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Examination of the Gateway Hypothesis in a rat model

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ABSTRACT

The Gateway Hypothesis is based on epidemiological data and states there is a progression of drug use from use of a softer drug (e.g., nicotine) to use of a harder drug (e.g., morphine). It has been suggested that this sequence is causal and is relevant to drug prevention policies and programs. The present experiment used an animal model to investigate whether the Gateway Hypothesis involves a causal progression. Subjects were 16 female and 16 male Sprague-Dawley rats with ages comparable to late adolescence/emerging adulthood in humans. Subjects received nicotine (6 mg/kg/day) or saline for 21 days SC via osmotic minipump and subsequently were allowed to self-administer IV morphine (0.5 mg/kg/injection, 3 h/day) for 10 days. Results did not confirm the Gateway Hypothesis. In fact, rats pre-exposed to nicotine self-administered significantly less morphine than did rats pre-exposed to saline. These findings may be relevant to future drug use prevention policies and programs.

1. Introduction

The Gateway Hypothesis described a developmental progression in the use of drugs (Kandel, 1975, 2002; Kandel and Kandel, 2014) such that individuals use "softer or lower" drugs (e.g., nicotine, alcohol, marijuana) followed by use of "harder or higher" drugs (e.g., cocaine, morphine, heroin) (Kandel, 2003). This hypothesis does not purport inevitability moving from a softer drug to a harder drug, but instead indicates that soft drug use increases likelihood of subsequent hard drug use. Although the Gateway Hypothesis does not indicate causality, it is widely interpreted in this way.

This causal presumption (or misinterpretation) about the developmental progression of drug use has influenced public policy and drug prevention programs for the past 50 years, including President Richard Nixon's "War on Drugs"; President Ronald Reagan's "zero tolerance" program for possession and use of drugs (Newman, 2016); and First Lady Nancy Reagan's "Just Say No" campaign (Evans, 2002). Additionally, in 1984, legal drinking age in the U.S. was raised from 18 to 21 and in 1987 tobacco purchasing age was raised from 16 to 18 (Apollonio and Glantz, 2016; National Minimum Drinking Age Act, 1984). These legislative and public policy changes reflected acceptance of the Gateway Hypothesis as alcohol and tobacco were categorized as "Gateway Drugs" (Torabi et al., 2010). Yet, sequencing of initiation of use from softer to harder drugs was based on epidemiological data that is correlational, not causal. Therefore, many drug policies have been influenced by an unproven hypothesis.

Recently, opioid use has increased dramatically in the U.S. and worldwide (National Institute of Drug Abuse, 2015; Rudd et al., 2016; United Nations Office on Drugs and Crime, 2012). Increased availability of prescription opioids has been followed by alarming increases in negative consequences associated with their abuse (Substance Abuse and Mental Health Services Administration, 2013; Substance Abuse and Mental Health Services Administration Center for Behavioral Health Statistics and Quality, 2015) and overdose deaths from prescription opioid pain relievers have more than tripled in the past 20 years (Centers for Behavioral Health Statistics and Quality, 2016). Additionally, morphine was utilized in the current study rather than heroin because: (a) heroin is a Schedule I drug with no indicated medical use, whereas Morphine is a Schedule II drug with some indicated medical use, and (b) the Centers for Disease Control and Prevention (2018) reported lifetime prevalence rates of heroin for individuals ages 18–25 (human age equivalent of the rats in the current study) to be 0.60%,whereas lifetime prevalence rates of prescription opioid use in this same age group is 7.2%. If, according to the Gateway Hypothesis, use of nicotine increases likelihood of morphine use, then more focus should be directed toward prevention of tobacco use.

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However, if use of nicotine does not increase opioid use, then focus should be on prevention and treatment of harder drugs.

It is unethical to conduct experiments that manipulate addictive drug exposure in humans. Therefore, reasons for initiation of use and maintenance of use are restricted to self-report measures. Human studies of the Gateway Hypothesis are unable to determine causality between lower drugs and higher drugs because of multiple variables that cannot be controlled (e.g., availability, legal issues, cultural factors). Animal models can contribute meaningfully to the study of the Gateway Hypothesis and evaluate causal mechanisms (Grunberg and Faraday, 2002). By utilizing a rat model, potential confounding variables are controlled that may influence the relationship between nicotine and subsequent morphine use, such as economic factors, social pressure, and/or availability. A rat model can examine whether prior use of one drug (e.g., nicotine) increases subsequent use of another drug (e.g., morphine).

The Gateway Hypothesis has been addressed in three controlled experiments (Frederiksson et al., 2017; Klein, 1997; Levine et al., 2011). Unfortunately, the results of these studies do not provide a clear answer. Klein (1997) examined effects of 19 days of saline, 6 mg/kg/day nicotine, or 12 mg/kg/day SC nicotine administration via osmotic minipump on subsequent oral fentanyl (50 μg/mL) self-administration (SA) by adolescent female and male Wistar rats for 4 weeks. Only 6 mg/kg/day of nicotine exposure increased subsequent fentanyl oral SA in non-stressed male rats; 12 mg/kg/day dosage did not result in increased fentanyl SA for male rats; neither dosage of nicotine changed fentanyl SA in female rats; and immobilization stress prior to fentanyl access attenuated or reversed the effects of 6 mg/kg/day nicotine on subsequent fentanyl SA in male rats. Klein (1997) concluded that the findings partially supported the Gateway Hypothesis. Levine et al. (2011) examined 1 and 7 days of nicotine SA (50 μg/mL) on subsequent effects of 4 days of one IP cocaine administration (20 mg/kg) on locomotor activity and conditioned place preference of male C57BL/6J mice. Levine et al. (2011) reported that 7 days of nicotine exposure (but not 1 day of nicotine exposure) increased locomotor response to subsequent cocaine administration and these mice showed an increase in conditioned place preference for the cocaine-coupled chamber compared to mice exposed only to cocaine for 7 days. This priming of the cocaine response by nicotine occurred after > 1 day of exposure to nicotine and concurrent exposure to nicotine and the first exposure to cocaine. The authors argued that these findings support the Gateway Hypothesis in that exposure of one drug increased the effects of the second, subsequently administered drug. However, it is important to note a co-author of this study was the author of the Gateway Hypothesis (i.e., Denise Kandel); the drugs were all sympathomimetics that generally increase activity; and no experimental condition self-administered the higher order drug, cocaine. Frederiksson et al. (2017) examined effects of alcohol exposure on subsequent cocaine SA and reinstatement. The authors exposed Wistar, or alcohol-preferring rats, to 20% alcohol or water in home cages for 7 weeks (three, 24-h sessions/week) or exposed Wistar rats to intoxicating levels of alcohol in vapor chambers for 14 h/day for 7 weeks. Following alcohol exposure, acquisition of IV cocaine SA was tested using 0.125, 0.25, 0.5, and 1.0 mg/kg cocaine. Subsequently, lever pressing was extinguished and reinstatement of cocaine-seeking was induced by cocaine-paired cues and priming (0, 2.5, 5, 10 mg/kg IP injections of cocaine) (Frederiksson et al., 2017). Pre-exposure to alcohol had no effect on cocaine SA or relapse, inconsistent with the Gateway Hypothesis. The present experiment examined if rats pre-exposed to nicotine subsequently self-administered more morphine compared to rats that were not pre-exposed to nicotine.

2. Material and methods

The current experiment was conducted as a 2 (saline, 6 mg/kg/day nicotine) × 2 (female, male), repeated-measures (morphine self-administration [MSA] days 1–10) full-factorial, mixed design. This experimental design resulted in four conditions with eight subjects in each treatment condition. Number of subjects per condition was based on previous research that used similar subjects, independent and dependent variables, and methods (Acri et al., 1991; Amit et al., 1976; Elliott et al., 2005; Faraday et al., 2003; Grunberg, 1982; Grunberg et al., 1986; Hamilton et al., 2009; Lee et al., 2017; Moosey, 2014; Nishida et al., 2016; Yarnell, 2012; Yarnell et al., 2013). Additionally, a repeated-measures Analysis of Variance (rANOVA) power analysis was conducted using G*Power 3.1 (Faul et al., 2009; Faul et al., 2007). Power analyses indicated a total sample size requirement of 24 subjects (for four groups [nicotine/saline and female/male] and ten repeated measures [MSA days 1–10]) for power of 0.95. Effect size (f) was estimated to be 0.3 based on previous literature on MSA in rats that found effect sizes ranging from 0.24 to 0.44 (Bardo et al., 1995; Le et al., 2014). In addition, previous literature regarding behavioral measures and nicotine administration have yielded moderate to large effect sizes, $\eta^2 = 0.06–0.14$ (Acri et al., 1991; Amit et al., 1976; Elliott et al., 2005; Faraday et al., 2003; Grunberg, 1982; Grunberg et al., 1986; Hamilton et al., 2009).

A total of 32 subjects were included in four separate, counter-balanced within-sex cohorts of 8 subjects per condition. Experimental procedures and environmental conditions were identical across the four cohorts.

2.1. Subjects

Subjects were 16 female and 16 male Sprague-Dawley (SD) rats from Charles River Laboratories (Wilmington, Massachusetts). Rats were approximately 54 days old upon arrival to model emerging adulthood in humans (Yarnell et al., 2013). Adolescence of SD rats ends around 42 and 55 days for females and males, respectively (Ojeda and Urbanski, 1994; Spear and Brake, 1983), and adulthood begins around 60 days (Lewis et al., 2002).

2.2. Housing

Subjects were individually housed in standard polycarbonate shoebox cages (42.5 × 20.5 × 20 cm) with hardwood chip bedding (Pine-Dri). Subjects were single housed to prevent effects of social and environmental enrichment on activity or drug SA (Elliott et al., 2005; Prager et al., 2011; Rosenzweig and Bennett, 1996). Cage bedding was changed two times/week for animal health and to minimize any effects of unclean housing conditions. Subjects had free and continuous access to standard, bland laboratory chow (Harlan Teklad 4% Mouse/Rat Diet 7001) and water. Housing rooms were maintained at approximately 23 °C with 40% relative humidity. Lighting was programed to a 12-h reverse light cycle (0600 lights off, 1800 lights on) because rats are nocturnal creatures (Prager et al., 2011). Prior to data collection, rats were numbered and gentled 7 days prior to behavioral testing to allow subjects to become accustomed to the touch, sounds, and odors of the experimenters (Prager et al., 2011). The work was conducted under an approved protocol by USUHS Institutional Animal Care and Use Committee in compliance with the NIH Guide for Care and Use of Laboratory Animals (National Research Council of the National Academies, 2011).

2.3. Independent variables

There were three independent variables: nicotine, sex, and time.

2.3.1. Nicotine

Nicotine bitartrate (Sigma Pharmaceuticals; dissolved in physiological saline) solution was administered via osmotic mini-pump (Alzet Model 2002, Durect Corporation). Minipumps were filled with either a nicotine solution (6 mg/kg/day) or saline (0 mg/kg/day) with a

delivery rate of 0.42 μL/h (Theeuwes and Yum, 1976). Nicotine dosages were prepared based on average weights of subjects within each of the two nicotine conditions. The 0 mg and 6 mg nicotine bitartrate/kg/day dosages represent a non-smoker and a 1/2- to 1-pack per day smoker, respectively (Grunberg, 1982; Grunberg et al., 1984; Grunberg et al., 1986; Hamilton et al., 2009; Winders et al., 1998) and was based on previous studies (Grunberg et al., 1985; Grunberg et al., 1988; Grunberg et al., 1987). This drug administration paradigm and dosage have been used extensively in rats and has reported results comparable with effects of tobacco smoking by humans (Acri et al., 1995; Acri et al., 1991; Grunberg, 1982, 1992; Grunberg et al., 1984; Riley et al., 1998; Winders and Grunberg, 1989). Richardson and Tizabi (1994) indicated that 6 mg nicotine/kg/day minipump administration, in male rats, yielded plasma nicotine levels of approximately 100 ng/mL, which is similar to the average (i.e., 1/2 to 1 pack of cigarettes per day) human smoker.

2.3.2. Sex

Females and males were included because sex differences are relevant to nicotine and opioid use among females and males (Ailes et al., 2015; Centers for Disease Control and Prevention, 2013; Gerdle et al., 2008; Moosey, 2014; United States Department of Health and Human Services, 2014; Weisbrod, 2015; Yarnell, 2012; Yarnell et al., 2013) and in accordance with NIH guidelines for animal studies (Clayton and Collins, 2014).

2.3.3. Time

Time was included as a within-subject independent variable to allow for observation of changes in dependent variables over time (i.e., MSA, inactive lever presses, horizontal activity as measured via the Open Field Activity [OFA]) and verification of nicotine delivery to the rat by body weight assessments. Behavioral measures were collected at six time points: baseline (Day −2), time 1 (Day 11), time 2 (Day 20), baseline 2 (Day 30), time 3 (Day 36), and time 4 (Day 43). Prior to formal data collection at baseline, a 60-min acclimation period was conducted to the OFA environment and thereby minimize potential stress effects on horizontal activity measures (Day −4).

2.4. Dependent variables

There were four dependent variables: MSA, inactive lever presses, horizontal activity, and body weight.

2.4.1. Morphine self-administration

Morphine sulfate was self-administered IV by lever press at a rate of 0.5 mg/kg/0.1 mL/5 s infusion based on previous literature (Thomsen and Caine, 2005; Yoon et al., 2010; Yoon et al., 2007). Amount of morphine consumed was measured as a continuous dependent variable measured by number of morphine injections by subjects over a period of 10 days (3 h per day, 5 days per week, Monday–Friday, for 2 weeks).

2.4.2. Operant conditioning chamber

Eight operant conditioning chambers (Med Associates Inc., St. Albans, VT) were used for the MSA phase. Each chamber was equipped with two levers, an infusion pump, and a 10-mL glass syringe connected to a fluid swivel (Instech, Plymouth Meeting, PA) by Teflon tubing (Lee et al., 2017; Nishida et al., 2016). After 10 days of recovery from catheter surgery (see below), animals were placed in the operant conditioning chambers and allowed to self-administer IV morphine (0.5 mg/kg/injection, 0.1 mL across 5 s) on one lever FR 1 press/injection in a daily 3-h session (5 days/week, Monday–Friday) for 2 weeks. During each drug infusion, a cue light above the active lever was illuminated, and the house light was extinguished for an additional 20-s (time-out) period during which the cue light above the active lever was turned off. Lever press responses during the time-out period were recorded but had no programmed consequences (Lee et al., 2017; Nishida et al., 2016). Drug intake was assessed while rats were in the operant conditioning chambers 5 days/week (Monday–Friday) for 2 weeks (Days 31–42) and body weight was measured on each MSA day at 0800.

2.4.3. Inactive lever presses

Inactive lever presses were recorded and analyzed to assess whether MSA as measured by the active lever presses was the result of drug-seeking behavior or changes in locomotor activity or exploratory behavior (Lee et al., 2017; Nishida et al., 2016).

2.4.4. Horizontal Activity (HA)

HA is a measurement of general health and gross motor movement (Elliott et al., 2005; Hamilton et al., 2009; Hamilton et al., 2012; Lenoir et al., 2013; Yarnell, 2012). HA was measured to determine whether MSA was affected by overall levels of activity and to determine if there were differences in activity that might contribute to MSA.

2.4.5. Body weight

Body weights were measured throughout the experiment. Body weight was utilized as a measure of overall general health and as a manipulation check during the nicotine pre-exposure phase to ensure rats were receiving nicotine or saline. Rats exposed to nicotine do not gain weight as quickly as do rats not exposed to nicotine (Grunberg et al., 1984; Grunberg et al., 1986; Grunberg et al., 1987; Winders and Grunberg, 1989). Additionally, body weight was used to determine appropriate amounts of morphine sulfate during the MSA phase.

2.5. Equipment and procedures

2.5.1. Open Field Activity (OFA)

OFA measures unconditioned locomotor activity when placed into an environment and left undisturbed and has been used in investigations of nicotine, morphine, stress, and affect with rats (Elliott et al., 2005; Elliott and Grunberg, 2005; Faraday et al., 1999; Hamilton et al., 2009; Yarnell et al., 2013). OFA was measured using Accuscan Superflex Sensor Version 2.2 infrared photocell system in Accuscan Instruments Standard Animal Cages (measuring 40 × 40 × 30 cm; Accuscan Instruments Incorporated, Columbus, OH) located in a separate room from the animal housing room. Data from the activity chambers were processed and aggregated by Accuscan Fusion Software (Version 3.4) (Moosey, 2014; Weisbrod, 2015; Yarnell, 2012; Yarnell et al., 2013). Data were collected during the rats' active cycle for 60 min at each time point.

2.5.2. Minipump surgery

On day −1, rats were assigned to either the nicotine or saline groups based on body weights to ensure that all group members had a similar starting average body weights. On day 0, all rats were anesthetized individually in a plastic chamber using a 5% isoflurane/oxygen mixture (flow rate: 0.5 to 1.0 L/min) and were maintained in their anesthesia-induced unconscious state throughout the surgical implantation of minipumps. One dose of buprenorphine (0.05–0.1 mg/kg) was given SC for antinociception. Minipump equations and implantation procedures were based on previous literature (Grunberg, 1982; Grunberg et al., 1984; Grunberg et al., 1986; Winders et al., 1998).

2.5.3. Catheter surgery

On day 22, rats underwent surgery to remove minipumps and implant a catheter for subsequent MSA. Rats were anesthetized with a ketamine/xylazine (100 mg/kg and 10 mg/kg) IP solution. Catheters were implanted in the jugular vein following procedures described previously (Pomerleau and Pomerleau, 1984; Thomsen and Caine, 2005). Rats were observed during recovery from surgery until the anesthesia wore off and they were ambulatory. Rats were allowed 10 days

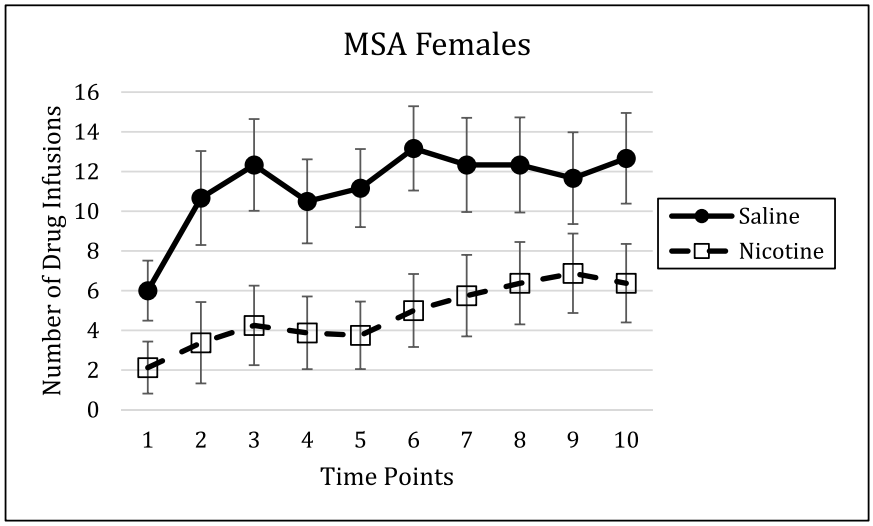

Fig. 1. Effects of nicotine on morphine self-administration for females.
Morphine self-administration of the animals throughout the experiment. Using a repeated-measures analysis of variance (rANOVA), female rats in the saline condition significantly self-administered more morphine (M = 11.28, SEM = 2.00) than did female rats in the nicotine condition (M = 4.78, SEM = 1.73; p = .030), and MSA significantly increased over time (p = .001).

to recover from catheter surgery prior to MSA. During this 10-day period, catheters were flushed with saline/heparin solution, one time/day, and Neosporin was applied to incisions.

2.6. Procedure

The experiment was conducted in four phases: (a) baseline; (b) nicotine or saline exposure; (c) recovery from catheter surgery/cessation of nicotine or saline; and (d) MSA.

2.6.1. Baseline (BL)

Rats were handled for 10 min/day and body weight was measured on days (−8), (−6), (−4), (−2) and (−1). Days were determined based on day of minipump surgery, recorded as Day 0. On day (−4) rats were placed into the OFA chamber for acclimation with BL OFA measured on day (−2).

2.6.2. Groups

Body weights from rats were assessed for two consecutive days (−2 and −1). Rats were assigned to treatment groups such that body weight was comparable.

2.6.3. Nicotine or saline exposure

Following BL, osmotic minipumps containing either saline or nicotine were implanted (day 0) based on procedures reported in the literature (1; 2; 48; 52; 55; 57). Nicotine exposure lasted 21 days (days 0–21) with OFA trials (i.e., HA) measured on days 11 (Time 1 [T1]) and 20 (Time 2 [T2]).

2.6.4. Recovery from catheter surgery

Following catheter surgery, animals were kept in home cages for 10 additional days (days 23–29) to allow for recovery from the catheter surgery prior to MSA. OFA was measured on day 30 (Baseline 2 [BL2]).

2.6.5. Morphine self-administration

Following 10 days of recovery, animals were placed in the operant conditioning chambers and allowed to SA morphine (0.5 mg/kg/infusion, 0.1 mL across 5 s) on FR 1 reinforcement schedules in a daily 3-h session (5 days/week, Monday–Friday) for 2 weeks (days 31–42). OFA was measured on day 36 (Time 3 [T3]) and day 43 (time 4 [T4]).

2.7. Statistical analyses

Data were analyzed using SPSS. Tests were two-tailed using $\alpha = 0.05$. Analyses for dependent variables (MSA, HA, inactive lever press, and body weight) were: independent samples t-tests to examine BL differences; omnibus repeated-measures Analysis of Variance (rANOVA) to measure change over time (BL, T1, T2, BL2, T3, T4) for each group for each dependent variable and rANOVAs split for sex (female, male). Analyses were split for sex based on an a priori hypothesis regarding sex differences in morphine self-administration for females and males. Greenhouse-Geisser corrections were applied if a violation of sphericity was detected. Planned-comparisons of nicotine groups are presented as pairwise comparisons. Data presented in the text include only significant results.

3. Results

3.1. Data management

Thirty-two rats were included in the analyses for the nicotine/saline exposure phase. Two female rats in the saline condition died during catheter surgery; 14 female rats (eight in the nicotine condition and six in the saline condition) were included in the analyses following catheter surgery (i.e., MSA, inactive lever presses, HA, and body weight). During MSA, three male rats in the saline condition had clogged catheters (MSA day 5, day 6, and day 7; respectively), so were unable to SA morphine from the day of the clog to the end of the MSA days. Thirteen male rats (eight in the nicotine condition and five in the saline condition) were included in the analyses during MSA.

3.2. Morphine self-administration

3.2.1. Morphine self-administration repeated-measures analysis of variance

Rats in the saline condition self-administered more morphine (M = 10.68, SEM = 1.43) than did rats in the nicotine condition (M = 4.64, SEM = 1.18; F[1, 23] = 10.63, p = .003, η_p^2 = 0.30), and MSA increased over time (F[3.41, 85.14] = 7.63, p < .001, η_p^2 = 0.23).

3.2.2. Morphine self-administration repeated-measures analysis of variance split by sex females

Female rats in the saline condition self-administered more morphine (M = 11.28, SEM = 2.00) than did female rats in the nicotine condition (M = 4.78, SEM = 1.73; F[1, 12] = 6.05, p = .030, η_p^2 = 0.34), and MSA increased over time (F[2.95, 35.42] = 7.28, p = .001, η_p^2 = 0.38; See Fig. 1).

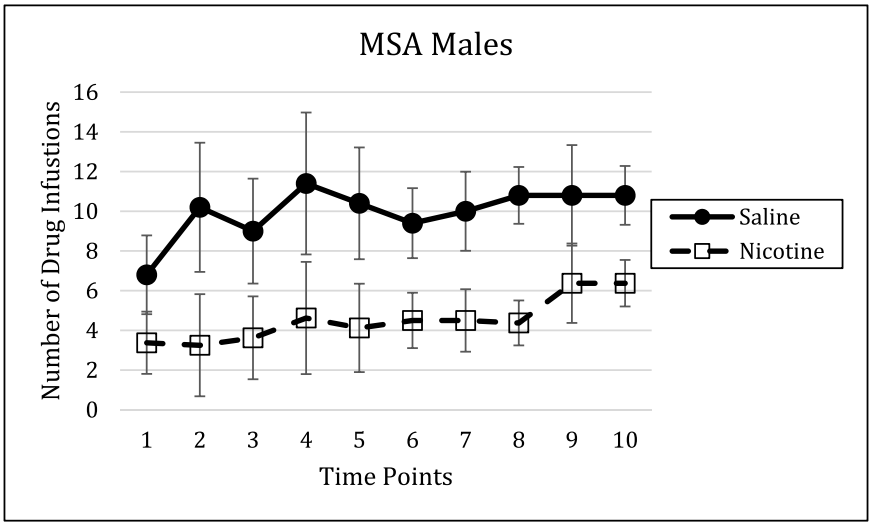

Fig. 2. Effects of nicotine on Morphine self-administration for males.
Using a repeated-measures analysis of variance (rANOVA), there were no significant main effects or interactions for male rats.

3.2.3. Morphine self-administration repeated-measures analysis of variance split by sex males

For male rats, there were no main effects or interactions (see Fig. 2).

3.2.4. Summary of morphine self-administration

Rats in the saline condition self-administered more morphine than did rats in the nicotine condition and MSA increased over time.

3.2.5. Inactive lever repeated-measures analysis of variance

Inactive lever presses decreased over time (F[4.51, 108.13] = 7.03, $p < .001$, $\eta_p^2 = 0.23$).

3.2.6. Inactive lever repeated-measures analysis of variance split by sex. Female

Female rats' inactive lever presses decreased over time (F[3.38, 40.60] = 4.84, $p = .004$, $\eta_p^2 = 0.29$).

3.2.7. Inactive lever repeated-measures analysis of variance SPLIT BY SEX males

Male rats' inactive lever presses decreased over time (F[3.26, 32.59] = 3.48, $p = .024$, $\eta_p^2 = 0.26$).

3.2.8. Summary of inactive lever activity

For female and male rats, inactive lever presses decreased over time.

3.3. Horizontal activity

3.3.1. Horizontal activity repeated-measures analysis of variance

There were no significant differences in baseline HA for either females or males between the treatment groups. Overall, HA was greater for rats in the saline condition than for rats in the nicotine condition (F [1, 26] = 5.90, $p = .022$, $\eta_p^2 = 0.19$). There was a sex × time interaction (F[2.59, 62.06] = 4.99, $p = .005$, $\eta_p^2 = 0.17$), such that females exhibited greater HA activity over time compared to males.

3.3.2. Horizontal activity repeated-measures analysis of variance split by MSA phase

For HA pre-MSA (BL, T1, T2, BL2), there were no significant main effects or interactions. For HA during-MSA (T3, T4), rats in the saline condition (M = 14,415.04, SEM = 1643.90) exhibited greater HA compared to rats in the nicotine condition (M = 9372.19, SEM = 1529.25; F[1,24] = 5.05, $p = .034$, $\eta_p^2 = 0.17$). HA was greater at T3 (M = 12,484.37, SEM = 1353.17) compared to T2 (M = 10,302.86, SEM = 1205.87; F[1, 24]) = 6.62, $p = .017$, $\eta_p^2 = 0.22$). There was a sex × time interaction (F[1, 24] = 12.764, $p = .002$, $\eta_p^2 = 0.35$), such that females exhibited greater HA pre-MSA compared to males.

3.3.3. Horizontal activity repeated-measures analysis of variance split by sex females

For female rats, activity at T3 (6602.21, SEM = 1249.85) was less than at all other time points (BL [M = 10,806.34, SEM = 749.29], T1 [M = 11,539.63, SEM = 909.07], T2 [M = 11,264.63, SEM = 811.64], BL2 [M = 10,412.79, SEM = 745.97], and T4 [M = 14,201.98, SEM = 2073.25]; F[2.00, 26.00] = 5.18, $p = .013$, $\eta_p^2 = 0.29$; See Fig. 3).

3.3.4. Horizontal activity repeated-measures analysis of variance split by sex males

For male rats, there were no main effects or interactions (see Fig. 4).

3.3.5. Horizontal activity repeated-measures analysis of variance split by sex and MSA phase females

For female rats pre-MSA, there were no significant main effects or interactions. For female rats during MSA, HA at T3 (M = 14,201.98, SEM = 2073.25) was greater than horizontal activity at T2 (M = 6602.21, SEM = 1249.85; F[1, 13] = 15.89, $p = .002$, $\eta_p^2 = 0.55$).

3.3.6. Horizontal activity repeated-measures analysis of variance split by sex and MSA phase males

For male rats pre-MSA and during MSA, there were no significant main effects or interactions.

3.3.7. Summary of horizontal data

Rats in the nicotine condition and rats in the saline condition did not significantly differ on HA during the pre-MSA phase when they were receiving nicotine or saline. Rats in the saline condition had greater HA compared to rats in the nicotine condition during MSA when they no longer were receiving nicotine or saline. For female rats, HA at T3 was less than all other time points (BL, T1, T2, BL2, T4). There also was a sex × time interaction, such that female rats increased in HA over time, but male rats did not.

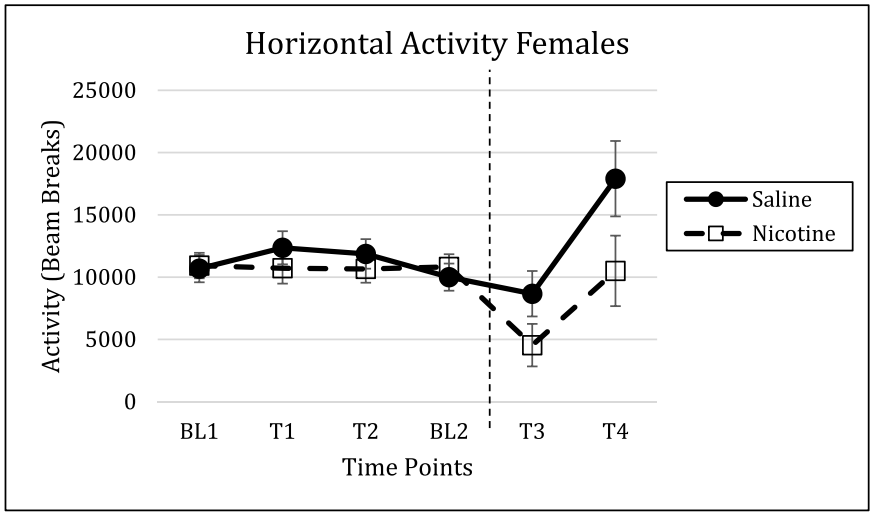

Fig. 3. Effects of nicotine on general health and activity for females.
Horizontal activity (HA; measure of general health and movement) of the animals throughout the experiment. rANOVA analyses revealed that HA at T3 (6602.21, SEM = 1249.85) was significantly less than at all other time points (BL [M = 10,806.34, SEM = 749.29], T1 [M = 11,539.63, SEM = 909.07], T2 [M = 11,264.63, SEM = 811.64], BL2 [M = 10,412.79, SEM = 745.97], and T4 [M = 14,201.98, SEM = 2073.25]; $p = .013$).

3.4. Body weight

3.4.1. Body weight repeated-measures analysis of variance

There were no significant differences in baseline body weight between experimental groups for either females or males within sex. Body weight increased over time ($F[37, 1036] = 107.29$, $p < .001$, $\eta_p^2 = 0.79$). Males ($M = 368.95$, $SEM = 5.49$) weighed more than females ($M = 241.96$, $SEM = 5.49$; $F[1, 26] = 268.03$, $p < 001$, $\eta_p^2 = 0.91$). Body weight increased over time ($F[37, 962] = 573.47$, $p < .001$, $\eta_p^2 = 0.96$). There was a drug × time interaction ($F[37, 962] = 2.83$, $p < .001$, $\eta_p^2 = 0.10$), such that rats assigned to the saline group gained more weight over time compared to rats assigned to the nicotine group, and a sex × time interaction ($F[37, 962] = 121.89$, $p < .001$, $\eta_p^2 = 0.82$), such that males gained more weight over time compared to females.

3.4.2. Body weight repeated-measures analysis of variance split by sex females

For female rats, body weight increased over time ($F[37, 481] = 187.32$, $p < .001$, $\eta_p^2 = 0.94$).

3.4.3. Body weight repeated-measures analysis of variance split by sex males

For male rats, body weight increased over time ($F[37, 481] = 396.80$, $p < .001$, $\eta_p^2 = 0.97$). There was a drug × time interaction ($F[37, 481] = 2.31$, $p < .001$, $\eta_p^2 = 0.15$), such that rats in the saline condition gained more weight over time than did rats in the nicotine condition.

3.4.4. Body weight repeated-measures analysis of variance split by phase

3.4.4.1. Pre-nicotine/saline exposure. Males weighed more than females ($F[1, 28] = 148.35$, $p < .001$, $\eta_p^2 = 0.84$), and weight increased over time ($F[1.51, 42.31] = 139.29$, $p < .001$, $\eta_p^2 = 0.83$). There was a sex × time interaction ($F[1.51, 42.31] = 62.92$, $p < .001$, $\eta_p^2 = 0.69$), such that male rats gained more weight over time compared to the female rats.

3.4.4.2. During nicotine/saline exposure. Males weighed more than females ($F[1, 28] = 260.29$, $p < .001$, $\eta_p^2 = 0.90$), and weight increased over time ($F[2.54, 71.16] = 359.93$, $p < .001$, $\eta_p^2 = 0.93$). There was a sex × time interaction ($F[2.54, 71.16] = 85.28$, $p < .001$,

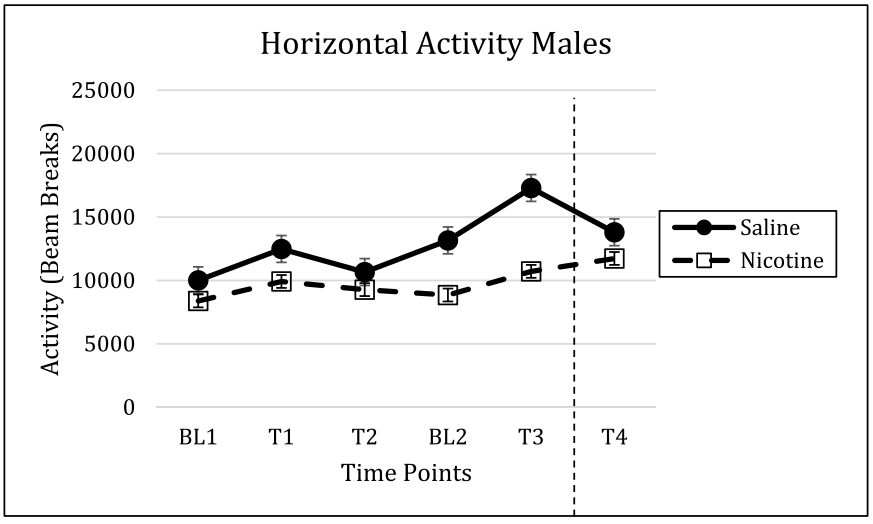

Fig. 4. Effects of nicotine on general health and activity for males.
Using a repeated-measures analysis of variance (rANOVA), there were no significant main effects or interactions for male rats.

η_p^2 = 0.75), such that male rats gained more weight over time compared to the female rats.

3.4.4.3. Post-nicotine/saline exposure/pre-morphine self-administration. Males weighed more than females (F[1, 27] = 282.94, $p < .001$, $\eta_p^2 = 0.91$), and weight increased over time (F[3.43, 92.65] = 58.49, $p < .001$, $\eta_p^2 = 0.68$). There was a drug × time interaction (F[3.43, 92.654] = 8.65, $p < .001$, $\eta_p^2 = 0.24$), such that rats assigned to the saline condition gained more weight over time compared to rats assigned to the nicotine condition, and a sex × time interaction (F[3.43, 92.65] = 3.70, $p = .011$, $\eta_p^2 = 0.12$), such that male rats gained more weight over time compared to the female rats.

3.4.4.4. During morphine self-administration. Males weighed more than females (F[1, 26] = 294.75, $p < .001$, $\eta_p^2 = 0.92$), and weight increased over time (F[3.61, 93.76] = 21.37, $p < .001$, $\eta_p^2 = 0.45$). There was a sex × time interaction (F[3.61, 93.76] = 3.16, $p = .021$, $\eta_p^2 = 0.11$), such that male rats gained more weight over time compared to the female rats.

3.4.5. Body weight repeated-measures analysis of variance split by sex and phase females

Body weight increased over time (F[2.41, 33.68] = 24.76, $p < .001$, $\eta_p^2 = 0.64$).

3.4.5.1. During nicotine/saline exposure. Body weight increased over time (F[3.86, 54.00] = 80.24, $p < .001$, $\eta_p^2 = 0.85$).

3.4.5.2. Post nicotine/saline exposure/pre-morphine self-administration. Body weight increased over time (F[9, 117] = 21.56, $p < .001$, $\eta_p^2 = 0.62$).

3.4.5.3. During morphine self-administration. Body weight increased over time (F[4.09, 53.14] = 9.55, $p < .001$, $\eta_p^2 = 0.42$).

3.4.6. Body weight repeated-measures analysis of variance split by sex and phase males

3.4.6.1. Pre-nicotine/saline exposure. Body weight increased over time (F[1.31, 18.40] = 116.28, $p < .001$, $\eta_p^2 = 0.89$).

3.4.6.2. During nicotine/saline exposure. Body weight increased over time (F[1.70, 23.83] = 288.42, $p < .001$, $\eta_p^2 = 0.95$).

3.4.6.3. Post-nicotine/saline exposure/pre-morphine self-administration. Body weight increased over time (F[1.36, 19.09] = 45.03, $p < .001$, $\eta_p^2 = 0.76$). Additionally, there was a drug × time interaction (F[1.36, 19.09] = 11.04, $p = .002$, $\eta_p^2 = 0.44$), such that male rats in the nicotine condition gained more weight over time compared to male rats in the saline condition.

3.4.6.4. During morphine self-administration. Body weight increased over time (F[2.76, 35.92] = 13.99, $p < .001$, $\eta_p^2 = 0.52$).

4. Discussion

The purpose of the present experiment was to experimentally examine the Gateway Hypothesis. The results did not confirm that pre-exposure to nicotine increases subsequent morphine self-administration (MSA). In fact, pre-exposure to nicotine lowered subsequent MSA for female and male rats. These findings are consistent with a report that pre-exposure to moderate, high, or intoxicating levels of alcohol had no effect on subsequent cocaine self-administration or relapse to cocaine seeking (Frederiksson et al., 2017).

MSA was utilized as a measure of higher order drug intake with nicotine pre-exposure utilized as a lower order drug. Lever presses on an active lever (i.e., the lever that administered the morphine) were operationalized as morphine intake. In addition to measuring active lever presses as morphine intake and inactive lever presses which has no programmed consequences, horizontal activity (HA) in the open field apparatus was measured to determine whether MSA was the result of increased activity or the result of intentional self-administration of morphine.

For females and males, across four separate cohorts, MSA was less for rats previously exposed to nicotine than for rats in the saline condition. These results are in direct contradiction with the Gateway Hypothesis. Because nicotine may alter activity, HA was measured to determine if MSA may have been the result of nicotine-induced changes in activity. The two conditions did not differ significantly on HA during the pre-MSA phase when rats were receiving nicotine or saline. During the MSA period (when rats were no longer receiving nicotine or saline), rats that had previously received saline exhibited greater HA compared to rats that had previously received nicotine. The saline pretreated rats self-administered more morphine than did nicotine pretreated rats. Because repeated morphine administration increases locomotor activity, greater HA in the saline pretreated group may have been the result of more morphine intake compared to the nicotine pretreated group (Badiani et al., 2000). Inactive lever presses were measured to determine if MSA may have been the result of increased activity or haphazard lever pressing. Again, the two experimental groups did not differ significantly on inactive lever presses.

There are several aspects of the methodology to indicate that these findings are stable. First, MSA cannot be explained by activity level and secondly, there was no significant difference between the nicotine and saline group on inactive lever presses. Therefore, any changes in MSA were not due to general activity level or lever presses. In addition, body weight was measured to assess general health and ensure the rats were appropriately gaining weight, and to confirm whether or not the rats received nicotine. Based on the slower rate of weight gain for rats assigned to the nicotine condition, the rats did receive nicotine and the rats assigned to the saline condition did not. Additionally, the experiment was conducted across four separate cohorts which found the same results each time. The replication of the findings further increase confidence in the results.

4.1. Limitations

4.1.1. Independent variables

To deliver nicotine, osmotic minipumps (non-contingent exposure) were used instead of SA. Minipumps are efficient and reliable to administer nicotine in animals and have resulted in biobehavioral effects parallel to human tobacco smoking (Malin, 2001). However, contingent drug exposure (i.e., cigarette smoking and nicotine self-administration) and non-contingent exposure have different psychological and physiological effects (Dworkin et al., 1995; Markou et al., 1993).

4.1.2. Methodology

Rats were pre-exposed to either nicotine or saline for 21 consecutive days and to morphine for 10 non-consecutive days. These are relatively short time durations of exposure. Rats live about three years (Quinn, 2005), and several studies have identified a correspondence of 30 days of human life to 1 day in a rat's life (Gittes, 1986; Iandoli et al., 2000; Klee et al., 1990; Peckham, 1979). Therefore, 3 weeks in a rat is roughly equivalent to a year and a half in a human. This exposure period was chosen to parallel nicotine use by adolescents/emerging adults because the Gateway Hypothesis was based on studies of younger people (Kandel, 1975).

Notably, the exposure of nicotine or saline and morphine did not overlap in the present experiment. Following pre-exposure to either nicotine or saline, there was a 10-day recovery period from the catheter surgery in which the rats were not exposed to nicotine, saline, or morphine. Following this 10-day period, rats were allowed to self-administer morphine. Therefore, the current findings in MSA may have

been influenced by nicotine withdrawal. In humans, individuals typically do not stop using a lower drug when they begin using a harder drug. When individuals progress to use of a higher drug, such as morphine, individuals are still typically using cigarettes or alcohol, rather than stopping use and switching to another substance.

Further, only a single dosage of morphine was utilized during self-administration. Therefore it is unknown whether difference in drug intake reflects a decrease or increase in drug sensitivity.

4.2. Future directions

Building upon the limitations that were present during this study, future research should be conducted to continue to examine the relationship between nicotine pre-exposure and morphine self-administration. Future studies could examine different dosages as a dose-response curve to examine potential differences in self-administration at different dosages. Additionally, motivation for morphine self-administration could be examined by self-administration of morphine on a progressive ratio schedule of reinforcement. This examination could add support for the conclusions found in the present study. The inclusion of additional behavioral measures would also provide further information regarding the association between nicotine pre-exposure and morphine self-administration. Further, because non-contingent (e.g., minipump) drug exposure and contingent drug exposure often have different effects, future research should include a group of rats self-administering nicotine prior to morphine self-administration. Comparing nicotine self-administration (i.e., contingent drug exposure) with nicotine delivered via an osmotic minipump (i.e., non-contingent drug exposure) would provide more information regarding effects of nicotine pre-exposure on subsequent morphine self-administration. Although prior research has established that 5–12 subjects per condition is sufficient to detect effects of nicotine and/or morphine drug exposure on naïve rats (Acri et al., 1991; Amit et al., 1976; Elliott et al., 2005; Faraday et al., 2003; Grunberg, 1982; Grunberg et al., 1986; Hamilton et al., 2009; Lee et al., 2017; Moosey, 2014; Nishida et al., 2016; Yarnell, 2012), future research should include greater numbers of rats per condition to increase reliability of findings. Finally, future studies also might consider the inclusion of biomarkers that focus on relevant molecular, biological, and/or neurochemical actions relevant to reinforcement of each drug.

5. Conclusions

The purpose of the present experiment was to examine effects of nicotine pre-exposure on subsequent morphine self-administration in female and male rats. The experiment did not confirm the a priori hypotheses based on the Gateway Hypothesis that rats previously exposed to nicotine will self-administer greater amounts of morphine compared to rats previously exposed to saline. In fact, the experiment found results in the opposite direction. That is, rats previously exposed to nicotine self-administered significantly less morphine compared to rats previously exposed to saline. The use of animal models provided the ability to experimentally evaluate the Gateway Hypothesis and future research should seek to replicate the current findings, vary the method of nicotine administration, and overlap the nicotine and morphine exposures to determine whether or not means of administration or overlap of drugs are important considerations in the Gateway Hypothesis. If the present findings are true and generalize to the human condition, then a nicotine agonist might be useful to prevent or to attenuate morphine use.

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