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Brief communication

Nicotine induces a conditioned place preference in male Japanese quail (*Coturnix japonica*)

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HIGHLIGHTS

- A conditioned place preference procedure was used to test for nicotine reward.
- An avian species was used to establish the importance of visual cues in addiction.
- Nicotine produced a dose dependent place preference in male Japanese quail.
- Nicotinergic mechanisms of drug addiction may be conserved in birds.

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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.

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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. 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

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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 each 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 (s) 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.