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Nicotine Induces a Conditioned Place Preference in Male Japanese Quail (*Coturnix japonica*)

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Abstract

Visual stimuli may play an important role in the development and maintenance of addiction in humans. Research with a visually oriented animal model such as Japanese quail (*Coturnix japonica*) may provide insight into how visual cues contribute to the addiction process. The aim of the current study was to investigate the rewarding properties of nicotine in male Japanese quail using a biased conditioned place preference (CPP) procedure. Adult male quail (N = 30) were allowed to freely explore the entire CPP apparatus during a place preference pre-test and time spent in each chamber was measured. During nicotine conditioning sessions, quail were administered nicotine (0.5, 1.0, or 2.0 mg/kg) or saline and were then confined to their initially least preferred chamber. On alternating days, all quail received saline and were confined to their initially preferred chamber. Locomotor activity was assessed in both chambers. The conditioning chambers had yellow or green walls to enhance the visual salience of each context. Following 8 conditioning sessions (4 nicotine; 4 saline), quail were allowed to explore the entire apparatus during a CPP post-test and time spent in each chamber was measured. The results indicated that quail treated with 0.5 and 1.0 mg/kg nicotine significantly increased the amount of time they spent in the nicotine-paired chamber compared to saline controls, suggesting that nicotine produced a CPP. Furthermore, quail treated with 0.5 mg/kg nicotine showed a significant increase in locomotor activity with repeated treatments. The current findings suggest that nicotine may have a rewarding effect in quail and may tentatively suggest that the neuropharmacological mechanisms that mediate CPP for nicotine are conserved in birds.

Keywords

Nicotine; CPP; Avian; Visual cues; Drug Reward

1. Introduction

A common behavioral procedure used to assess both the rewarding and aversive properties of drugs is the conditioned place preference (CPP) test. In this behavioral test, an animal is injected with a drug and confined to a distinct compartment. Vehicle or saline is administered on alternate days with confinement in opposite distinct compartments.

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Following repeated conditioning sessions, animals are given free access to the entire apparatus in a drug-free state. If subjects spend significantly more time in the drug-paired compartment, it may be inferred that the drug possesses rewarding properties. Conversely, if animals spend significantly more time in the vehicle-paired chamber, it may be inferred that the drug was aversive [1].

The rewarding properties of nicotine have been examined extensively in preclinical models. However, reports on whether nicotine induces a place preference, place aversion, or neither, are mixed and inconclusive [2–4]. Several methodological differences may contribute to these conflicting results including strain, dose, number of conditioning trials, and whether a biased or unbiased procedure was used. Briefly, in a biased CPP procedure the drug effect is repeatedly paired with the initially least preferred compartment and vehicle is paired with the initially preferred compartment. Conversely, if experimental subjects do not exhibit an overt preference for one compartment, an unbiased procedure may be used and the drug-associated compartment is randomly assigned [4]. The majority of studies that utilized a biased procedure have demonstrated a CPP in Sprague-Dawley rats with relatively low doses of nicotine (0.1–1.4 mg/kg, subcutaneously; sc) [5]. Other studies, primarily those using other strains of rodent and/or an unbiased procedure have reported no effect of nicotine or a conditioned place aversion [6–8]. Collectively, these studies indicate inconsistencies in the ability of nicotine to elicit a CPP.

The majority of preclinical research has been conducted with rodent species but there may be additional benefits to using species other than rodents. Japanese quail (*Coturnix japonica*) are a visually oriented avian species with color vision and high visual acuity [9]. Previous research has shown that visual stimuli have ecological significance for Japanese quail. In particular, sexually dimorphic plumage patterns are critical for the identification of mating opportunities and the initiation of copulatory behavior in quail [9]. In humans, visual cues are postulated to play a key role in the addiction process. The results of several human laboratory studies have demonstrated that videos that display cigarette smoking scenes enhance self-reported cigarette craving compared to neutral scenes [10–12]. Given the importance of visual stimuli in drug abuse in humans, use of a visually-oriented animal model may provide a unique contribution to our understanding of the mechanisms of addiction.

Nicotine acts on the nicotinic acetylcholine receptors and these receptors have been autoradiographically localized in the zebra finch [13] and in the forebrain and midbrain of the chicken [14]. Nicotine has also been shown to improve performance on a sustained-attention task in pigeons [15,16]. Therefore, it is evident that nicotine has a physiological effect in avian species. However, it is not known whether nicotine has a rewarding effect in aves.

In the current experiment, a biased design was used to test nicotine for its ability to produce a CPP in male Japanese quail. It was hypothesized that nicotine would elicit a dose-dependent CPP in male Japanese quail.

2. Methods

2.1. Subjects

Thirty (N = 30) adult male Japanese quail (2–3 months old) served as subjects. Two subjects were excluded from data analysis because they failed to sample both chambers of the CPP apparatus. Quail were obtained from a colony at the University of Kentucky (eggs supplied by Northwest Gamebirds, Kennewick, WA), where they were maintained in mixed sex groups until approximately 4–5 weeks of age. Birds were then housed in individual wire-

mesh cages (supplied by GQF Manufacturing, Savannah, GA) and were placed on a 16:8 h light/dark cycle with food and water available *ad libitum*. All subjects were drug naïve prior to experimentation and all experimental procedures and animal care were consistent with guidelines established by the Institutional Animal Care and Use Committee at the University of Kentucky.

2.2. Drugs

Nicotine bitartrate (NIC; provided by the National Institute on Drug Abuse, Bethesda, MD) was dissolved in isotonic physiological saline (SAL) solution and pH was adjusted to 7.4. NIC was injected intraperitoneally (ip) at a volume of 1 ml/kg. Doses of NIC were 0.5 mg/kg, 1.0 mg/kg and 2.0 mg/kg.

2.3. Apparatus

Eight, three-chambered CPP boxes measuring approximately 68 cm long × 21 cm wide × 21 cm deep (ENV-013; Med Associates Inc., St. Albans, VT) were used. The two outermost chambers (28.6 cm long × 21.2 cm wide × 21.2 cm deep) had green or yellow walls and transparent ceilings with wire-mesh floors. Each chamber of the apparatus was equipped with six photobeams approximately 6.4 cm apart and 3.2 cm from the floor. The smaller central chamber (10.8 cm long × 21.2 cm wide × 21.2 cm deep) had gray walls and three photobeams also approximately 6.4 cm apart and 3.2 cm from the floor. Green and yellow were used to cover the walls of the conditioning chambers to enhance the visual salience of each context. Quail are capable of color vision and demonstrate innate preferences for certain colors (e.g., green > yellow > red) [9]. White noise was used throughout each phase of the experiment to attenuate extraneous noise.

2.4. Procedure

Similar CPP procedures have been used in previous work with male Japanese quail [17].

2.4.1. Habituation—Subjects were allowed to freely explore the entire CPP apparatus once a day for 3 days, 30 min per day.

2.4.2. Pre-test—A place preference pre-test was conducted to determine subject's initial preference. During the pre-test, subjects were allowed free access to the entire CPP apparatus for 30 min and time spent in each chamber was measured. Initial place preference was defined as spending more time in one chamber (e.g., green) of the CPP apparatus compared to the others (e.g., yellow or middle).

2.4.3. Conditioning—During conditioning, quail were injected (ip) with either 0.5 mg/kg (n = 8), 1.0 mg/kg (n = 8), 2.0 mg/kg (n = 8) of NIC or SAL (n = 6) and were immediately restricted to one of the outer green or yellow chambers of the CPP apparatus. A biased CPP procedure was used in the current experiment. Most subjects initially preferred the green chamber of the CPP apparatus (59%) over the yellow one (25%) during the pre-test. Thus, the least preferred chamber (as determined by the pre-test) was designated as the NIC-paired chamber for the nicotine groups or the SAL-paired chamber for the saline group. Quail that did not spend significantly more time in the green or yellow chamber during the pretest were randomly assigned to a green or yellow NIC-paired chamber (or SAL-paired chamber in the case of saline controls). On alternate days, quail were given an injection of saline and placed into the opposite chamber. Saline controls received saline in each chamber on alternating days. Conditioning sessions were carried out for 8 days, once per day for 30 min and locomotor activity was measured during each session.

2.4.4. Post-test—Following the conditioning phase, quail were given a place preference post-test. Subjects were allowed free-access to the entire CPP apparatus for 30 min and time spent in each chamber was recorded. (Note that quail were given the preference test in a drug-free state.)

2.5. Statistical Analysis

A difference score was calculated from the raw place preference data by subtracting the amount of time (sec) spent in the least preferred chamber as determined by the pre-test from the time spent in that chamber during the post-test (i.e., [time in initially least preferred chamber after conditioning] – [time in initially least preferred chamber before conditioning]). Difference scores were analyzed using a one-way analysis of variance (ANOVA) with Treatment as the between subjects factor. Locomotor activity data were analyzed during drug conditioning days using a one-way ANOVA for repeated measures. Fisher's Protected LSD post-hoc tests were used to examine statistically significant effects where appropriate. Statistical significance was set at the $p < 0.05$ level.

3. Results

Figure 1 illustrates CPP difference scores for quail treated with 0.5, 1.0, or 2.0 mg/kg NIC or SAL. Male quail that received NIC at the 0.5 and 1.0 mg/kg doses significantly increased the amount of time they spent in the drug-paired chamber compared to those treated with saline or 2.0 mg/kg. A one-way ANOVA revealed a significant main effect of Treatment, $F(3, 26) = 11.56$; $p = 0.0001$. Post-hoc analysis revealed that quail that received the 0.5 and 1.0 mg/kg doses of NIC had a greater difference score compared to those that received saline or 2.0 mg/kg nicotine. Quail that received 0.5 and 1.0 mg/kg NIC were not statistically different from each other and quail that received 2.0 mg/kg NIC did not differ compared to saline controls.

It should be noted that male quail spent similar amounts of time (sec) in the smaller middle chamber during the pre-test ($M = 283.97$, $SEM = 36.99$) as during the post-test ($M = 258.95$, $SEM = 26.64$), and there were no differences between treatment groups in time spent in the middle chamber across tests, $F(3, 26) = 0.95$. Therefore, animals that showed a shift in preference did not shift their time spent in the middle chamber to the drug-paired chamber.

Figure 2 illustrates the number of photobeam breaks across drug conditioning trials for all treatment groups. (The saline group served a control since it did not receive nicotine during these trials). Treatment groups differed significantly in their activity across trials, resulting in an Treatment $\times$ Trials interaction, $F(9, 78) = 2.07$; $p = 0.0419$. Post-hoc analyses indicated that the group that received 0.5 mg/kg of nicotine had increased activity compared to the other groups and this activity was greater on the 3rd and 4th drug days compared to the 1st.

4. Discussion

The current results indicate that nicotine produced a CPP in male quail. Specifically, a significant shift in place preference was observed in quail treated with 0.5 and 1.0 mg/kg nicotine. The highest dose of nicotine (2.0 mg/kg) tested in the current experiment failed to produce CPP or conditioned place aversion (CPA) in quail. The findings are consistent with rodent studies that have observed nicotine CPP using a biased design [5,18]. The present experiment is the first study, to date, to demonstrate a nicotine-induced CPP in an avian species. The findings suggest that the neuropharmacological mechanisms that mediate nicotine-induced CPP in mammalian species may be conserved in birds.

While the findings of the current study are consistent with those found in rodents, it is possible that NIC-induced CPP is not a robust effect in male Japanese quail. Using slightly different procedures, we failed to find NIC-induced CPP in a previous unpublished experiment conducted in our laboratory. However, inconsistencies and replicability issues appear to be common across nicotine CPP studies in general. Le Foll and Goldberg [5] reported that of 19 studies that investigated nicotine CPP, six reported no effect, nine reported a significant NIC-induced CPP, and four reported aversion. The inconsistent nature of NIC-induced CPP may be somewhat unique to the effects of nicotine relative to other drugs of abuse. Reports of significant CPP for cocaine and amphetamine in rodents are relatively consistent across similar drug doses [4]. In addition, CPP for cocaine has been consistently demonstrated in previous studies with male Japanese quail and are in agreement with previous findings in studies with rodents [17,19].

A biased CPP procedure was used in the current experiment and the results of CPP studies that employ a biased design should be interpreted cautiously. Bardo and Bevins [1] have suggested that one potential limitation of the biased CPP procedure may be that pairing drug effects with an initially non-preferred chamber may enhance approach to that environment by reducing aversion to that chamber rather than producing a true CPP via positive effects of the drug. Conversely, it has been suggested that biased designs may be more effective in studies that investigate CPP produced by nicotine specifically. Calcagnetti and Schechter [20] repeatedly paired 0.8 mg/kg nicotine with both the initially preferred and initially non-preferred environments. Their results indicated that when nicotine was paired with the initially least preferred environment, a significant shift in time spent in the least preferred environment was observed but when paired with the initially preferred environment no shift was apparent [20]. More recently, Le Foll and Goldberg [5] reported nicotine-induced CPP across a range of doses (0.1–1.4 mg/kg sc) when paired with the initially least preferred side but a significant CPP was not observed with the highest dose of nicotine (2.0 mg/kg). Interestingly, when paired with the initially preferred side, 2.0 mg/kg nicotine produced a significant CPA [5]. Taken together, these findings suggest that biased CPP procedures may in fact be a more sensitive measure of not only the positive or rewarding effects of a drug but also the negative or aversive effects of drugs relative to unbiased procedures.

The use of a biased CPP procedure in the current experiment may also lend itself to an alternative interpretation of NIC-induced CPP that is consistent with the rodent literature. The apparatus used in the current study appeared to have some inherent bias in which quail initially preferred a chamber with green walls over a chamber with yellow walls. It is possible that this bias was due to the green chamber having a lower light intensity compared to the yellow chamber. Previous CPP research has shown that rodents typically prefer a black chamber relative to a white chamber unless the light intensity in each chamber is similar. Roma and Riley [21] demonstrated that the expression of an unconditioned preference for a black chamber (versus white) in rats may be eliminated by removing ambient white light from the experiment room. It is therefore plausible that the unconditioned preference for the green chamber in the current study may have been due to a lower light intensity (as green reflects a smaller range of the light spectrum relative to yellow). As such, it is possible that the initial bias in the current experiment was due to anxiogenic effects of the putatively brighter chamber and that nicotine may have served to reduce that bias by reducing the anxiogenic effects. There is considerable evidence that nicotine administration produces anxiolytic effects. For example, following acute administration of nicotine, mice have been shown to spend increased time in the light side of a light-dark box following acute nicotine injections [22] and rodents have been shown to reduce anxiety-like behaviors during a social interaction test [23]. Therefore, similar to other rodent studies [e.g., 18] nicotine may have produced a CPP via a conditioned reduction in aversion to the initially non-preferred chamber rather than for its rewarding properties.

In the current experiment, repeated nicotine administration (0.5 mg/kg) resulted in increased locomotor activity on the 3rd and 4th days of place conditioning. Similarly, Janhunen and colleagues [24] found that nicotine (0.5 mg/kg) increased locomotor activity on the 3rd place conditioning day and also on the 1st day. This nicotine-induced increase in locomotor activity has been suggested to be due to enhanced mesolimbic dopaminergic transmission [25,26]. A common mechanism for NIC on both CPP and increased locomotion may reside in the mesolimbic dopamine system. However, increased locomotor activity was not evident in the current experiment at the 1 mg/kg dose of nicotine, a dose that produced a significant CPP. The establishment of a CPP appears to be dependent on dopaminergic mechanisms in most cases, but has also been shown to occur independently of drug-induced locomotor effects [4,27]. Importantly, plasma concentration and pharmacokinetic information for NIC in are currently unavailable in avian species. However, the current findings tentatively suggest that mechanisms other than dopaminergic neurotransmission may play a role in nicotine CPP in avian species.

The present findings extend the existing literature by using visual stimuli to distinguish the conditioning chambers from one another as opposed to olfactory or tactile stimuli typically used in studies with rodents [1]. Yellow and green walls were used in the conditioning chambers in the current experiments as quail are sensitive to visual stimuli and color [9]. Given the hypothesized importance of visual cues in the human addiction process and relapse [28], the use of quail to examine the conditioned rewarding properties of drugs may be of additional benefit with regard to how visual stimuli become associated with drug-seeking behavior [1].

4.1. Conclusions

In sum, the current study is the first to demonstrate a place preference for a visually distinct nicotine-paired context in an avian species. These findings suggest that nicotine may have rewarding effects and/or anxiolytic effects in male Japanese quail. They tentatively suggest that dopamine function plays a similar role in the development of nicotine-induced CPP in birds as well as mammals. Furthermore, the current findings provide some insight into the role of visual cues in the development of nicotine addiction.

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Figure 1. Mean (± SEM) CPP Difference Scores for quail treated with NIC 0.5, NIC 1.0, NIC 2.0 or Saline. * indicates a significant difference from quail treated with Saline ($p < 0.05$). † indicates a significant difference from quail treated with NIC 2.0 mg/kg ($p < 0.05$).

Figure 2.

Mean ($\pm$ SEM) total photobeam breaks for quail treated with NIC 0.5, NIC 1.0, NIC 2.0 or Saline. * indicates a significant difference from quail treated with NIC 0.5 mg/kg on Trial 1 ($p < 0.05$). † indicates a significant difference from quail treated with Saline ($p < 0.05$).