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Delta-9-THC exposure during zebra finch sensorimotor vocal learning increases cocaine reinforcement in adulthood

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ABSTRACT

Zebra finches are songbirds that learn vocal patterns during a sensitive period of development that approximates adolescence. Exposure of these animals to a cannabinoid agonist during their period of sensorimotor vocal learning alters song patterns produced in adulthood. Thus, songbirds have unique value in studying developmental effects of drug exposure on a naturally learned behavior. A missing feature of this animal model has been a method to study drug reinforcement of behavior. To address this gap we have adapted place conditioning methods, used previously to determine that singing behavior is rewarding, to study cocaine reinforcement of behavior. We have found that cocaine dose-dependently reinforces both place conditioning and aversion at potencies consistent with those observed in mammalian species. Use of this place conditioning method has allowed us to determine that, when administered during periods of sensorimotor vocal learning, delta-9-THC, but not nicotine persistently increases sensitivity to cocaine through adulthood. Establishment of this method significantly expands the songbird drug exposure model, and holds promise for better appreciation of mechanisms important to sensorimotor learning that is dependent upon successful progress through sensitive periods of CNS development.

1. Introduction

Zebra finches learn vocal patterns during a critical period of development (Eales, 1985). Because of this they represent an animal model suitable for studying neurobiology underlying maturational stage-dependent learning ability (Mello, 2014).

We have previously found that the critical period for vocal learning in these animals is associated with distinct sensitivity to CNS-active drugs (Soderstrom and Johnson, 2003). For example, exposure to a cannabinoid agonist during distinct sub-periods of sensorimotor vocal learning have differing effects on the relative numbers of syllables improvised vs. learned from adult male tutors (Soderstrom and Tian, 2004). Thus, this songbird model is suitable for investigating effects of drugs to alter behavior (and underlying neural circuitry) dependent upon learning during distinct, sensitive periods of development. Ability to study naturally learned vocal behavior is unique to songbirds, and is not possible with more common rodent models.

A missing feature of the zebra finch model is the ability to study drug reinforcement of behavior (Spealman and Goldberg, 1978; Tzschentke, 1998). This is important as developmental exposure to multiple classes of abused drugs are associated with increased self-administration and sensitivity to reinforcement in adulthood (reviewed by Spear, 2016). As drug exposures during similar periods of development cause both persistently-altered vocal learning and learning related to reinforcement, both may arise from similarly-altered neurophysiology. Testing this hypothesis requires ability to study both types of learning within the same animal model. To fill this gap we have developed methods to measure cocaine-conditioned reinforcement of place conditioning in zebra finches. The method we have employed is adapted from that described previously to demonstrate rewarding effects of undirected singing (Riters and Stevenson, 2012).

We have used the resulting place conditioning method developed to test ability of both delta-9-THC (THC) and nicotine administered during vocal learning periods to persistently alter cocaine reinforcement in adulthood.

Abbreviations: CB1, cannabinoid receptor 1; CB2, cannabinoid receptor; THC, delta-9-tetrahydrocannabinol; HVC, used as a proper name; Area X, Area X of striatum; lMAN, lateral magnocellular nucleus of the anterior nidopallium; RA, robust nucleus of the arcopallium; VTA, ventral tegmental area

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2. Methods

2.1. Subjects

Subjects were 118 zebra finches raised in our breeding aviary. Both male (56 adult and 32 juvenile [50 +/− 3 days of age]) and female (30 adult) animals were used as indicated. Birds were housed under 14:10-h light/dark cycles and provided ad libitum food (Sun Seed VitaFinch) and water. Animal experiments were approved by the East Carolina University Animal Care and Use Committee that follows the National Institutes of Health guide for the care and use of Laboratory animals. Note that we use the terms: reward as an intrinsically positive state; aversion as an intrinsically negative state and; reinforcement as a change in behavior prompted by association with either rewarding or aversive stimuli (per Nestler et al., 2008).

2.2. Chemicals and reagents

Except where noted all supplies, chemicals and reagents were purchased from Sigma or Fisher Scientific. The phytocannabinoid partial agonist THC (used at 3 mg/kg IM once daily) was obtained from the NIDA drug supply program.

Drugs were suspended in vehicle for injections from concentrated stocks in DMSO (for THC), 50 mM sodium citrate in 0.45% sodium chloride (nicotine, 0.4 mg/kg IM once daily) and phosphate buffered saline (pH = 7.4, cocaine, 1.25, 2.5, 5 and 10 mg/kg IM). Nicotine and cocaine dosages were calculated as free base amounts.

2.3. Drug treatments

Drugs were delivered in 50 µl via IM injection into pectoralis using 30 ga needles. For chronic exposures, injections were given once daily in the morning for a period of 25 days. For developmental exposures, treatments were given from 50 to 75 days of age during a period of sensorimotor vocal learning. Following developmental treatments, animals were allowed to mature to adulthood at 100 days of age. For adult exposures, mature animals (≥100 days of age) were given 25 daily injections and were unmanipulated for an additional 25 days to simulate the maturation period in the developmental group. Following the maturation or simulated maturation period, animals underwent cocaine-reinforced place conditioning testing as described below.

2.4. Conditioning apparatus

The two-chamber conditioning apparatus used was constructed from two standard wire 16 × 16 × 18″ finch cages with right or left side panels removed. Cages were joined at the missing panels by steel cage rings. An opening for a removable panel was created between the two cages. This removable panel was constructed from stiff poster board with bright yellow construction paper glued to one side, and bright green construction paper glued to the opposing side. The same yellow or green construction paper was affixed with zip ties to the sides, roof and floor of respective cages. A standard wooden perch was used in the green cage, and an aluminum foil-covered perch was used in the yellow cage. Thus, chambers were designed to be distinguished by color and perch texture, although we do not know if these intended cues were those used for discrimination.

2.5. Cocaine conditioning

Experiments were conducted in the afternoon. During the first five days of experiments, the panel separating the two chambers of the conditioning apparatus was removed and animals were allowed to freely explore both sides for a period of 15 min (900 s). On the last of these five days, behavior was video recorded and time spent in each chamber documented. The chamber that animals spent the least amount of time in during the pre-conditioning test was designated as the least-preferred chamber. Mean times spent in least-preferred chambers were calculated and compared across treatment groups: no significant differences were observed for any experiment as described in detail in the Results section below. Given the biased design of our place-conditioning paradigm we cannot distinguish between reward or aversion as stimuli responsible for chamber preferences. For clarity, without attributing differences to reward or aversion we have designated the chamber that animals spent the most pre-conditioning test time in as the “most-preferred”, and the other the “least-preferred”.

For conditioning, the chamber-dividing panel was installed and animals were randomly assigned to receive cocaine or vehicle as their first treatment. For the biased place-conditioning design employed, cocaine was administered prior to placement in least-preferred chambers. Vehicle or cocaine injections were given IM and unless specified otherwise, animals were confined in a chamber 5 min after injections for a period of 15 min. Vehicle and cocaine treatments were made on alternating days for a total of eight days. The day following this conditioning period, animals were given a conditioning test by removing the dividing panel and allowing free exploration of both sides of the apparatus for 15 min (900 s). The amount of time spent in the least-preferred, cocaine-paired chamber was recorded. For chamber placement delay (Fig. 2) and developmental experiments (Fig. 3) conditioning scores were calculated (post-conditioning seconds spent in the cocaine-paired chamber minus pre-conditioning seconds spent in the cocaine-paired chamber, note that maximum conditioning score = 900).

Preliminary experiments demonstrated that zebra finches exhibit either strong initial pre-conditioning preference for, or aversion to, particular chambers. The green chamber with wood perch was preferred by more birds than the yellow chamber with foil perch (65% preferred green vs. 35% yellow, mean green preconditioning time = 713 s vs. yellow = 188 s). No attempt to counterbalance these initial preferences was made which is a limitation of our approach.

2.6. Cocaine dose-response experiment

To determine an appropriate dosage of cocaine to use in chronic treatment studies, a dose response experiment was done to evaluate efficacy of 0, 1.25, 2.5, 5 and 10 mg/kg dosages. Groups consisted of n = 4–7 animals. As described in 2.5 above, some preference was exhibited for the green chambers over yellow, however both chambers were represented in initial preferences. For conditioning, animals were randomly assigned to receive either vehicle or cocaine first and placed in the appropriate chamber 5 min after injections (vehicle was paired with most-preferred chambers, cocaine least-preferred).

2.7. Timing of opposing cocaine responses

When studied in rodents, cocaine initially produces positive reinforcement shortly after administration that is later followed by a negative affective state (reviewed by Ettenberg, 2004). Therefore, a complete characterization of cocaine reinforcement in our songbird model system depended upon understanding the timing of these responses in zebra finches. Zebra finches (n = 6) were randomly assigned to timing groups to receive 1, 5, 10, 15 and 30 min delays after cocaine injections before introduction to least preferred chambers. As this study was not related to singing behavior that is only produced by male zebra finches, these experiments provided an opportunity to employ adult females (≥100 days of age).

2.8. Chronic treatment with THC and nicotine

Developing (50 +/− 3 days of age) and adult (> 100 days of age) animals were randomly assigned to subgroups (n = 7–8) to receive either vehicle, THC (3 mg/kg suspended in vehicle) or nicotine (0.4 mg/

kg suspended in vehicle). The vehicle for THC was DMSO:Alkamuls EL-620 [Rhodia, Cranberry, NJ]:PBS, 1:1:18. The vehicle for nicotine was (ethanol:Alkamuls EL-620 [Rhodia, Cranberry, NJ]:PBS, 1:1:18). Treatments were given by IM injection of 50 µl into pectoralis. Injections were made once daily, in the morning, over a period of 25 days. Following chronic exposures, developing animals were allowed to mature to adulthood (100 +/− 3 days of age). Adult animals underwent the same 25 day treatment regimen followed by 25 days of no manipulation to simulate the maturation period in developing birds. After the maturation period or its simulation, animals were evaluated for cocaine-reinforced place conditioning as described above.

3. Statistics

Results of the dose-response experiment were evaluated by 2-way ANOVA with cocaine dosage and chamber conditioning as main effects. Results of chamber placement delay experiments were evaluated by 1-way ANOVA with delay time as the main effect. Results of chronic treatment studies were evaluated using two-way ANOVA with drug (vehicle vs. THC or nicotine) and treatment period (treatment during development vs. as adults) as main effects. The Bonferroni correction was used for multiple comparisons. Statistics were calculated using either GraphPad Prism 7 or SigmaStat 3.1 software running on a Windows XP emulation. Means +/− standard error or 95% confidence interval are reported as indicated.

4. Results

4.1. Cocaine dose-response

A dose-response experiment was done to determine an appropriate cocaine dosage to employ following developmental treatments. Groups of 5 adult males were assigned to receive 0, 1.25, 2.5, 5 or 10 mg/kg cocaine. In pre-conditioning tests 65% of animals preferred green chambers with standard wooden perches, 35% preferred yellow with foil-covered perches. As mentioned above, birds demonstrated strong chamber pre-conditioning preferences, and spent an average of 82.2 +/− 2.9% of the total pre-conditioning exploration time in their preferred chamber. Results of the dose-response experiment are summarized in Fig. 1. Two-way ANOVA indicated that both dosage and pre- vs. post-conditioning test periods had significant effects on time in least-preferred chambers, and that these factors interacted. Thus indicating that differences between pre- and post-conditioning time spent in cocaine-paired chambers depended upon dosage (dosage F [4,40] = 7.28, $p = 0.0002$; pre- vs. post-conditioning F[1,40] = 69.19, $p < 0.0001$; interaction F[4,40] = 7.17, $p = 0.0002$). Post-hoc tests revealed that 2.5 mg/kg cocaine was the lowest dosage to produce a significant pre- vs. post-conditioning difference in preference score (by 702.8 +/− 140.3 s, t = 5.01, Bonferroni's multiple comparisons test). Thus, the 2.5 mg/kg cocaine dosage was employed in following experiments.

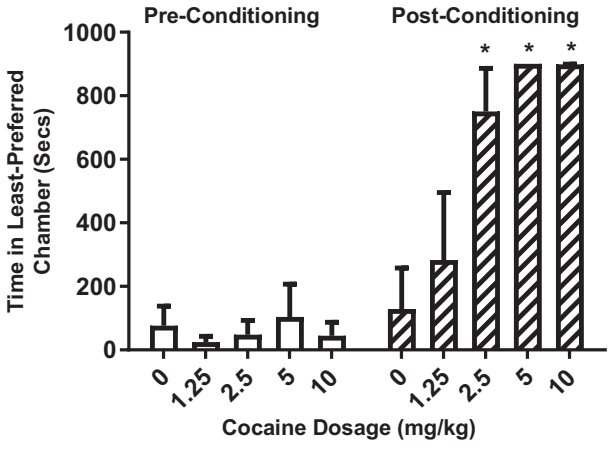

Fig. 1. Cocaine dose-dependently reinforces place conditioning in zebra finches. Groups were assigned to receive 0, 1.25, 2.5, 5 or 10 mg/kg cocaine (n = 5). Significant effects of both cocaine dosage and pre-vs. post-conditioning time spent in cocaine-paired chambers were confirmed by 2-way ANOVA.

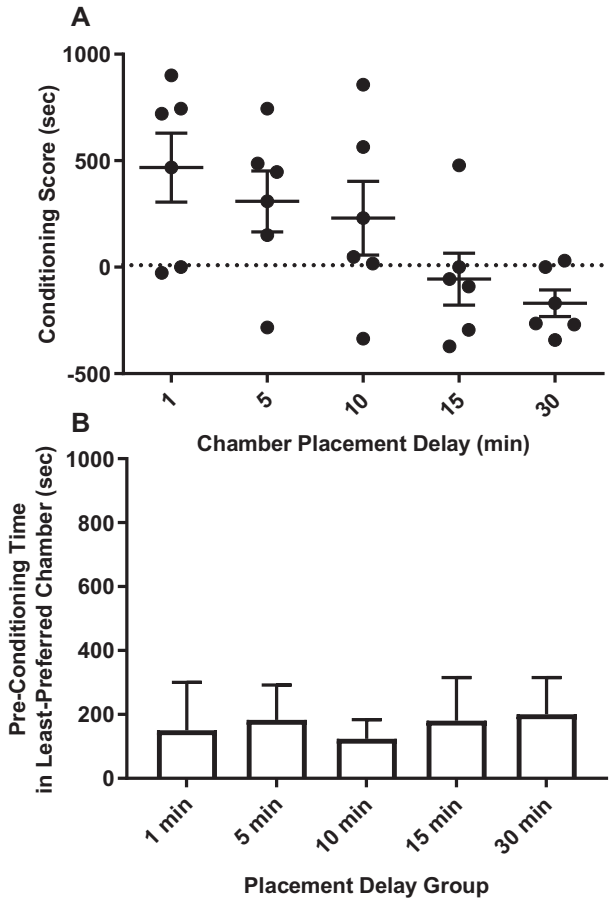

Fig. 2. Cocaine produces opposing conditioned responses that depend upon post-administration chamber placement delays. A, the valence of opposing responses depends upon the duration of intervals between cocaine injection and placement into least-preferred chambers (1-way ANOVA, $p = 0.019$). These variable results suggest that the system may be useful for evaluation of both place-preference and place-aversion in songbirds. B, there were no differences across placement delay groups in the amount of pre-conditioning time spent in least-preferred chambers (1-way ANOVA, $p = 0.99$).

4.2. Timing of opposing cocaine responses

Similar to what is observed following IV administration of 0.75 mg/kg cocaine to rats (Ettenberg et al., 1999) we found that 2.5 mg/kg cocaine delivered IM resulted in increased time spent in cocaine-paired chambers when introduction was delayed 1, 5 and 10 min following injections (Fig. 2A). A reduced amount of time spent in cocaine-paired chambers consistent with place aversion was observed after 15 and 30 min delays. One-way ANOVA confirmed a significant effect of chamber placement delay on conditioning scores (F[4,25] = 3.61, $p = 0.019$). There were no differences across treatment groups for the amount of time spent in least-preferred chambers (Fig. 2B, 1-way ANOVA F[4,25] = 0.08, $p = 0.99$). These results indicate that this system may be useful for studying both place preference and aversion in songbirds. As our dose-response study employed a 5 minute chamber placement delay following cocaine injections, for consistency we chose

to continue to use this time in following experiments.

4.3. Persistent effects of chronic THC and nicotine treatments

In rats, nicotine administered during periadolescence, but not adulthood, persistently increases both nicotine self-administration (Adriani et al., 2002) and sensitivity to cocaine reinforcement (McMillen et al., 2005) following maturation. Persistently-altered rodent behavior following adolescent exposure to multiple abused drug classes has now been established (reviewed by Spear, 2016). To test the hypothesis that a similar phenomenon occurs in songbirds, we evaluated effects of periadolescent THC and nicotine treatments to persistently alter sensitivity to cocaine reinforcement.

Animals (n = 8) were treated once daily for 25 days with either vehicle, the cannabinoid partial agonist THC (3 mg/kg, in a DMSO-containing vehicle) or nicotine (0.4 mg/kg, in an ethanol-containing vehicle). Groups of both developing (50 days of age) and adult (> 100 days of age) animals were compared. Following the 25-day treatment period, animals were allowed to mature without further treatment for an additional 25 days. Thus, at the time of cocaine conditioning, all animal were adults (≥100 days of age) and any drug effects observed must have persisted for at least 25 days. Results of these chronic exposure experiments are summarized in Fig. 3.

Least-preferred chambers were determined through pre-conditioning tests as described above. The amount of time spent in least-preferred chambers did not differ across treatment period (development vs. adult) and drug groups (vehicle vs. drug) for either THC (mean development-vehicle = 82.5 +/− 45.3, development-THC = 90.0 +/− 30.0, adult-vehicle = 132.5 +/− 49.3, adult-THC = 144.0 +/− 46.0, one-way ANOVA, $p = 0.86$) or nicotine experiments (mean development-vehicle = 280.9 +/− 51.7, development-nicotine = 158.8 +/− 54.2, adult-nicotine = 198.4 +/− 58.1, one-way ANOVA, $p = 0.61$).

When administered during development, 3 mg/kg THC significantly increased conditioning scores compared to vehicle-treated controls (Fig. 3A, difference between mean conditioning scores = 540, 95% CI = 25.9–1054, $p = 0.038$, two-way ANVOA with Bonferroni's correction). No significant conditioning score differences were observed between vehicle- and THC-treated adults (Fig. 3A, mean difference = 157.1, 95% CI = −357–671.2, $p = 0.73$, two-way ANVOA with Bonferroni's correction). These results suggest that developmental, but not adult THC treatments increase sensitivity to the reinforcing effects of cocaine. This increased sensitivity is persistent, lasting at least 25 days.

Seven adult animals were treated with nicotine, in all other groups n = 8. Although there was a trend for 0.4 mg/kg nicotine treatments delivered during development to increase conditioning scores, the difference from the vehicle group was not significant (Fig. 3B, mean difference = 222.5, 95% CI = −304–749, $p = 0.547$, two-way ANVOA with Bonferroni's correction). Daily nicotine treatment of adults did not result in conditioning scores different from the vehicle-treated group (Fig. 3B, mean difference = 14.3, 95% CI = −540.8–512.3, $p = 0.997$, two-way ANVOA with Bonferroni's correction). Although direct comparisons cannot be made across different experiments, conditioning scores in animals treated with the ethanol-containing vehicle used in the nicotine experiments (Vehicle, Fig. 3B) appeared higher than those treated with the DMSO-containing vehicle in THC experiments (Vehicle, Fig. 3A). These apparent differences may be due to aversive effects of the DMSO-containing vehicle or positive reinforcing effects of the ethanol-containing vehicle. Thus, the apparent lack of expected developmental nicotine efficacy to increase sensitivity to cocaine reinforcement may be related to elevated conditioning scores in animals treated with the ethanol-containing vehicle.

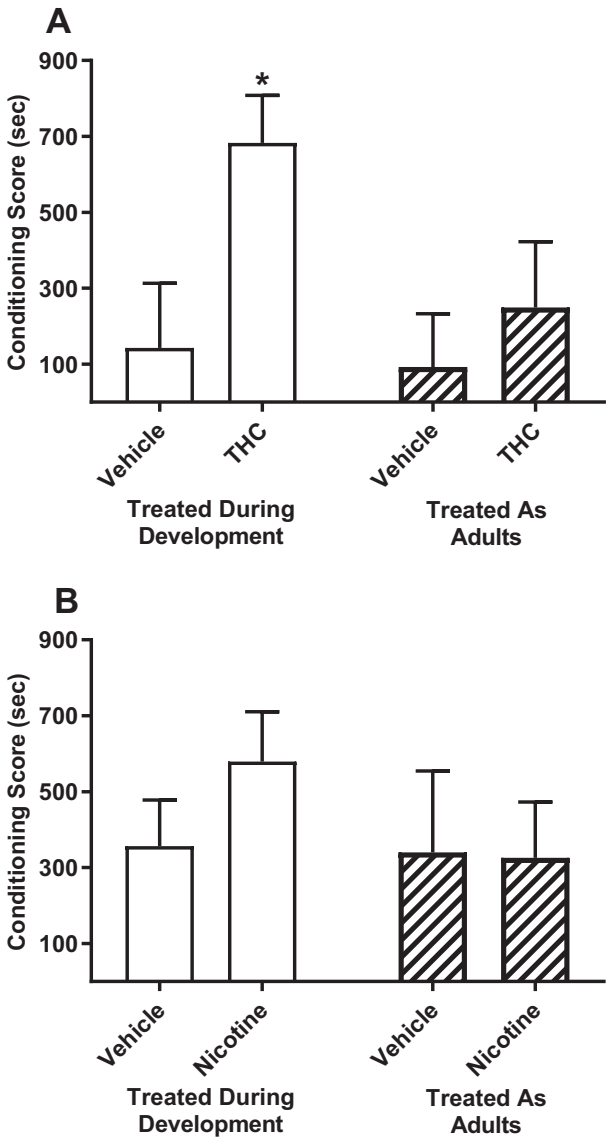

Fig. 3. THC (3 mg/kg, A) but not nicotine (0.4 mg/kg, B) increases sensitivity to cocaine when delivered during a sensitive period of vocal learning, but not in adulthood. Daily treatments were administered for 25 days from 50 to 75 days in developing animals or to adults. Animals were allowed to mature an additional 25 days following treatments to adulthood. Animals in the adult treatment group were not manipulated for 25 days post-treatment to simulate the maturation period of the developmental group. Different vehicles were used for THC and nicotine (DMSO- and ETOH-containing, respectively). A potential increased cocaine sensitivity in the control animals treated with the ETOH-containing vehicle during development may have reduced the difference from nicotine-treated animals (panel B).

4.4. Similar responses in both sexes

As evidence suggests that human females may be more sensitive to cocaine than males (Elman et al., 2001), our use of females in timing experiments allows initial assessment of potential zebra finch sex differences. Results from females from the timing experiment (Fig. 2) and males from the dose-response experiment conditioned using 2.5 mg/kg cocaine with 5 min chamber placement delays (Fig. 1) were compared (Fig. 4). As expected, 2-way ANOVA (by sex and pre- vs. post-conditioning) confirmed a significant difference between pre- and post-conditioning times spent in least-preferred chambers ($F[1,18] = 20.1$, $p = 0.0003$). However, a sex difference was not observed ($F[1,18] = 2.8E-7$, $p = 0.99$). This may be consistent with subtle sex

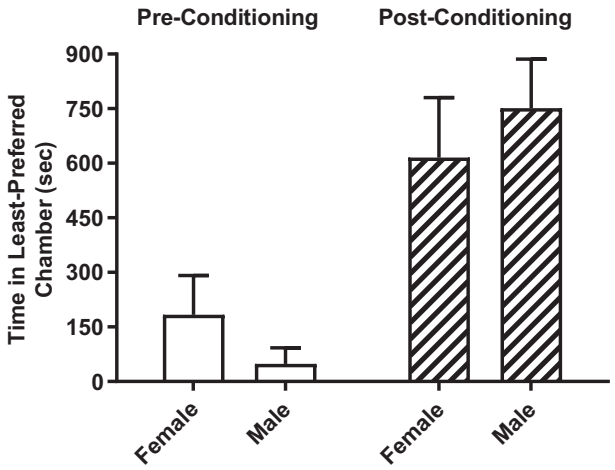

Fig. 4. Lack of sex differences in cocaine-induced place conditioning. Shown are results from adult animals conditioned by placement in least-preferred chambers 5 min after injection with 2.5 mg/kg cocaine. Although adult females tended to have higher pre-conditioning time in least-preferred chambers, this apparent difference from males was not statistically significant. Both sexes were subject to significantly increased post-conditioning time in least-preferred chambers.

differences in cocaine place-preference observed only at low dosages in C57BL/6J mice (Hilderbrand and Lasek, 2014).

5. Discussion

Consistent with effects observed in other vertebrates, cocaine dose-dependently produced place conditioning in zebra finches, with a potency similar to what is characteristically-produced in rodents (McMillen et al., 2005; Mueller and Stewart, 2000). In addition, initial reward followed later by an opposing aversive response was observed over a time frame similar to that reported in rats (Ettenberg et al., 2015; Su et al., 2013). Taken together, these results support the validity of the procedure we have developed to study cocaine reinforcement of songbird behavior.

It has been recently reported that the principal mammalian target of cocaine, the dopamine transporter (DAT) is absent in birds and reptiles (Lovell et al., 2015). This interesting report convincingly demonstrates that the ortholog of the norepinephrine transporter (NET) is expressed in the avian dopaminergic system and substitutes for DAT. This genetic difference explains earlier pharmacological results where NAT-selective drugs were efficacious in zebra finches while unexpectedly high DAT-selective inhibitor concentrations were required to potentiate dopamine signaling (Gale and Perkel, 2005; Sasaki et al., 2006). Mammalian NET transports both dopamine and norepinephrine with similar efficiency (Gu et al., 1994) a characteristic that appears to be shared by the avian protein, although functional expression studies are required to conclusively demonstrate this. Similar cocaine potency in mammals and DAT-deficient zebra finches is consistent with lack selectivity for human and mouse DAT, NET and serotonin transporters (Han and Gu, 2006).

5.1. Persistent effects of developmental drug exposure in songbirds

We have previously employed zebra finches as a vocal learning model to study effects of CNS-active drugs administered during late-postnatal development. These songbirds have the advantage of naturally learning vocal patterns during a sensitive period of late-postnatal development that approximates mammalian adolescence. Prior work has shown that repeated cannabinoid agonist exposure during the sensorimotor stage of vocal learning reduces the stereotypy of song motifs and the number of distinct note types produced in adulthood (Soderstrom and Johnson, 2003). Agonist exposure during this learning period also results in persistent changes to increase dendritic spine densities and expression of synaptic markers - physiological effects similar to those reported following developmental drug exposure in mammals (Gilbert and Soderstrom, 2011, 2014). Our current results contribute to this prior work by demonstrating developmental cannabinoid exposure not only alters vocal learning, but also persistently increases sensitivity to cocaine reinforcement. This is important as elements of similar neural circuitry may play a role in both effects.

For example, it has long been established that activation of dopaminergic projections from mammalian VTA to both ventral and dorsal regions of striatum are important to drug reward (Krebs et al., 2012). Initial drug abuse is associated with activation of thalamo-cortical circuits through ventral striatum (i.e. nucleus accumbens) that, with repeated exposure, are followed by recruitment of more dorsal striatal regions relevant to adoption of addiction-related reflexive motor responses (Belin and Everitt, 2008; Everitt and Robbins, 2005; Koob and Volkow, 2010). These circuits share features with songbird midbrain dopaminergic projections to regions of striatum including Area X (a region of basal ganglia). Positive reinforcement of singing behavior that is dependent upon this circuit is important to the process of vocal learning (Hoffmann et al., 2016). Optogenetic activation of this songbird dopaminergic pathway promotes, and inactivation inhibits, vocal learning (Xiao et al., 2018). Thus, addiction-related motor programming may share behavioral homology with stereotyped Area X-dependent learned vocalizations. If so, then similar neurodevelopmental changes responsible for altered vocal learning may also persistently increase sensitivity to cocaine reinforcement. The songbird model represents a uniquely useful system within which to study such potential convergent developmental effects on a naturally learned, reward-dependent behavior.

5.2. Similar acute cocaine responses in both sexes

Based upon our current finding that both adult male and female zebra finches are subject to acute cocaine-reinforced place conditioning (Fig. 4), we suspect that similar midbrain dopaminergic projections to striatal regions homologous to nucleus accumbens are involved. This is because female zebra finches lack a substantial Area X (Nottebohm and Arnold, 1976) and striatal regions outside of Area X are more similar in gene expression patterns to reward-relevant mammalian accumbens-containing ventral striatum (Petkov and Jarvis, 2012). If effects of developmental cannabinoid treatments to both alter song learning (Soderstrom and Johnson, 2003; Soderstrom and Tian, 2004) and cocaine sensitivity (Fig. 3) involve learning-essential Area X, then similar chronic, developmental cannabinoid treatment of female zebra finches should be less, or even ineffective. This is an interesting possibility that we plan to test.

5.3. Unanticipated findings

An unexpected result of this study was inability to clearly document an effect of developmental nicotine exposure to increase cocaine sensitivity. Similar nicotine treatment during rat development clearly increased cocaine reinforcement in adulthood (McMillen et al., 2005). A trend toward such an effect in zebra finches may indicate that increasing group sizes will confirm it (Fig. 3B). However, it may be the case that songbirds are less sensitive to cholinergic modulation than are rodent species. The apparently higher sensitivity to effects of THC during development may be related to distinctly high-level CB1 receptor expression in relevant brain regions that notably peak during sensorimotor vocal learning (Soderstrom and Tian, 2006).

A second unexpected observation was contrasting effects of 2.5 mg/kg cocaine that produced clear place-conditioning in the dose-response experiment (Fig. 1) but not in vehicle-treated animals that participated

in the developmental study (Fig. 3). Preliminary dose-response work that employed an unbiased design (wherein half of animals receive cocaine in most- and least-preferred chambers, respectively) suggested that 2.5 mg/kg cocaine approximated an ED50 for place-conditioning (data not shown). In the biased dose-response results presented in Fig. 1 (where cocaine was exclusively paired with least-preferred chambers), 2.5 mg/kg was the lowest effective dosage used, but appeared to exceed ED50 potency. Taken together, these findings suggest that 2.5 mg/kg is close to the linear dose-response range for cocaine place-conditioning of zebra finches. Small procedural differences associated with different experimenters conducting the different experiments may have systematically influenced dosage. Modest dosage differences within this linear response range are subject to large efficacy differences. This type of experimenter factor may have played a role. Another (perhaps more likely) factor is related to pre-conditioning differences. In the dose-response experiment, animals were not subjected to the 25-day chronic treatment regimen. This regimen that involved repeated daily manipulations and injections during the developmental study may have resulted in conditioning to the treatment procedure itself (capture, injection and release, daily for 25 days). This may have produced an aversive association with which cocaine had to compete. This implies that chronic THC injections prevented development of this association, consistent with analgesic and/or anxiolytic efficacy, and impaired learning and memory. Differences between effects of developmental vs. adult THC treatments suggest developing animals are more- and/or adult animals are less- sensitive to or capable of manifesting these THC effects.

Finally, a weakness of our approach is skew that was present in pre-conditioning chamber preference wherein 65% of animals spent most time in the green chamber with standard wood dowel perch while 35% spent most time in the yellow chamber with foil perch. Given the sensitivity with which zebra finches distinguish color (Caves et al., 2018), we suspect but do not know for certain that this feature was attended to in the conditioning we observed. We have not tested the potential discriminative value of perch texture or appearance, and therefore also do not know if this was a feature attended to. Ideally there should be no differences across pre-conditioning preferences, and it may be possible to modify environments or to counterbalance for this effect. Such steps should be considered in adapting the approach we describe to new studies. A second issue with this method is that (as mentioned above) few of the animals used in our experiments spent significant pre-conditioning time exploring both chambers. This is apparently a behavioral characteristic of zebra finches. Given this problem, developing a two-chamber apparatus wherein each chamber is actively explored by zebra finches may prove challenging – if not impossible.

5.4. Conclusions

We have developed a songbird model to study drug-reinforced place conditioning. This model has allowed us to demonstrate that behavior of both songbird sexes is reinforced by cocaine in a manner consistent with rodent species. Use of this model has also allowed us to determine that exposure of males to THC during periods of sensorimotor vocal learning persistently increases sensitivity to cocaine in adulthood. Because vocal learning and drug reinforcement involve dopaminergic projections from midbrain to ventral striatum, the songbird model provides an opportunity to study how developmental drug exposure may generally alter processes of incentive-driven sensorimotor learning in a persistent manner.

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Declaration of competing interest

None.

References

Adriani, W., Macrì, S., Pacifici, R., Laviola, G., 2002. Peculiar vulnerability to nicotine oral self-administration in mice during early adolescence. Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol. 27, 212–224. https://doi.org/10.1016/S0893-133X(02)00295-6.

Belin, D., Everitt, B.J., 2008. Cocaine seeking habits depend upon dopamine-dependent serial connectivity linking the ventral with the dorsal striatum. Neuron 57, 432–441. https://doi.org/10.1016/j.neuron.2007.12.019.

Caves, E.M., Green, P.A., Zipple, M.N., Peters, S., Johnsen, S., Nowicki, S., 2018. Categorical perception of colour signals in a songbird. Nature 560, 365–367. https://doi.org/10.1038/s41586-018-0377-7.

Eales, L.A., 1985. Song learning in zebra finches: some effects of song model availability on what is learnt and when. Anim. Behav. 33, 1293–1300. https://doi.org/10.1016/S0003-3472(85)80189-5.

Elman, I., Karlsgodt, K.H., Gastfriend, D.R., 2001. Gender differences in cocaine craving among non-treatment-seeking individuals with cocaine dependence. Am. J. Drug Alcohol Abuse 27, 193–202.

Ettenberg, A., 2004. Opponent process properties of self-administered cocaine. Neurosci. Biobehav. Rev. 27, 721–728. https://doi.org/10.1016/j.neubiorev.2003.11.009.

Ettenberg, A., Raven, M.A., Danluck, D.A., Necessary, B.D., 1999. Evidence for opponent-process actions of intravenous cocaine. Pharmacol. Biochem. Behav. 64, 507–512.

Ettenberg, A., Fomenko, V., Kaganovsky, K., Shelton, K., Wenzel, J.M., 2015. On the positive and negative affective responses to cocaine and their relation to drug self-administration in rats. Psychopharmacology 232, 2363–2375. https://doi.org/10.1007/s00213-015-3873-6.

Everitt, B.J., Robbins, T.W., 2005. Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. Nat. Neurosci. 8, 1481. https://doi.org/10.1038/nn1579.

Gale, S.D., Perkel, D.J., 2005. Properties of dopamine release and uptake in the songbird basal ganglia. J. Neurophysiol. 93, 1871–1879. https://doi.org/10.1152/jn.01053.2004.

Gilbert, M.T., Soderstrom, K., 2011. Late-postnatal cannabinoid exposure persistently elevates dendritic spine densities in area X and HVC song regions of zebra finch telencephalon. Brain Res. 1405, 23–30. https://doi.org/10.1016/j.brainres.2011.06.019.

Gilbert, M.T., Soderstrom, K., 2014. Developmental but not adult cannabinoid treatments persistently alter axonal and dendritic morphology within brain regions important for zebra finch vocal learning. Brain Res. 1558, 57–73. https://doi.org/10.1016/j.brainres.2014.02.039.

Gu, H., Wall, S.C., Rudnick, G., 1994. Stable expression of biogenic amine transporters reveals differences in inhibitor sensitivity, kinetics, and ion dependence. J. Biol. Chem. 269, 7124–7130.

Han, D.D., Gu, H.H., 2006. Comparison of the monoamine transporters from human and mouse in their sensitivities to psychostimulant drugs. BMC Pharmacol. 6, 6. https://doi.org/10.1186/1471-2210-6-6.

Hilderbrand, E.R., Lasek, A.W., 2014. Sex differences in cocaine conditioned place preference in C57BL/6J mice. Neuroreport 25, 105–109. https://doi.org/10.1097/WNR.0000000000000053.

Hoffmann, L.A., Saravanan, V., Wood, A.N., He, L., Sober, S.J., 2016. Dopaminergic contributions to vocal learning. J. Neurosci. 36, 2176–2189. https://doi.org/10.1523/JNEUROSCI.3883-15.2016.

Koob, G.F., Volkow, N.D., 2010. Neurocircuitry of addiction. Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol. 35, 217–238. https://doi.org/10.1038/npp.2009.110.

Krebs, R.M., Boehler, C.N., Roberts, K.C., Song, A.W., Woldorff, M.G., 2012. The involvement of the dopaminergic midbrain and cortico-striatal-thalamic circuits in the integration of reward prospect and attentional task demands. Cereb. Cortex N. Y. N. 1991 (22), 607–615. https://doi.org/10.1093/cercor/bhr134.

Lovell, P.V., Kasimi, B., Carleton, J., Velho, T.A., Mello, C.V., 2015. Living without DAT: loss and compensation of the dopamine transporter gene in sauropsids (birds and reptiles). Sci. Rep. 5, 14093. https://doi.org/10.1038/srep14093.

McMillen, B.A., Davis, B.J., Williams, H.L., Soderstrom, K., 2005. Periadolescent nicotine exposure causes heterologous sensitization to cocaine reinforcement. Eur. J. Pharmacol. 509, 161–164. https://doi.org/10.1016/j.ejphar.2005.01.002.

Mello, C.V., 2014. The zebra finch, Taeniopygia guttata: an avian model for investigating the neurobiological basis of vocal learning. Cold Spring Harb Protoc 2014https://doi.org/10.1101/pdb.emo084574. pdb.emo084574.

Mueller, D., Stewart, J., 2000. Cocaine-induced conditioned place preference: reinstatement by priming injections of cocaine after extinction. Behav. Brain Res. 115, 39–47.

Nestler, E.J., Hyman, S.E., Malenka, R.C., 2008. Reinforcement and addictive disorders, chapter 15. In: Molecular Neuropharmacology: A Foundation for Clinical Neuroscience, second edition. McGraw Hill Professional. McGraw-Hill Education.

Nottebohm, F., Arnold, A., 1976. Sexual dimorphism in vocal control areas of the songbird brain. Science 194, 211–213.

Petkov, C.I., Jarvis, E.D., 2012. Birds, primates, and spoken language origins: behavioral phenotypes and neurobiological substrates. Front. Evol. Neurosci. 4. https://doi.org/

10.3389/fnevo.2012.00012.
Riters, L.V., Stevenson, S.A., 2012. Reward and vocal production: song-associated place preference in songbirds. Physiol. Behav. 106, 87–94. https://doi.org/10.1016/j.physbeh.2012.01.010.
Sasaki, A., Sotnikova, T.D., Gainetdinov, R.R., Jarvis, E.D., 2006. Social context-dependent singing-regulated dopamine. J. Neurosci. 26, 9010–9014. https://doi.org/10.1523/JNEUROSCI.1335-06.2006.
Soderstrom, K., Johnson, F., 2003. Cannabinoid exposure alters learning of zebra finch vocal patterns. Dev. Brain Res. 142, 215–217. https://doi.org/10.1016/S0165-3806(03)00061-0.
Soderstrom, K., Tian, Q., 2004. Distinct periods of cannabinoid sensitivity during zebra finch vocal development. Brain Res. Dev. Brain Res. 153, 225–232. https://doi.org/10.1016/j.devbrainres.2004.09.002.
Soderstrom, K., Tian, Q., 2006. Developmental pattern of CB1 cannabinoid receptor immunoreactivity in brain regions important to zebra finch (Taeniopygia guttata) song learning and control. J. Comp. Neurol. 496, 739–758. https://doi.org/10.1002/cne.20963.
Spealman, R.D., Goldberg, S.R., 1978. Drug self-administration by laboratory animals: control by schedules of reinforcement. Annu. Rev. Pharmacol. Toxicol. 18, 313–339. https://doi.org/10.1146/annurev.pa.18.040178.001525.
Spear, L.P., 2016. Consequences of adolescent use of alcohol and other drugs: studies using rodent models. Neurosci. Biobehav. Rev. 70, 228–243. https://doi.org/10.1016/j.neubiorev.2016.07.026.
Su, Z.-I., Santoostaroam, A., Wenzel, J., Ettenberg, A., 2013. On the persistence of cocaine-induced place preferences and aversions in rats. Psychopharmacology 229, 115–123. https://doi.org/10.1007/s00213-013-3086-9.
Tzschentke, T.M., 1998. Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. Prog. Neurobiol. 56, 613–672.
Xiao, L., Chattree, G., Oscos, F.G., Cao, M., Wanat, M.J., Roberts, T.F., 2018. A basal ganglia circuit sufficient to guide birdsong learning. Neuron 98, 208–221.e5. https://doi.org/10.1016/j.neuron.2018.02.020.