Published in final edited form as:
Pharmacol Biochem Behav. 2011 September ; 99(3): 459–467. doi:10.1016/j.pbb.2011.04.018.

Self-administered and Yoked Nicotine Produce Robust Increases in Blood Pressure and Changes in Heart Rate with Modest Effects of Behavioral Contingency in Rats

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Abstract

Experimenter-administered nicotine produces reliable increases in blood pressure and changes in heart rate. However, an extensive literature demonstrates that the effects of psychoactive drugs are dependent on whether administration is contingent on behavior. The present study assessed the cardiovascular effects of nicotine and whether those effects vary as a function of whether nicotine was self-administered or response-independent. Rats were divided into three groups according to a yoked design. The pattern of infusions for each triad was determined by the animal self-administering nicotine; the other two animals received either yoked nicotine or saline. Heart rate and blood pressure were measured during eighteen daily, 1 hour drug sessions by radiotelemetry. Each session was preceded and followed by a 20 minute period during which cardiovascular function was monitored in the operant chambers, but drug was not available. Acute exposure to yoked nicotine produced a rapid rise in blood pressure that was larger than the increase observed with self-administered nicotine. Additional infusions during the first session resulted in a similar sustained elevation in blood pressure in the nicotine groups. Over subsequent sessions, self-administered nicotine produced a larger effect on systolic blood pressure particularly early in each session, although for both self-administered and yoked nicotine the hypertensive effects waned partially with repeated test sessions. This decrease was fully accounted for by a pre-session decrease in pressure; relative to pre-session levels the strong hypertensive effects of nicotine persisted. Initial exposure to nicotine produced a short-lived bradycardia that in subsequent sessions was replaced with a longer-lasting nicotine-induced tachycardia; neither effect was related to the behavioral contingency of nicotine delivery. Together, these data provide a rich picture of the cardiovascular effects of nicotine. Effects of behavioral contingency were observed,

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but differences were limited. Other non-pharmacological factors such as baseline shifts potentially related to nicotine-associated cues deserve further attention.

1. Introduction

There is a large literature detailing the effects of nicotine in experimental animals. Literally hundreds of studies have shown that administration of nicotine produces neural, hormonal, cardiovascular, gastrointestinal, metabolic, and behavioral changes. Although the experimental design varies, there is a common design feature in almost all of the research to date: *the experimenter administers a set dose of nicotine at a fixed time, independent of the animal's behavior*. The pharmacological profile of nicotine has been based almost entirely on such studies.

This experimental approach, and data that it generates, may not accurately reflect the effects of nicotine in smokers. Smokers self-administer nicotine and a sizable literature on other drugs of abuse has shown that the effects of drugs vary as a function of behavioral contingency. That is, the very act of taking a drug – making drug delivery a consequence of behavior – can alter the effects of that drug. Behavioral-contingency has been shown to modify the effects of a wide range of psychoactive drugs across many response domains (Smith et al. 1982; Kiyatkin et al. 1993; Dworkin et al. 1995; Di Ciano et al. 1996; Mutschler and Miczek, 1998; Markou et al. 1999; Suto et al. 2010). For example, response independent cocaine is more likely to be lethal in rats than the same dose of cocaine when it is self-administered (Dworkin et al. 1995).

Neurochemically, rats self-administering cocaine demonstrate a greater increase in extracellular dopamine and glutamate in the core of the accumbens than rats passively receiving cocaine (Suto et al. 2010). Behaviorally, withdrawal from non-contingent cocaine produces more ultrasonic vocalizations than self-administered cocaine (Mutschler and Miczek, 1998). If the purpose of animal research is to model the human condition, assessing the effects of behaviorally-contingent nicotine is critical for understanding its pharmacological actions.

In the present study we examined the role of behavioral-contingency as a determinant of the effects of nicotine on blood pressure (BP) and heart rate (HR). We have chosen the cardiovascular effects of nicotine for this study for three reasons. First, it is widely-accepted that nicotine produces reliable changes in HR and BP. Systemic injection of nicotine (administered independent of the animal's behavior) reliably increasess BP in non-anesthetized rats (Kiritsy-Roy et al. 1990). Likewise, many studies have also reported nicotine-induced changes in HR, although the direction of the effect is variable [tachycardia: (Cruz and Vidrio, 1997; Marano et al. 1999); bradycardia: (Aceto et al. 1986; Kiritsy-Roy et al. 1990)]. However, the cardiovascular effects of self-administered nicotine in rats is unknown. Second, we have previously shown that nicotine-induced activation of the sympathetic nervous system, as indexed by the release of peripheral catecholamines, is modulated by behavioral-contingency (Donny et al. 2000). On the initial day of drug exposure, animals receiving nicotine independent of their behavior demonstrated the expected elevation of plasma epinephrine and norepinephrine, whereas animals that self-administered nicotine, failed to show a change relative to yoked saline controls (Donny et al. 2000). Given the role of the sympathetic nervous system in the cardiovascular effect of nicotine (Marano et al. 1999), this work suggests that the acute cardiovascular effects of nicotine may be greater when nicotine is delivered independently of the animal's ongoing behavior. Finally, the development of radiotelemetry technology allows a continuous and precise assessment of nicotine's effects on HR and BP over many days in freely moving

rats. This contrasts with most previous studies on behavioral-contingency that have been limited to relatively few time points (Donny et al. 2000; Dworkin et al. 1995; Mutschler and Miczek, 1998). To date, little is known about how the influence of behavioral control may change over repeated exposures.

In order to address the role of behavioral contingency we employed a classic yoked experimental design. In this design, animals are divided into triads in which one animal controls the timing of nicotine infusions by responding on an operant (i.e., self-administered nicotine) while the other two animals receive infusions of either nicotine or saline that are yoked to the first animal (i.e., delivered at the same time). The strength of this design, which is critical to the questions being addressed, is that both the dose and timing of nicotine exposure are identical between animals that received behaviorally-contingent and non-contingent nicotine.

Here we present a detailed analysis of the effects of nicotine on HR and BP in groups of rats self-administering nicotine (SA-N), receiving yoked nicotine (Y-N), and receiving yoked saline (Y-S). We asked three questions: 1) Does nicotine as it is self-administered by rats affect HR and BP?, 2) Do the cardiovascular effects of nicotine change as a function of repeated administration within a session or across sessions?, 3) Are the acute and chronic cardiovascular effects of behaviorally-contingent nicotine different from nicotine administered independent of the animal's ongoing behavior?

2. Methods

2.1 Subjects

Thirty male, Sprague-Dawley rats (Zivic Miller, Zelienople, PA), 41–44 days old and weighing between 200 and 225 g at the start of the experiment, were individually housed in a temperature-controlled environment on a 12 hour reverse light/dark cycle (lights off from 7:00 AM to 7:00 PM) with unlimited access to water. All animals were habituated to the colony room for a minimum of 7 days prior to any experimental procedures during which they received unlimited food.

2.2 Apparatus

All training and experimental sessions took place in a 25.4 × 30.5 × 27.9 cm operant chamber with an inactive lever, an active lever with a cue light directly above it, a house light, and a pellet trough. Active responses, inactive responses and infusions were recorded throughout the experimental sessions for all three groups by an interfaced computer and software (Med Associates, MED-PC 2.0, St. Albans, VT). All infusions were given with a motor driven infusion pump (Med Associates model 100-10RPM, St. Albans, Vermont). BP and HR assessments were transmitted via a radio wave to a receiver located just outside the operant chamber.

2.3 Procedures

All data were collected prior to the year 2000. Therefore, the procedures described below are consistent with the standard procedures for nicotine self-administration at that time (Donny et al. 1995) rather than the procedures recently published by this laboratory (e.g., Donny et al. 2003; Palmatier et al. 2006; Palmatier et al. 2007a). Most notably, all infusions were paired with a compound visual stimulus that was subsequently shown to support behavior by itself and for that behavior to be enhanced by non-contingent nicotine (see Discussion for more detail; Donny et al. 2003; Palmatier et al. 2006; Palmatier et al. 2007a)

2.3.1 Study Overview—The experimental procedures are detailed below. Briefly, animals were first implanted with jugular catheters and a radiotelemetry transmitter. After recovery from surgery all animals were trained to lever press for food reinforcement, further habituated to transportation and placement in the operant chambers, and run through a single additional food reinforcement session. Yoking sessions were then initiated and continued each weekday for a total of 18 sessions. All procedures took place during the dark phase of the light/dark cycle.

2.3.2 Surgery—Each rat was implanted with jugular venous catheter for nicotine self-administration and a telemetric transmitter (TA1PA-C40, Data Science International, St. Paul, MN) for recording BP and HR. These two implantations were performed as a single surgery while rats were anesthetized with halothane (1– 2 % in medical grade Oxygen). A midline incision of 4–5 cm long was made on the abdomen. The descending aorta was exposed below the renal arteries. A curved 21 gauge needle was used to puncture the aorta rostral to the bifurcation. The catheter of the transmitter was inserted to a distance of about 2 cm into the aorta and glued with a drop of tissue adhesive (Vetbond 3M Animal Care Products, St. Paul, MN). The body of the transmitter was sutured to the internal surface of the abdominal musculature, which was then sutured closed. The skin wound was closed with autoclips. An incision was made in the ventral surface of the neck to expose the right jugular vein and the IV catheter was inserted to a distance of 3.8 cm into the vein; the other end of the silastic tubing passed subcutaneously to exit the skin at the midscapular area. IV catheters were constructed of a 16-cm piece of Silastic tubing (0.020-in ID × 0.037-in OD) attached to a plastic pedestal (C313G-L20-3UP, Plastics One, Roanoke, VA). The dead volume of the catheters was 16 μl.

Catheters were flushed once daily with 0.1 ml of a solution containing Timentin (66.67 mg/ml), 30 U of heparin, and 8,333 U of streptokinase for 2 weeks after surgery. On days when animals were tested this solution was given post-session and catheters were flushed prior to session with 0.1 ml of saline with heparin (10 U/ml). Starting two weeks after surgery each animal's catheter was flushed twice daily with 0.1 ml sterile heparinized saline (10 U/ml pre-session and 30 U/ml post-session). At the end of the experiment, all rats were sacrificed, the radio transmitters were removed, and catheter patency was verified by infusing saline containing dye.

2.3.3 Training—All animals were trained to lever press for food reinforcement over a 3 day period. Training consisted of a 20 minute habituation session, a session in which they learned that the sound of the pellet dispenser predicted food delivery in the pellet trough and a session in which animals were first hand-shaped to respond on the active lever and then reinforced on a continuous reinforcement schedule (fixed ratio 1) for a total of 75 45-mg food pellets. Following food training, animals were fed 20 g at the end of each day (approximately 5 PM) for the remainder of the study. This feeding schedule results in a gradual weight gain of approximately 15 g/week (Donny et al. 1995).

2.3.4 Habituation—Since the procedure of transporting the animal to the experimental room and/or attaching it to the swivel mechanism may produce a cardiovascular response, two additional days of habituation were used to minimize the likelihood of this response affecting cardiovascular measurements. On two consecutive days animals were transported from their home cage to the experimental chambers and attached to leashes that allowed practically unrestricted movement in the chamber. Access to the levers was blocked with a metal barrier; retractable levers were not available for this experiment.

2.3.5 Food Session—On the day following the second habituation session, all animals were run through a single 1 hour session for food reinforcement with the identical

reinforcement parameters used for nicotine self-administration (fixed ratio 1 with a 60 second time out after each reinforcer; same cue conditions as described below). The purpose of this session was to ensure high response rates immediately prior to initiating yoked sessions and to expose animals to the removal and replacement of a metal barrier that would be used to enable pre-session and post-session assessment periods. Animals were placed in the operant chambers with access to the levers blocked by a metal wall. After 20 minutes the wall was removed, providing access to the levers for 60 minutes during which responding on the active lever was reinforced by a food pellet. After 60 minutes, access to the levers was blocked and the rats remained in the operant chambers for an additional 20 minutes.

2.3.6 Yoking Sessions—Following recovery from surgery, lever training, habituation, and the food reinforcement session, animals were randomly divided into three groups based on a yoked design (Dworkin et al. 1995). All animals were drug-naïve prior to the start of the first session. The first group (SA-N) received nicotine bitartrate (0.06 mg/kg/infusion; dose reported as free base; 0.1 ml/kg over approximately 1 second) contingent upon a single active lever press (fixed ratio 1; 60 second time out). This dose has previously been found in our laboratory to produce robust and reliable nicotine self-administration in rats (Donny et al. 1995). A fixed ratio 1 schedule of reinforcement was utilized to minimize differences in response rates between SA-N and the other two groups. Animals in the second group (Y-N) received the same number of infusions at identical times during each session as compared to their SA-N partner. Individuals in the third group (Y-S) were also yoked to the SA-N group but received saline infusions instead of nicotine. Responding on the previously active lever (i.e., during food reinforced training), was no longer reinforced in the two yoked groups, constituting extinction. Responding on the inactive lever had no consequence for any of the groups.

All changes in the cue and house lights were identical for the three groups and based upon the responses of the SA-N individual in each cohort. All infusions were paired with a 1 second cue light followed by a 1 minute time out period during which the chamber light was turned off and responding was recorded, but not reinforced.

Eighteen daily yoking sessions were conducted. All animals were placed in the operant chambers for a total of 100 minutes. Telemetry data were collected for 20 minutes prior to and immediately following a 60 minutes period in which animals either self-administered nicotine or received yoked nicotine or saline. During the 20 minute pre-session and post-session period access to the levers was blocked with a metal wall. Diastolic blood pressure (DBP) data from Day 11 were lost due to experimenter error.

2.4 Statistical Analysis

The data presented in this paper represent the consolidation of four separate cohorts (each cohort contained only complete triads of animals). A total of 10 triads completed the experiment. One triad from the second cohort was not included in the analyses because of a catheter failure in the self-administration animal. Data were defined as outliers and deleted from subsequent analyses if outside a pre-determined range [DBP: <60 mmHg or >140 mmHg; systolic blood pressure (SBP): <80 mmHg or >180 mmHg; HR: <200 beats per minute (bpm) or >550 bpm] indicative of error in measurement.

Data from the pre-session (20 minutes), session (60 minutes) and post-session (20 minutes) periods were collapsed into means for each consecutive 1 minute interval. These data give a real time account of HR and BP across the session with the infusion rate and timing matched across groups by design. However, each triad was administered infusions at different times within the session. Therefore, additional analyses organized according to when infusions

were administered were also conducted and are available in the Supplemental Material. The results of these "By Infusion" analyses were consistent with the data presented below.

Telemetry data were analyzed using Proc Mixed (SAS, Cary, NC) with an autoregressive covariance structure. Restricted maximum likelihood was used to avoid potential case-wise deletion related to missing data. Behavioral data were analyzed in PASW 18 (SPSS, Somers, NY) using the general linear model. To characterize change over repeated measures linear and quadratic (indicated by a superscript "2"; e.g., Day^2 or $Time^2$) parameters were estimated for Day and Time; no higher order parameter estimates were included in the models. Orthogonal contrasts compared the two groups receiving nicotine (SA-N & Y-N) to Y-S and the two nicotine conditions to each other (SA-N vs. Y-N). To more thoroughly characterize the acute effects of nicotine, additional analyses were conducted on the first 10 minutes of the first session (By Time). Statistical significance was set at $p<.05$.

To simplify the presentation, the results focus on the main and interaction effects of Group; results for other effects (e.g., main effect of Day) are omitted. Table 1 summarizes the F values and degrees of freedom for all significant effects for the primary analyses. All statistics not represented in Table 1 are presented in the text. Degrees of freedom exceeding 9999 are rounded to the nearest thousand. Figure 1 presents only Days 1, 9, and 18 to illustrate changes over repeated testing.

3. Results

Given the dense and complex nature of the data being reported, a brief summary is presented first; this summary is followed by a more detailed description of the major findings.

3.1 Overview of the findings

Nicotine elevated both DBP and SBP by up to 20 mmHg. This increase in BP dissipated with repeated testing relative to Y-S, but not relative to pre-session levels. The effect of nicotine on BP was also impacted by whether the infusions were self-administered or independent of the animal's behavior. On Day 1, Y-N animals displayed a greater increase in BP early in the session compared to the SA-N group, but over days this effect became more pronounced in SA-N group. Upon initial exposure, nicotine produced a short-lived bradycardia; however, relatively little effect on HR was observed with repeated infusions during the first few sessions. Thereafter, nicotine-induced tachycardia predominated and this effect was similar in SA-N and Y-N animals.

3.2 Blood pressure

3.2.1 Day 1—Shortly after the start of the first session, nicotine markedly elevated DBP and SBP relative to yoked saline (Figure 1, Table 1). The differences between nicotine and saline treated animals grew over the remainder of the session as the two nicotine groups diverged from Y-S; this Group by Time interaction reached statistical significance for SBP, but not DBP. The initial elevation in BP was greater in Y-N than SA-N; analyses focused exclusively on the first 10 minutes of the first session revealed a differential response in the Y-N and SA-N groups as indicated by a Group by $Time^2$ interaction for both DBP ($F_{1,158}=4.6$, $p<.05$) and SBP ($F_{1,156}=7.2$, $p<.01$). Analyses of the post-session period revealed that the elevation in blood pressure (both DBP and SBP) caused by nicotine persisted for at least 20 minutes.

3.2.2 Repeated testing—With repeated testing, pre-session DBP and SBP markedly decreased in the nicotine groups relative to Y-S as indicated by a significant Group by Day interaction (Figure 1; Table 1). Small, but reliable differences in the rate of decline in BP

during the 20 minute period also resulted in significant interactions between Group and Time and between Group, Time and Day^2. In addition, the decrease in both SBP and DBP over the 20 minute pre-session period was significantly greater in the Y-N than the SA-N group; however, these effects were small and not readily observed upon visual inspection.

The hypertensive effects of nicotine during the 60 minute session continued to be evident with repeated testing, but decreased in magnitude relative to Y-S controls as indicated by a Group by Day interaction. The differences between the nicotine and saline conditions were greatest in the middle to latter part of the session due to a curvilinear change in pressure over time in the Y-S group compared to the relatively stable pressure over the session in nicotine treated animals (Figure 1).

In contrast to Day 1, the hypertensive effects of nicotine were greater in the SA-N group than the Y-N group with repeated testing. These differences were most apparent early in the session. Analyses confirmed a significant overall increase in SBP (but not DBP) in SA-N compared to Y-N. Group by Time interactions indicated that SBP was elevated in SA-N compared to Y-N animals in the early part of the session (differences most evident on Days 8–18). DBP in Y-N animals continued to be above SA-N animals early in the session up to Day 7; however, on subsequent days, DBP was higher in the SA-N animals early in the session, resulting in both Group by Time and a Group by Time by Day^2 interactions.

During the post-session period, the predominant effect with repeated testing was a prolonged elevation in BP in nicotine groups relative to Y-S (Figure 1). Analyses also revealed significant interactions between Group and Time; however, visual inspection of the data failed to reveal any clear relationships. Differences between SA-N and Y-N during the post-session period were small and depended on both Day^2 and Time.

3.2.3 Change from baseline—To examine whether the diminished effects of nicotine with repeated testing could be accounted for by pre-session group differences that emerged over days (see Figure 1), a final set of analyses focused on BP during the 60 minute session after adjusting for the mean levels during the last 5 minutes of the pre-session period (i.e., difference scores; see Supplemental Material for Figure representing difference scores). After controlling for pre-session pressure, the effects of nicotine were strong (~10 mmHg; DBP: $F_{1,25}$=676.5, p<.001; SBP: 907.8, p<.001) and either remained unchanged over days (SBP) or increased as indicated by a Group by Day interaction (DBP: $F_{1,27000}$=14.9, p<.001). For both SBP and DBP, SA-N was significantly elevated over Y-N (DBP: $F_{1,16}$=7.8, p<.05; SBP: $F_{1,16}$=9.8, p<.01); however, this difference was small (<1 mmHg) relative to the effects of nicotine.

3.3 Heart rate

3.3.1 Day 1—There were no significant differences between the groups in HR prior to the start of the first session. There was a short-lived decrease in HR in the two nicotine-treated conditions compared to Y-S after the start of the session, but there was little evidence of an effect of nicotine thereafter. Analyses focused on the first 10 minutes of the session confirmed the differences between nicotine and saline treated animals in the effect of Time^2 ($F_{1,239}$=9.1, p<.01) and failed to reveal any differences between SA-N and Y-N.

3.3.2 Repeated testing—Few group differences in HR were observed during the pre-session period. Statistical analyses indicated a reliable interaction between Group and Time; however, upon inspection no clear patterns were observed (Figure 1; also see Supplementary Materials).

In contrast to the initial bradycardia observed early in the first session, subsequent sessions revealed an increase in HR in nicotine-treated compared to Y-S animals. These effects emerged over days (i.e., by Day 4), peaking on Day 7, and diminishing somewhat thereafter, resulting in a main effect of Group and a Group by Day^2 interaction. Group by Time and Group by Time by Day^2 interactions also indicated that the increase was most evident late in the session during the first few days, but throughout the session during later days. Few differences between SA-N and Y-N were observed. Y-N animals tended to have a higher HR through Day 11; thereafter, HR tended to be higher in the SA-N animals as indicated by a Group by Day interaction.

Similar results were obtained during the post-session period with the nicotine-treated animals having a faster HR than Y-S animals, particularly during the latter part of the post-session period. No reliable differences between SA-N and Y-N were observed during the post-session period.

3.3.3 Change from baseline—As described above for BP, across session analyses were repeated after adjusting for heart rate during the last 5 minutes of the pre-session period (Figure 1; Supplemental Material). Analyses of the change from pre-session levels revealed a consistent nicotine-induced increase in HR relative to Y-S, particularly after Day 2. The average effect of nicotine was approximately 25 bpm ($F_{1,25}$=291.0, p<.001) and this effect was related to both Day ($F_{1,29000}$=13.9, p<.001) and Day^2 ($F_{1,29000}$=17.2, p<.001), indicative of an increased response during the first few days of the study. The only significant difference in HR between SA-N and Y-N was in the interaction with Day ($F_{1,19000}$=5.0, p<.001) in which HR increased more in the SA-N animals with repeated testing; again, however, these statistically significant differences in slope were small.

3.4 Operant behavior

On Day 1, the average (±SEM) time of the first, second, third, and last infusions of the session were 1.0±0.2, 4.2±2.0, 8.8±3.2, and 50.5±2.3 minutes, respectively. All triads earned their first infusion on Day 1 within 1.6 minutes. All but one triad received their second infusion within 3.0 minutes and their third infusion within 11.8 minutes of the start of session; the remaining triad did not earn these infusions until 20.3 and 33.5 minutes after the session started. Across the entire study, the average (±SEM) time of the first, second, third, and last infusions of the session were 1.8±0.4, 4.2±0.6, 7.5±0.9, and 52.5±0.8 minutes, respectively.

Analysis of active lever responding on Day 1 failed to reveal any significant differences between groups (Figure 2). Across the 18 day period, responding was significantly higher in nicotine-treated than Y-S animals ($F_{1,25}$=16.0, p<.001) and this difference did not change significantly over days. Comparison of SA-N to Y-N failed to reveal a main effect of Group on active responses, but there was a trend for a Group by Day interaction ($F_{1,16}$=3.8, p<.10). From Day 9 on, SA-N animals averaged approximately 10 more active responses per hour than Y-N animals. Inactive responses were low and similar between groups on Day 1 (SA-N: 5.56±1.77, Y-N: 5.56±1.99, Y-S: 6.22±2.25) and throughout the study (overall; SA-N: 6.78±0.54, Y-N: 3.94±0.31, Y-S: 2.72±0.36).

4. Discussion

4.1 Initial exposure to nicotine

The effects of nicotine observed here are consistent with previous studies of acute, experimenter-administered nicotine (e.g., Kiritsy-Roy et al. 1990). Nicotine rapidly increased BP which remained elevated throughout the first session. Nicotine produced a

short-lived decrease in HR that dissipated despite additional infusions. These effects were qualitatively similar in the SA-N and Y-N conditions, although some quantitative differences were observed.

Relative to yoked nicotine, response-contingent nicotine failed to produce the same boost in BP in drug-naïve animals. These latter data are consistent with our earlier report on the effects of yoked nicotine on activation of the sympathetic nervous system (Donny et al. 2000); yoked, but not self-administered nicotine elevated blood epinephrine and norepinephrine 15 minutes after the first nicotine infusion in the first testing session. The sympathetic nervous system, and specifically stimulation of α-adrenergic receptors, plays a critical role in mediating nicotine's pressor effects (Marano et al. 1999). Greater sympathetic activation in the yoked animals may result in an increase in both circulating catecholamines and BP compared to animals self-administering nicotine. Interestingly, given the emergence of a greater SBP response from self-administered nicotine in later sessions (discussed below), one might predict a heightened sympathetic response as nicotine is self-administered chronically.

Both animal (Aceto et al. 1986; Dong et al. 1991) and human studies (Perkins et al. 1989; Perkins et al. 1994; Perkins et al. 1995; Fattinger et al. 1997; Houlihan et al. 1999) have demonstrated the development of acute tolerance to nicotine's effects on BP and HR with experimenter-administered nicotine. This tolerance tends to be pronounced with closely spaced, repeated infusions (Aceto et al. 1986; Kiritsy-Roy et al. 1990). Consistent with this literature we found additional infusions of non-contingent nicotine within the first session failed to produce the same increases in BP; however, there was a sustained elevation of BP related to both self-administered and yoked nicotine relative to the Y-S group. The bradycardia produced by both self-administered and yoked nicotine was also only observed early in the first session and there was little evidence of any effect of nicotine on HR thereafter. Together, these data support the conclusion that acute tolerance develops to some of the rapid cardiovascular effects of nicotine, but indicate that hypertensive effects persist throughout a one-hour period of exposure regardless of whether nicotine was self-administered or yoked.

4.2 Chronic nicotine

The cardiovascular effects of nicotine changed with repeated sessions. The rapid increase in BP from yoked nicotine was diminished during subsequent sessions. In contrast, the more prolonged hypertensive response to nicotine remained relatively stable for the first 8–10 days, after which the magnitude decreased somewhat relative to saline. Although the timeline varied, the loss of both the rapid increase in BP and the prolonged elevation suggest the development of partial chronic tolerance to these effects. These changes were generally similar in the SA-N and Y-N groups, although SBP was higher in the SA-N group overall. Interestingly, nicotine-induced tachycardia was only seen after repeated sessions and was similar in both groups receiving nicotine. These data suggest that nicotine-induced tachycardia is either baseline-dependent (i.e., only observed after prolonged habituation to the testing chambers) or sensitizes over repeated administrations. Regardless, these data are consistent with the observation that regular smokers continue to demonstrate a nicotine-induced tachycardia despite years of smoking (Perkins, 2002).

4.3 The importance of behavioral contingencies and context

Previous research has shown that the effects of nicotine depend on the behavioral and environmental circumstances surrounding nicotine delivery. For example, acute tolerance to the cardiovascular effects of smoking occurs when consistent environmental cues predict nicotine delivery, but not when these cues change with each administration (Epstein et al.

1991; Goulden et al. 2000). Similarly, chronic tolerance to nicotine in animals is, in large part, determined by the presence of drug-predictive stimuli (Caggiula et al. 1991; Caggiula et al. 1995; Cepeda-Benito et al. 1998; Cepeda-Benito et al. 2000). In the present study, the 20 minute pre-session period was a reliable cue for nicotine administration. Interestingly, a decrease in BP during the pre-session period paralleled the decrease in BP response to nicotine observed during the session. Indeed, these pre-session shifts seemed to account for all of the observed effect; the increase in BP from pre-session to session remained stable (SBP) or slightly increased (DBP) with repeated days of testing. Compensatory changes in response to drug-predictive cues have been described by others (Siegel et al. 2000) as a mechanism underlying conditioned tolerance; however, to our knowledge, there are no published data relevant to this hypothesis for nicotine. While the present data are consistent with a conditioned compensatory response, the design of the present study does not provide an adequate test of this hypothesis. It is possible that a history of nicotine exposure leads to greater habituation to the environment or a shift in resting BP that is unrelated to Pavlovian conditioning. Nevertheless, these data are intriguing and support further research into this hypothesis.

The experimental design presented here closely resembles classic studies of stress in which an aversive stimulus produces a greater physiological response in animals that lack control over the stimulus (Laudenslager et al. 1983; Shors et al. 1989). Furthermore, some researchers have argued that drugs can also be viewed as foreign, stressful stimuli (Antelman, 1988). In this light, the smaller increase in BP observed after self-administered, compared to yoked, nicotine may represent a blunted stress response in animals that have control over administration. This is consistent with the failure of self-administered nicotine to elevate peripheral catecholamines in our previous study (Donny et al. 2000). The fact that these differences emerged early in the first session raises interesting questions about the source of these differences. Simply performing an approach behavior, even when that behavior is associated with a different reinforcer (i.e., here, food reinforcement), may interact with the effects of nicotine as a foreign, stressful stimulus. We cannot disentangle whether this history is necessary in the current study; however, future studies could distinguish the history from the simple performance of the behavior by testing animals that are not pre-trained.

Conversely, participation in an approach response may potentiate drug effects that are related to reward. There is direct evidence that important features of a reinforcing stimulus change when it is non-contingently experienced. For example, self-administered brain stimulation becomes aversive when non-contingently experienced (Steiner et al. 1969). Likewise the effects of drugs such as cocaine and heroin on neurophysiological mechanisms hypothesized to be related to their reinforcing and addictive properties are increased in animals self-administering the drug (Lecca et al. 2007a; Lecca et al. 2007b). This would suggest that the elevated hypertensive response that emerged after repeated session in animals self-administering nicotine may be related to nicotine reinforcement.

When comparing the effects of response-contingent and response-independent drug it is critical to ask whether differences between the groups can be accounted for by activity related to bar pressing. The present study was designed to minimize the effects of bar pressing by utilizing a fixed ratio 1 schedule of reinforcement. Although some small group differences emerged, the rate of bar pressing was low for all groups and unlikely to account for the cardiovascular differences observed. In addition, the magnitude of the cardiovascular effects did not precisely parallel changes in operant responding. On days when there were clear differences in BP between SA-N and Y-N, there were no differences in response rates (e.g., Day 9). Nevertheless, it is impossible to determine whether the differences between

SA-N and Y-N that were apparent late in the study were related to the small differences in response rates on those sessions.

Responding was slightly increased in Y-N relative to Y-S. This effect of non-contingent nicotine could be related to the locomotor activating effects of nicotine. Alternatively, yoked nicotine could have been intermittently and incidentally paired with responding, resulting in partial reinforcement. This effect could limit differences between self-administered and non-contingent nicotine compared to paradigms that explicitly unpair behavior from the stimulus. Finally, the cue light could have acquired conditioned incentive properties as a consequence of a Pavlovian association with nicotine. Such properties would be expected to elicit approach behavior and, consequently, could have resulted in incidental responses (the lever was located directly below the cue light).

It is important to note that multiple reinforcers were present in this study. We have recently described the moderate unconditioned reinforcing effects of the visual stimuli used here (Palmatier et al. 2006; Palmatier et al. 2007b). Furthermore, nicotine can enhance responding for this visual stimulus even when its delivery is not associated with either the stimulus or lever pressing (Donny et al. 2003; Palmatier et al. 2006; Palmatier et al. 2007a). Indeed, when the two reinforcers are behaviorally dissociated by making nicotine infusions contingent on one lever and the visual stimulus contingent on a second lever, response rates for nicotine tend to be lower than those observed in animals self-administering them as a compound reinforcer (Palmatier et al. 2006). Consequently, two behaviorally relevant stimuli were presented in the study and self-administration of nicotine was likely the result of reinforcement both from nicotine and the visual stimulus. Therefore, both stimuli could, conceivably, have contributed to the differential effects of self-administered and yoked nicotine. In contrast, it is unlikely that the visual stimulus accounts for the effects of nicotine as even the yoked saline condition received non-contingent presentations of the visual stimulus.

5. Conclusions

These data provide a rich picture of the cardiovascular effects of both acute and chronic nicotine. They also add to a relatively scant literature on the importance of non-pharmacological factors as determinants of the effects of nicotine. The present study, as well as numerous others (Smith et al. 1982; Moolten and Kornetsky, 1990; Ator and Griffiths, 1993; Kiyatkin et al. 1993; Dworkin et al. 1995; Stefanski et al. 1999), illustrate that the effects of drugs, including the cardiovascular effects of nicotine, are dependent on the behavioral and contextual circumstances surrounding drug administration.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the National Institutes of Health National Institute on Drug Abuse [Grant DA-10464]. All studies were conducted in accordance with EC Directive 86/609/EEC and approved by the University of Pittsburgh Institutional Animal Care and Use Committee. The manuscript also meets the uniform requirements for manuscripts submitted to biomedical journals as outlined by the International Committee of Medical Journal Editors.

Non-standard abbreviations

SA-N	group self-administering nicotine
Y-N	group receiving yoked nicotine
Y-S	group receiving yoked saline
BP	blood pressure
DBP	diastolic blood pressure
SBP	systolic blood pressure
HR	heart rate
bpm	beats per minute

References

Aceto MD, Tucker SM, Ferguson GS, Hinson JR. Rapid and brief tolerance to (+)- and (−)-nicotine in unanesthetized rats. Eur J Pharmacol. 1986; 132:213–8. [PubMed: 3816976]

Antelman SM. Time-dependent sensitization as the cornerstone for a new approach to pharmacotherapy: drugs as foreign/stressful stimuli. Drug development research. 1988; 14:1–30.

Ator NA, Griffiths RR. Differential sensitivity to midazolam discriminative-stimulus effects following self-administered versus response-independent midazolam. Psychopharmacology (Berl). 1993; 110:1–4. [PubMed: 7870866]

Caggiula, AR.; Epstein, LH.; Antelman, SM.; Knopf, S.; Perkins, KA.; Saylor, S., et al. Conditioned tolerance to nicotine in rats. In: Clarke, P.; Quik, M.; Thurau, K.; Adlkofer, F., editors. Effects of nicotine on biological systems II. Verlag: Birkhauser; 1995. p. 101-16.

Caggiula AR, Epstein LH, Antelman SM, Saylor SS, Perkins KA, Knopf S, et al. Conditioned tolerance to the anorectic and corticosterone-elevating effects of nicotine. Pharmacology, Biochemistry & Behavior. 1991; 40:53–9.

Cepeda-Benito A, Reynoso J, Erath SA-N. Dose-response analyses of associative tolerance to nicotine analgesia in the rat: tail-flick and hot-plate tests. Exp Clin Psychopharmacol. 2000; 8:112–6. [PubMed: 10743911]

Cepeda-Benito A, Reynoso J, McDaniel EH. Associative tolerance to nicotine analgesia in the rat: tail-flick and hot-plate tests. Exp Clin Psychopharmacol. 1998; 6:248–54. [PubMed: 9725108]

Cruz SL, Vidrio H. Tachyphylaxis and sensitization to nicotine-induced tachycardiac and pressor effects after nicotine infusions. Arch Med Res. 1997; 28:189–94. [PubMed: 9204607]

Di Ciano P, Blaha CD, Phillips AG. Changes in dopamine oxidation currents in the nucleus accumbens during unlimited-access self-administration of d-amphetamine by rats. Behav Pharmacol. 1996; 7:714–29. [PubMed: 11224467]

Dong L, Houdi AA, Van Loon GR. Desensitization of central nicotinic cardiovascular effects by nicotine isomers and a quaternary analogue. Pharmacol Biochem Behav. 1991; 38:843–52. [PubMed: 1871199]

Donny EC, Caggiula AR, Knopf S, Brown C. Nicotine self-administration in rats. Psychopharmacology (Berl). 1995; 122:390–4. [PubMed: 8657839]

Donny EC, Caggiula AR, Rose C, Jacobs KS, Mielke MM, Sved AF. Differential effects of response-contingent and response-independent nicotine in rats. Eur J Pharmacol. 2000; 402:231–40. [PubMed: 10958889]

Donny EC, Chaudhri N, Caggiula AR, Evans-Martin FF, Booth S, Gharib MA, et al. Operant responding for a visual reinforcer in rats is enhanced by noncontingent nicotine: implications for nicotine self-administration and reinforcement. Psychopharmacology (Berl). 2003; 169:68–76. [PubMed: 12774186]

Dworkin SI, Mirkis S, Smith JE. Response-dependent versus response-independent presentation of cocaine: differences in the lethal effects of the drug. Psychopharmacology (Berl). 1995; 117:262–6. [PubMed: 7770601]

Epstein LH, Caggiula AR, Perkins KA, McKenzie SJ, Smith JA. Conditioned tolerance to the heart rate effects of smoking. Pharmacol Biochem Behav. 1991; 39:15–19. [PubMed: 1924497]

Fattinger K, Verotta D, Benowitz NL. PharmacodYNamics of acute tolerance to multiple nicotinic effects in humans. J Pharmacol Exp Ther. 1997; 281:1238–46. [PubMed: 9190859]

Goulden LC, Collins FL Jr, Hutchinson KE, Shields T. Effects of dose and interdose interval on conditioned heart rate tolerance to smoking. Exp Clin Psychopharmacol. 2000; 8:142–6. [PubMed: 10743915]

Houlihan ME, Pritchard WS, Robinson JH. A double blind study of the effects of smoking on heart rate: is there tachyphylaxis? Psychopharmacology (Berl). 1999; 144:38–44. [PubMed: 10379622]

Kiritsy-Roy JA, Mousa SA, Appel NM, Van Loon GR. Tolerance to nicotine-induced sympathoadrenal stimulation and cross- tolerance to stress: differential central and peripheral mechanisms in rats. Neuropharmacology. 1990; 29:579–89. [PubMed: 2385330]

Kiyatkin EA, Wise RA, Gratton A. Drug- and behavior-associated changes in dopamine-related electrochemical signals during intravenous heroin self-administration in rats. Synapse. 1993; 14:60–72. [PubMed: 8511719]

Laudenslager ML, Ryan SM, Drugan RC, Hyson RL, Maier SF. Coping and immunosuppression: inescapable but not escapable shock suppresses lymphocyte proliferation. Science. 1983; 221:568–70. [PubMed: 6603018]

Lecca D, Cacciapaglia F, Valentini V, Acquas E, Di Chiara G. Differential neurochemical and behavioral adaptation to cocaine after response contingent and noncontingent exposure in the rat. Psychopharmacology (Berl). 2007a; 191:653–67. [PubMed: 16932924]

Lecca D, Valentini V, Cacciapaglia F, Acquas E, Di Chiara G. Reciprocal effects of response contingent and noncontingent intravenous heroin on in vivo nucleus accumbens shell versus core dopamine in the rat: a repeated sampling microdialysis study. Psychopharmacology (Berl). 2007b; 194:103–16. [PubMed: 17541779]

Marano G, Ramirez A, Mori I, Ferrari AU. Sympathectomy inhibits the vasoactive effects of nicotine in conscious rats. Cardiovasc Res. 1999; 42:201–5. [PubMed: 10435011]

Markou A, Arroyo M, Everitt BJ. Effects of contingent and non-contingent cocaine on drug-seeking behavior measured using a second-order schedule of cocaine reinforcement in rats. Neuropsychopharmacology. 1999; 20:542–55. [PubMed: 10327424]

Mineka S, Hendersen RW. Controllability and predictability in acquired motivation. Annu Rev Psychol. 1985; 36:495–529. [PubMed: 3919637]

Moolten M, Kornetsky C. Oral self-administration of ethanol and not experimenter-administered ethanol facilitates rewarding electrical brain stimulation. Alcohol. 1990; 7:221–5. [PubMed: 2184835]

Mutschler NH, Miczek KA. Withdrawal from a self-administered or non-contingent cocaine binge: differences in ultrasonic distress vocalizations in rats. Psychopharmacology (Berl). 1998; 136:402–8. [PubMed: 9600587]

Palmatier MI, Evans-Martin FF, Hoffman A, Caggiula AR, Chaudhri N, Donny EC, et al. Dissociating the primary reinforcing and reinforcement-enhancing effects of nicotine using a rat self-administration paradigm with concurrently available drug and environmental reinforcers. Psychopharmacology (Berl). 2006; 184:391–400. [PubMed: 16249908]

Palmatier MI, Liu X, Caggiula AR, Donny EC, Sved AF. The role of nicotinic acetylcholine receptors in the primary reinforcing and reinforcement-enhancing effects of nicotine. Neuropsychopharmacology. 2007a; 32:1098–108. [PubMed: 17091131]

Palmatier MI, Matteson GL, Black JJ, Liu X, Caggiula AR, Craven L, et al. The reinforcement enhancing effects of nicotine depend on the incentive value of non-drug reinforcers and increase with repeated drug injections. Drug Alcohol Depend. 2007b; 89:52–9. [PubMed: 17240084]

Perkins KA. Chronic tolerance to nicotine in humans and its relationship to tobacco dependence. Nicotine Tob Res. 2002; 4:405–22. [PubMed: 12521400]

Perkins KA, Epstein LH, Stiller RL, Marks BL, Jacob RG. Chronic and acute tolerance to the heart rate effects of nicotine. Psychopharmacology (Berl). 1989; 97:529–34. [PubMed: 2498949]

Perkins KA, Grobe JE, Fonte C, Goettler J, Caggiula AR, Reynolds WA, et al. Chronic and acute tolerance to subjective, behavioral and cardiovascular effects of nicotine in humans. J Pharmacol Exp Ther. 1994; 270:628–38. [PubMed: 8071855]

Perkins KA, Grobe JE, Mitchell SL, Goettler J, Caggiula A, Stiller RL, et al. Acute tolerance to nicotine in smokers: lack of dissipation within 2 hours. Psychopharmacology (Berl). 1995; 118:164–70. [PubMed: 7617803]

Shors TJ, Seib TB, Levine S, Thompson RF. Inescapable versus escapable shock modulates long-term potentiation in the rat hippocampus. Science. 1989; 244:224–6. [PubMed: 2704997]

Siegel S, Baptista MA, Kim JA, McDonald RV, Weise-Kelly L. Pavlovian psychopharmacology: the associative basis of tolerance. Exp Clin Psychopharmacol. 2000; 8:276–93. [PubMed: 10975617]

Smith JE, Co C, Freeman ME, Lane JD. Brain neurotransmitter turnover correlated with morphine-seeking behavior of rats. Pharmacol Biochem Behav. 1982; 16:509–19. [PubMed: 6123120]

Stefanski R, Ladenheim B, Lee SH, Cadet JL, Goldberg SR. Neuroadaptations in the dopaminergic system after active self-administration but not after passive administration of methamphetamine. Eur J Pharmacol. 1999; 371:123–35. [PubMed: 10357249]

Steiner SS, Beer B, Shaffer MM. Escape from self-produced rates of brain stimulation. Science. 1969; 163:90–1. [PubMed: 5763497]

Suto N, Ecke LE, You ZB, Wise RA. Extracellular fluctuations of dopamine and glutamate in the nucleus accumbens core and shell associated with lever-pressing during cocaine self-administration, extinction, and yoked cocaine administration. Psychopharmacol. 2010; 211:267–75.

Research Highlights

The cardiovascular effects of nicotine were characterized using a yoked design.

Acute nicotine elevated blood pressure and decreased heart rate.

Repeated exposure reduced the hypertensive effects of nicotine relative to saline.

Baseline blood pressure decreased with days of nicotine exposure.

Small, but significant, differences were seen as a function of response contingency.

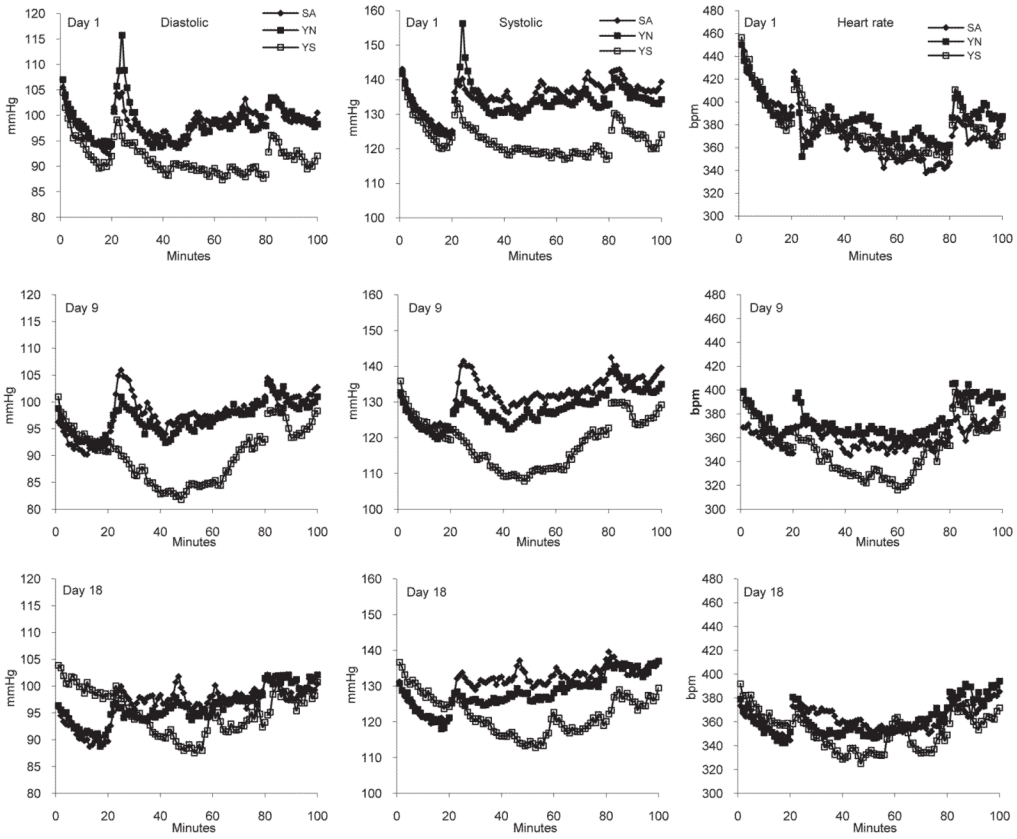

Figure 1.

Mean minute by minute diastolic blood pressure (left panels), systolic blood pressure (middle panels), and heart rate (right panels) on Days 1, 9 and 18. During the first (i.e., pre-session) and last (i.e., post-session) 20 minutes of each session access to the levers was blocked and no infusions were administered. Variance estimates were omitted for brevity; descriptively, the vast majority of SEMs ranged from 1.5–4.0 mmHg for both DBP and SBP and from 5–15 bpm for heart rate.

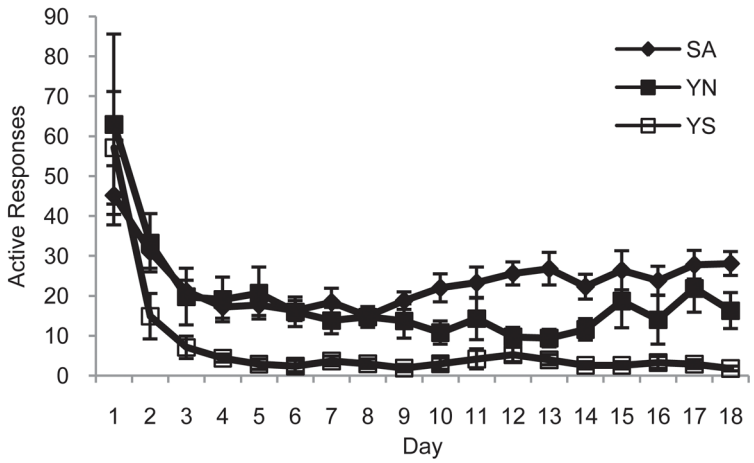

Figure 2.
Mean (±SEM) number of active lever responses for each of 18 days of testing.

Pharmacol Biochem Behav. Author manuscript; available in PMC 2012 September 1.

Table 1

	NICOTINE EFFECT (SA-N & Y-N) vs. Y-S			CONTINGENCY EFFECT SA-N vs. Y-N		
PRE-SESSION	**DBP**	**SBP**	**HR**	**DBP**	**SBP**	**HR**
Day 1 Only						
G						
GxT						
GxT^2						
All Days						
G	$F_{1,25}=5.5^{*}$					
GxD	$F_{1,9121}=15.2^{***}$	$F_{1,9668}=15.1^{***}$				
GxD^2						
GxT	$F_{1,9121}=127.9^{***}$	$F_{1,9668}=287.5^{***}$	$F_{1,9669}=110.4^{***}$	$F_{1,6078}=19.0^{***}$	$F_{1,6446}=52.9^{***}$	$F_{1,6446}=41.2^{***}$
GxDxT						$F_{1,6446}=3.9^{*}$
GxD^2xT	$F_{1,9121}=7.3^{**}$	$F_{1,9668}=7.0^{**}$				
GxT^2						
$GxDxT^2$						
GxD^2xT^2						
SESSION						
Day 1 Only						
G	$F_{1,25}=17.5^{***}$	$F_{1,25}=64.1^{***}$				
GxT		$F_{1,1587}=4.5^{*}$				
GxT^2						
All Days						
G	$F_{1,25}=180.2^{***}$	$F_{1,25}=519.7^{***}$	$F_{1,25}=117.8^{***}$		$F_{1,16}=9.2^{**}$	
GxD	$F_{1,27000}=11.0^{***}$	$F_{1,29000}=10.1^{**}$				$F_{1,19000}=5.1^{*}$
GxD^2			$F_{1,29000}=11.6^{***}$			
GxT	$F_{1,27000}=370.6^{***}$	$F_{1,29000}=708.2^{***}$	$F_{1,29000}=10.5^{**}$	$F_{1,18000}=5.7^{*}$	$F_{1,19000}=15.1^{***}$	
GxDxT	$F_{1,27000}=7.8^{**}$					

	NICOTINE EFFECT (SA-N & Y-N) vs. Y-S			CONTINGENCY EFFECT SA-N vs. Y-N		
PRE-SESSION	**DBP**	**SBP**	**HR**	**DBP**	**SBP**	**HR**
GxD^2xT			$F_{1,29000}=14.0^{***}$	$F_{1,18000}=4.5^{*}$		
GxT^2	$F_{1,27000}=23.4^{***}$	$F_{1,29000}=37.0^{***}$				
$GxDxT^2$						
GxD^2xT^2						
POST-SESSION						
Day 1 Only						
G	$F_{1,25}=10.7^{**}$	$F_{1,25}=27.8^{***}$				
GxT						
GxT^2						
All Days						
G	$F_{1,25}=33.4^{***}$	$F_{1,25}=202.7^{***}$	$F_{1,25}=23.5^{***}$			$F_{1,16}=24.7^{***}$
GxD			$F_{1,9671}=7.1^{**}$			
GxD^2						
GxT	$F_{1,9130}=81.4^{***}$	$F_{1,9671}=112.8^{***}$				$F_{1,6446}=7.9^{**}$
GxDxT						
GxD^2xT					$F_{1,6446}=9.2^{**}$	
GxT^2	$F_{1,9130}=12.0^{***}$	$F_{1,9671}=21.3^{***}$	$F_{1,9671}=7.8^{**}$	$F_{1,6085}=4.9^{*}$	$F_{1,6446}=7.8^{**}$	
$GxDxT^2$						
GxD^2xT^2						

G=Group, T=Time; D=Day; Quadratic effects of Time and Day are denoted as T^2 and D^2, respectively. Only main and interaction effects of Group are presented. Degrees of freedom exceeding 9999 are rounded to the nearest thousand.

* <.05;

** <.01;

*** <.001