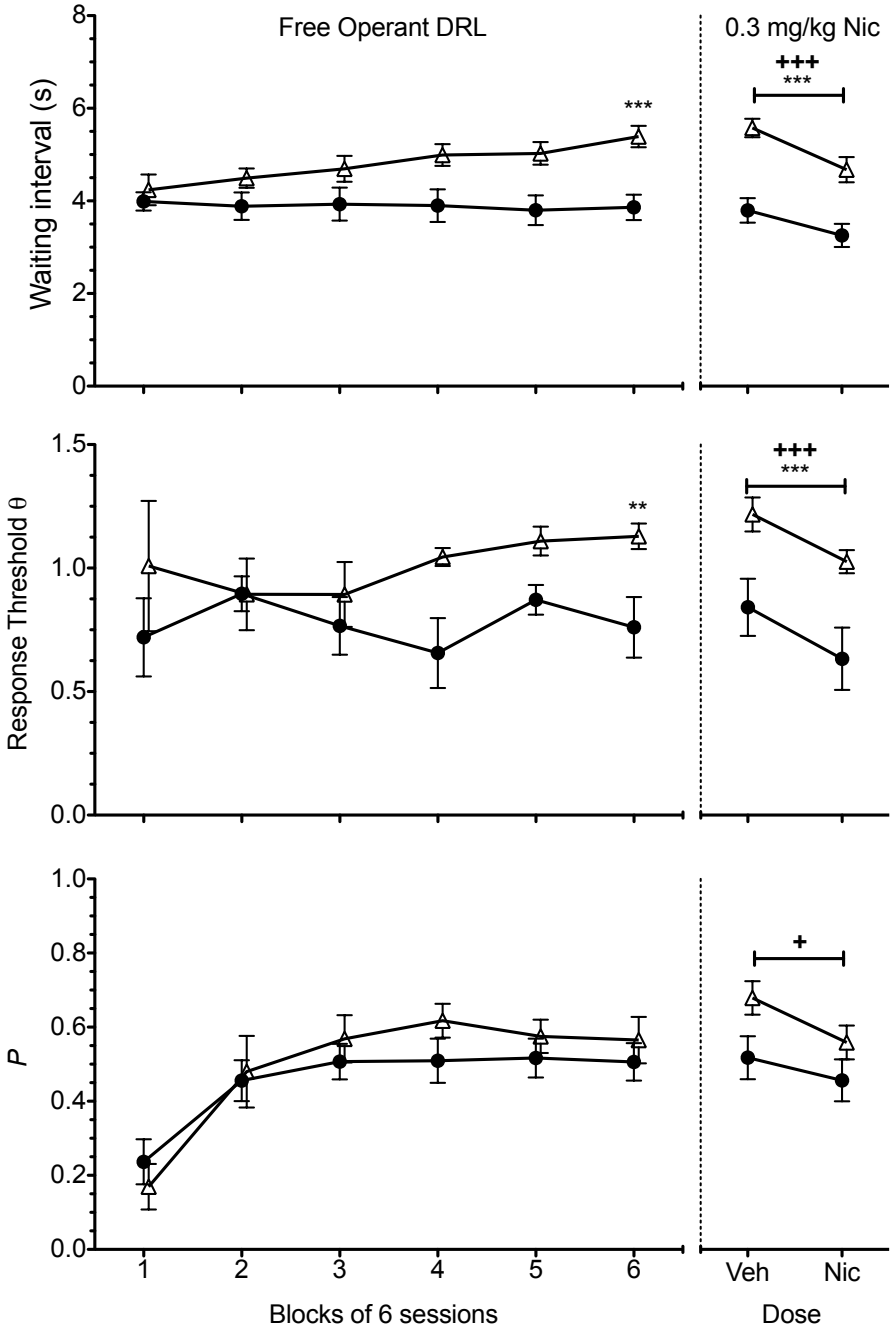


Figure 12. Left panels depict mean waiting intervals (top), mean estimates of θ (middle) and P (bottom) for free-operant DRL sessions. Right panels depict effects of veh and 0.3 mg/kg injections. *Denotes significant effect of group. +Denotes significant effect of drug. Symbols are repeated with lower p-values (i.e., $^{*}p < .050$, $^{**}p < .010$, $^{***}p < .001$)

Discussion

In both DRL schedules, SHR consistently produced shorter mean waiting intervals than WKY. This finding supports previous evidence from SHR and WKY on FO-DRL schedules (Bull et al., 2000; Evenden & Meyerson, 1999; Ferguson et al., 2007; Orduña et al., 2009; Sagvolden & Berger, 1996; Sanabria & Killeen, 2008; van den Bergh et al., 2006), but is contrary to the hypothesis that SHR would perform better in an externally paced DT-DRL task. Similarly, estimates of θ show that the response-withholding performance of SHR was significantly worse than WKY, regardless of DRL schedule. Strain differences in performance under the DT-DRL schedule were also reflected in estimates of *P* (Figure 11, bottom). This means that the shorter mean waiting intervals produced by SHR in DT-DRL were influenced by a significant increase in exponentially distributed responses (i.e., lower values of *P*). This effect is visible in the mean distribution of waiting intervals produced in DT-DRL (Figure 11, top). Notice the peak of the mean distribution of SHR waiting intervals is shorter than 2 s, and declines steadily with increasing durations. This exponential pattern, combined with a lack of a peak around the criterial waiting time suggests that SHR may have been less sensitive to the contingencies of the timing task than WKY. Although the majority of waiting intervals produced by WKY were below criterion, the mode of their distribution suggests that WKY were more sensitive to the contingencies of the task.

Difficulties in acquisition may have arisen from the fact that, in DT-DRL, waiting intervals were initiated by the program—not the rat—and terminal responses were produced by a head-entry beam break into the hopper. In this arrangement, the production of waiting intervals required rats to attend to cues signaling that the waiting

interval had been initiated by the program. If rats failed to attend to the cues, and instead checked the hopper for food at random intervals, the distribution of intervals would closely resemble the exponential pattern produced by SHR in the DT-DRL condition. While the differential effect on SHR performance is certainly interesting, it seems that a DT-DRL task in which the initiation and termination of waiting intervals is controlled by the subjects would be more appropriate for evaluating response inhibition.

An acute dose of nicotine (0.3 mg/kg) decreased mean waiting intervals and estimates of θ, regardless of strain or DRL schedule. This finding is consistent with prior evidence of the effects of nicotine on FO-DRL performance (Kirshenbaum et al., 2011, 2008, 2009; C. Morrison & Armitage, 1967; C. F. Morrison, 1968; Popke, Fogle, et al., 2000; Popke, Mayorga, et al., 2000). Acute nicotine decreases estimates of *P* in FO-DRL, suggesting a nicotine-induced increase in exponentially distributed responses. Mazur and colleagues (2014) reported that the same does of acute nicotine had the opposite effect on estimates of *P* obtained from FMI, where trials are initiated by the rat. Given that nicotine can increase response rates (Bovet & Bovet, 1965; Davis, Kensler, & Dews, 1973; Morrison, 1967), the opportunity to rapidly produce waiting intervals in FO-DRL (but not in FMI) may account for the paradoxical effect on *P*.

Note that deficient SHR performance relative to WKY was not detected by FMI schedules in Chapters 1 or 2, where no strain differences were observed. This suggests that some difference exists between FMI and DRL schedules that differentially affect one strain. In FMI schedules, waiting intervals are initiated with a lever press and terminated with a head entry into the food hopper (target response). In DRL schedules, the initial and terminal responses are co-located, and waiting intervals are typically produced using

either lever presses or head entries but not both. The separation of the initial and terminal response may facilitate longer waiting intervals in FMI schedules, either by the delay incurred from moving between operanda, or by removing the opportunity for rapid iterative responses.

Another important difference between FMI and DRL schedules is that, in the FMI task, rats control the beginning of a waiting interval independently from the end of an interval; in FO-DRL, each response is both the beginning of new interval and the end of the previous one, except the first response after a reinforcer (see Figure 9 for visual depiction). In the DT-DRL task, waiting intervals are initiated by the program. In DRL schedules, rats did not control the beginning of intervals. Thus, differences between FMI and DRL performance may be attributed to control over the beginning of intervals. To test this possibility, waiting intervals from the final non-drug session of FO-DRL were examined post-hoc. The mean of the first waiting interval produced after each reinforcer was delivered did not differ from the overall mean waiting intervals. The absence of significant differences between these waiting intervals suggests that these effects cannot be explained simply by control over the start of the waiting interval.

In conclusion, limiting the rate at which waiting intervals could be produced did not appear to improve the response-withholding performance of SHR. SHR generated significantly shorter mean waiting intervals than WKY in DT-DRL and FO-DRL, although in SHR performance in DT-DRL was influenced by a greater proportion of exponentially distributed responses. Further research is necessary to determine specific components of response inhibition schedules that may contribute to improved SHR performance, and the relation of those components to response inhibition in humans.

CHAPTER 5

GENERAL DISCUSSION

The primary purpose of Experiments 1 and 2 was first, to determine if nicotine improves response inhibition, and second, to determine if SHR are differentially affected by nicotine compared to their normotensive control strain, the WKY. We hypothesized that nicotine would rescue SHR deficits in FMI performance. Although previous research suggests that nicotine is detrimental to response inhibition, FMI was a novel approach to measuring response inhibition independent of motivational bias. However, acute and chronic doses of nicotine decreased response threshold (θ) for both SHR and WKY rats. Furthermore, SHR and WKY strains from both experiments (two separate cohorts) did not differ in their baseline response threshold. This finding was surprising, considering that SHR consistently perform worse than WKY on similar waiting tasks such as FO-DRL schedules.

Experiment 3 aimed at elucidating why we did not observe strain differences in response inhibition performance on FMI schedules. Although FMI and FO-DRL are purported to measure "waiting impulsivity," (Dalley et al., 2011) and require subjects to wait a specified interval between responses, subtle differences may be key to understanding the differential performance of WKY. FO-DRL permits animals to generate waiting intervals in rapid succession, whereas FMI schedules do not. Similarly, children with ADHD have been shown to perform worse in tasks where they are allowed to control the pace of trials, compared to tasks where the pace is controlled by the experimenter. Thus, a discrete-trials version of the DRL schedule (DT-DRL) was employed to test the possibility that externally-paced tasks facilitate improved

performance. Data from the DT-DRL task suggests that this is not the case. Both DRL schedules revealed robust response inhibition deficits in SHR, in the form of reduced mean waiting intervals. However, the distribution of waiting intervals suggests that animals were generating relatively few timed waiting intervals in DT-DRL. To properly test differences between self-paced and externally paced DRL tasks, modifications may need to be made to the DT-DRL arrangement used in Experiment 3.

The effects of nicotine on DRL performance agree with our findings from FMI schedules, and a number of previous studies (Kirshenbaum & Brown, 2008; Kirshenbaum et al., 2011, 2009; Mayorga, Popke, Fogle, & Paule, 2000; Popke, Fogle, et al., 2000). An acute s.c. injection of nicotine (0.3 mg/kg) significantly reduced mean waiting intervals and estimates of θ, regardless of strain or dose.

Taken together, our findings do not support the hypothesis that elevated rates of smoking in the ADHD population are due to nicotine's ability to ameliorate response inhibition deficits. In fact, the present study and the majority of existing research suggest the opposite—that nicotine is detrimental to response inhibition. Comparison data from FMI and DRL schedules indicate that FMI schedules may not be appropriate for studying response inhibition deficits in SHR. The main advantage of FMI schedules is the ability to dissociate measures of response inhibition from motivational bias. In DRL these two measures are confounded. However, SHR and WKY did not display any motivational differences in FMI schedules, so the emergence of strain differences in DRL schedules cannot be explained in terms of motivational bias. Further examination of these tasks may highlight critical components involved in the amelioration of response inhibition deficits.

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APPENDIX A

THE TEMPORAL REGULATION MODEL

'Waiting interval,' refers to the time elapsed between the initial lever press and the terminal head entry. The Temporal Regulation model was applied to estimate parameters of the distribution of waiting intervals (Mika et al. 2012; Sanabria and Killeen 2008). The model assumes that, at the beginning of every trial, rats either enter a *timing state* (with probability P) or they do not (with probability $1 - P$). When in a timing state, rats produce waiting intervals that are gamma-distributed, centered close to the criterial FMI interval (here, 6 s). When rats are not in a timing state, they produce intervals at a constant average rate, and as such, non-timing intervals are exponentially distributed. Thus, according to the Temporal Regulation model, a mixture of two distributions, one gamma and one exponential, underlie the distribution of waiting intervals:

$$\Pr(\text{IRT} = t \mid t < \delta) = 0$$

$$\Pr(\text{IRT} = t \mid t \geq \delta) = P\Gamma(t - \delta; n, c) + (1 - P)(1 / K) \exp(-(t - \delta) / K). \qquad (1)$$

In Equation 1, the probability of entering a timing state, P, is the mixture weight of a gamma distribution with shape parameter n and scale parameter c. Both distributions, gamma and exponential, are shifted rightwards to account for the minimum time required to complete the initial and terminal response, δ. Thus, the mean duration of timed waiting intervals is $nc + \delta$ and the mean duration of non-timed waiting intervals is $K + \delta$. Our analysis was primarily concerned with estimates of P and of the rescaled mean of the gamma distribution, $\theta = (nc + \delta) / 6$ s. If the mean timed waiting interval is shorter or longer than the 6-s criterial time, estimates of θ are, respectively, less than or greater than

1. Estimates of θ served as indices of response inhibition performance (Sanabria and Killeen, 2008).