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Fixed ratio discrimination training increases in vivo striatal dopamine in neonatal 6-OHDA-lesioned rats

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Abstract

Massed training in the conditional discrimination task, the fixed ratio discrimination (FRD) task led to elevated extracellular dopamine (DA) concentrations in the neonatal 6-hydroxydopamine (6-OHDA)-treated rat, a model of Lesch–Nyhan disease (LND). Rats neonatally treated with 6-OHDA or its vehicle were, as adults, implanted with microdialysis probes and assessed for basal pretraining concentrations of DA and its major metabolites. Subsequently, microdialysis samples were collected each day following three separate FRD training periods (trained group) or three separate periods of noncontingent food presentations (untrained group). The present study found that there were significant increases in extracellular DA in the caudate–putamen from basal pretraining concentrations in the repeated sample collections of trained 6-OHDA-lesioned animals but not in the samples of untrained 6-OHDA-lesioned animals. Consistent with previous studies [Brain Res. 508 (1990) 30.], there was an increase in the extracellular concentrations as compared to tissue concentrations of DA and 3,4-dihydroxyphenylacetic acid (DOPAC). Similar to our previous studies with long-term FRD training [Pharmacol. Biochem. Behav. 51 (1995) 861; Brain Res. 713 (1996) 246.], there was also an indication of an increase in cortical and striatal tissue concentration of DA in the trained 6-OHDA-lesioned animals as compared to the untrained 6-OHDA-lesioned animals. The elevations in striatal DA concentrations following operant performance in the present study illustrate how operant procedures of the behavior therapy used with individuals with LND and other mental retardation syndromes may interact with the modulation of dopaminergic function by the pharmaceutical application of DA antagonists to suppress aberrant behaviors.

Keywords: Neonatal 6-OHDA; Dopamine; Microdialysis; Striatum; Operant training; Lesch–Nyhan disease; Self-injurious behavior; Operant therapy

1. Introduction

In earlier studies, performance in a complex operant learning task, the fixed ratio discrimination (FRD) task, for 90 sessions led to an increase in neostriatal tissue concentrations of dopamine (DA) and its major metabolites in an animal model of Lesch–Nyhan disease (LND) (Tessel et al., 1995; Stodgell et al., 1996). In this animal model, rat pups receive bilateral intracerebroventricular injections of the neurotoxin 6-hydroxydopamine (6-OHDA). When assessed as 90-day-old adult rats, the animals are severely depleted of DA in the basal ganglia (Breese, 2002; Tessel et al., 1995). In the present study, we assessed whether short-term intensive operant training causes an elevation in DA release in adult animals that were depleted of brain DA as neonates. To do this, we evaluated the extracellular concentrations of DA and its metabolites prior to and following operant training sessions in the Lesch–Nyhan model using microdialysis procedures.

LND is an inherited disorder of purine metabolism dysfunction that results in mental retardation, motor dys-

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function, self-injurious behavior (SIB), and hyperuricemia (Lesch and Nyhan, 1964). Neurochemical evidence indicates that in the brains of LND patients, there is a reduction in DA (60–90%) in the caudate, putamen, and nucleus accumbens (Lloyd et al., 1981). An MRI study found that the basal ganglia are slightly smaller in LND patients (Harris et al., 1998). Furthermore, PET studies suggest a deficiency in the density of dopaminergic fibers projecting in the basal ganglia as indicated by both a decrease in radiolabelled fluoro-DOPA and a decrease in the binding of DA transporter ligand WIN-35428 (Ernst et al., 1996; Wong et al., 1996).

The 6-OHDA animal model of LND has been well characterized by Breese et al. (2002). As adults, neonatally 6-OHDA-treated animals show behavioral problems such as hyperactivity, aggressiveness, stereotypies, and slight motor and learning impairments. Neonatally 6-OHDA-treated animals also display postsynaptic receptor functional supersensitivity in response to L-DOPA or apomorphine challenges. Behavioral indicators of this receptor supersensitivity include the occurrence of aberrant behaviors such as intense sniffing, hyperactivity, taffy pulling (repeated drawing of the forepaws up to and away from the nose), and SIBs such as tail and limb biting (Breese et al., 1984; Stodgell et al., 1998). Inducement of SIB is thought to result primarily from activation of supersensitive DA D_1 receptors as only a D_1 receptor antagonist (SCH-23390) effectively suppresses the occurrence of such behavior. Multiple injections of SKF-38393, a selective D_1 agonist, resulted in "priming," a functional enhancement of the SIB-eliciting effects of DA agonists in neonatally DA-depleted animals that does not occur in animals bilaterally lesioned with 6-OHDA as adults (Moy et al., 1997). However, a selective D_1 agonist does not produce as large an effect in eliciting SIB as do mixed D_1/D_2 agonists such as L-DOPA or apomorphine.

Typically, tissue concentrations of DA in adult rats that were given *neonatal treatment* of 6-OHDA are reduced to 1–20% of the striatal tissue concentrations of vehicle-treated animals. Microdialysis studies on neonatal 6-OHDA unilateral-lesioned animals indicate that there may be a reduced amount of extracellular DA depletion. When administered to only the right ventricle of 3-day-old pups, 6-OHDA resulted in partial depletion of DA concentration (65%) in the lesioned side. However, extracellular concentrations of DA metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), were less than 5% that of nonlesioned sides and vehicle-treated controls (Herrera-Marschitz et al., 1994). Castaneda et al. (1990) found that while postmortem tissue assays indicated that there was a 99% depletion of DA, in vivo microdialysis measures indicated a 12–54% depletion of extracellular DA. For the metabolites, both microdialysis sampling and postmortem tissue measures indicated a greater than 97% depletion. These investigators additionally measured extracellular concentrations during treadmill performance and following amphetamine administration and found that in both cases, extracellular indices indicated that there was no increase in DA release or metabolite production in the 6-OHDA-treated animals.

Studies using microdialysis measures in non-6-OHDA-treated animals have shown that varying environmental experiences can lead to changes in extracellular catecholamine concentrations in rodent dopaminergic pathways. The present study assessed DA release 24 h after food-reinforced operant conditioning and thus was not measuring release during appetitive or consummatory food-related behaviors. Wilson et al. (1995) of HC Fibiger's laboratory found, using in vivo microdialysis, that there was an increase in DA release during the consummatory components, but not anticipatory components, of feeding behavior during conditioned food trials in the nucleus accumbens. Likewise, Bassereo and Di Chiara (1999) found that increases in DA transmission in the nucleus accumbens also occurred during consummatory, more so than appetitive, behaviors. Consummation of food alone during microdialysis sampling can cause increases in DA release in the nucleus accumbens and the prefrontal cortex and is positively influenced by food deprivation (motivation) and negatively influenced by habituation (Di Chiara et al., 1999). The relative increases in DA release in the basal ganglia may vary depending upon whether the animals were schedule-trained or were given free access to food. One study found increased striatal DA in food-deprived control rats during performance of a fixed time (1 min) schedule of food reinforcement but not when food-deprived animals were given free access to a food pile (Church et al., 1987). Another study found that rats, after receiving reinforcement for completing five lever presses in a (FR5) schedule of reinforcement, showed significant increases in DA and DA metabolites in the nucleus accumbens compared to food-deprived untrained controls (Salamone et al., 1994). In further analyses, rats with the FR5 schedule were divided into three groups based upon response rates. Rats with low response rates did not significantly differ from control rats, whereas rats with medium and high rates of responding showed significant increases in extracellular DA release in the nucleus accumbens relative to the control group. Rats that received massed presentation of food pellets or laboratory chow consumed large quantities of food, but showed no significant increases in DA release. A study on drinking in thirsty rats found that there was an increase in DA and its metabolites in both the nucleus accumbens and the caudate–putamen during a lick operant task (Young et al., 1992).

One study looked at the effects of 6-OHDA treatment in adult animals on DA concentrations following scheduled-maintained feeding or free food access (Cousins et al., 1993). 6-OHDA was administered bilaterally to the nucleus accumbens, medial striatum, or the ventrolateral striatum of 3-month-old rats. The animals were then given a choice of performing a FR5 for a preferred food or free access to rat chow. Depletion in the accumbens resulted in a decrease in lever pressing and spontaneous motor activity but led to an

increase in food consumption. Depletion in the medial striatum did not affect either lever pressing or food consumption. Depletion in the ventrolateral striatum resulted in decreased lever pressing and food consumption. The animals with depletions in the ventrolateral striatum also showed problems in home cage feeding. Tissue analyses indicated severe depletion levels of catecholamines in the nucleus accumbens, medial striatum, and the ventrolateral striatum following performance in the FR5 or free access to rat chow. For adult 6-OHDA-lesioned animals, it does not appear that performance in an FR5 schedule of reinforcement led to increases in striatal tissue concentrations of DA. These findings are similar to the results of another study by our laboratory that indicated no apparent increases in tissue concentrations of striatal DA or its major metabolites in adult 6-OHDA-lesioned animals after FRD training (Van Keuren et al., 1998). However, there was a change in dopaminergic function in the adult 6-OHDA-depleted animals, in that there was a significant normalization in the behavioral responsiveness of these animals to the mixed DA agonist, apomorphine, following the operant training procedures.

In the present study, we chose to evaluate the release of extracellular DA and its metabolites in the pallidal output of the striatum of the 6-OHDA-lesioned rats following intensive periods of operant training. The operant training procedures consisted of the training steps involved in the performance of the FRD task, a conditional discrimination task, or an arbitrary match-to-sample task. Conditional discrimination tasks, which require learning relations between physically different stimuli, are very difficult for individuals with severe and profound levels of mental retardation to perform and the ability to perform conditional discriminations is often a training step in developing the language and other adaptive abilities of these individuals (MacKay, 1991; Saunders and Spradlin, 1993). Many of the individuals who perform SIB have severe and profound levels of mental retardation. We chose a conditional discrimination for this study so that we could assess the performance of an animal model of LND in a task that is known to be difficult for individuals with mental retardation to perform and that many of these individuals engage in when they participate in operant learning therapies (MacKay, 1991). This will provide an assessment of whether the types of operant training tasks performed by individuals with mental retardation may affect the release of DA and its metabolites in the striatal pathways that are involved in the occurrence of SIB.

2. Materials and methods

2.1. Study design

This study assessed changes in the amount of DA concentration in the neonatal 6-OHDA-treated rat following periods of performance in operant training sessions. To do this, we implanted guide cannulas into the caudate–putamen brain regions of 90-day-old animals treated with 6-OHDA or vehicle as neonates and then measured basal (pretraining) catecholamine concentrations for 4 h on 1 day prior to the animals being shaped for operant training tasks. Following collection of the basal neurotransmitter concentrations, the animals were divided into training or nontraining groups. Animals in the training group received three periods of intensive operant training, each period lasting for approximately 3 days, 3 h/day, for a total of 9 h of training per period. During each of the three training periods, the complexity of the task requirements increased progressively. Nontraining groups were handled as much as trained animals and received reinforcement pellets in their home cages. On the day after each 3-day period of operant training or no training, microdialysis samples were collected for 4 h following a 3-h period of stabilization. In all, 4 days of microdialysis samples collections were conducted with the first being prior to training/nontraining conditions (basal collection period) and the remaining three occurring after each of the three training periods (denoted as second, third, and fourth microdialysis sampling periods in result figures). After the fourth microdialysis sampling period, the animals were sacrificed and HPLC determinations of catecholamine brain tissue concentrations were conducted.

2.2. Animals

Pregnant female Sprague–Dawley rats were purchased from Harlan Sprague–Dawley (St. Louis, MO) and housed with Purina breeder blocks and water available ad libitum until their pups were weaned. All animals were housed in the University of Kansas Animal Care Unit and all procedures were performed in accordance with an animal use statement approved by the University of Kansas Institutional Animal Care and Use Committee.

On Postnatal Day 3, rat pups were removed temporarily from their home cage and received intracerebroventricular bilateral injections of either 6-OHDA (50 μg of 6-OHDA HBr in 0.1% ascorbic acid in saline; RBI, Natick, MA) or vehicle. For the bilateral injections, animals were hypothermically anesthetized by covering the animal with approximately 6 cm of crushed ice for a duration of 1 min per gram body weight (Cunningham and McKay, 1993). They were then placed in a neonatal rat adaptor for a stereotaxic apparatus (Stoeling Instruments, Wooddale, IL). They were given two 5-μl injections of 6-OHDA or vehicle, each injection 2 mm on either side of the midsagittal line, 1.5 mm from lamba. After the injections, all animals were allowed to remain with their mothers until weaning at 21 days of age. At 90 days of age, the 6-OHDA- and vehicle-treated animals were reduced to 85% of their free feeding weight for the operant training and nontraining conditions.

2.3. Operant training

The animals were taught the same tasks as used in previous studies in our laboratory (Loupe et al., 1995; Tessel et al., 1995). The apparatuses used include $29 \times 25.3 \times 28$-cm experimental operant chambers housed in larger insulated shells with ventilation fans and operated via computer (Coulbourn Instruments, Allentown, PA). A center retractable lever and two side levers were located 2 cm from a grid floor on one side of the chamber. A three-diode cuelight panel was located above each lever. A food trough was located above the center retractable lever and cuelight, approximately 9.5 cm from the grid floor. A houselight was located 26.5 cm from the floor to the right of the food trough. Correct responses resulted in delivery of a 45-mg food pellet (PJ Noyes, Lancaster, NH) and incorrect responses resulted in a 5-s timeout period. In general, the animals participated in the training steps as described below; however, adjustments were made if necessary to make sure that each animal successfully completed a training step before progressing to a more difficult step. Within each 3-h daily training session, each animal was presented with approximately the same number of trials, usually 180 trials.

During the first training period, which lasted 3 days (540 trials), the animals pressed the center response lever for food reinforcement. The number of responses per reinforcement (FR1, FR2, FR4, FR8, FR16) was increased each time the animal met criterion of completing 60 trials of a FR schedule in 1 h.

During the second training period, the animals were reinforced for performing a chain of responses on two levers. They pressed the retractable lever located in the center of the chamber (i.e., center lever) several times before the cue light above the center lever extinguished and the center lever retracted. They then pressed a lever located to the side of the center lever once to receive reinforcement. A press on the left side lever was correct if the animal was required to press the center lever 16 times (FR16) and a press on the right side lever was correct if the animal was required to press the center lever 8 times (FR8). A center lever response schedule of FR16 was presented for approximately 270 sequential trials followed by a center lever response schedule of FR8 for approximately 270 trials.

During the third period of training, the animals performed the FRD task for 540 trials. The animals received food reinforcement for pressing the center lever until the lever retracted and then making a single response on the correct side lever. As in the second training period, correct responses involved pressing the right side lever following completion of a schedule of FR8 and pressing the left side lever following completion of a FR16. Incorrect responses resulted in a 5-s timeout period. A correction procedure was used such that if an animal made an incorrect response, trials involving the same side lever center lever ratio requirement contingency were sequentially presented until the animal pressed the correct side lever. After that, the occurrence of a FR16 or FR8 trial was randomly distributed across the training period.

2.4. Implantation of guide cannula

The neonatal 6-OHDA-treated and vehicle-treated rats were implanted with microdialysis guide cannulas (model number MD-2250; BAS, West Lafayette, IN) in the caudate–putamen. For surgery, rats were initially anesthetized with the inhalation of halothane and then given an intraperitoneal dose of 2 ml/kg body weight of an anesthetic cocktail (37.5 mg/ml ketamine, 1.8 mg/ml xylazine, and 0.37 mg/ml acepromazine). After anesthetization, the animal's head was shaved and the animal was placed in a stereotaxic frame in a sterile environment. A midsagittal incision was made from just behind the eyes to just in front of the ears and underlying tissue was cleared from the skull. Coordinates for the intersection of bregma and the midsagittal line were determined and used to calculate the stereotaxic coordinates of the caudate–putamen (AP $+0.7$ mm, ML $+2.7$ mm, VD -3.6 mm, relative to bregma and the midsagittal line; Paxinos and Watson, 1986). Anchor screws were placed in the three quadrants surrounding the location of the guide cannula and tightened to a depth of 1 mm. Using the stereotaxic arm, the guide cannula was placed in the drilled target hole perpendicularly so that the tip of the cannula was 3.5 mm ventral from the surface of the skull. Dental cement and sutures were used to hold the cannula and anchor screws in place. Accuracy of location was determined by microscope inspection at the time of tissue removal.

2.5. Microdialysis sample collection and analysis

For measurements of catecholamine extracellular concentrations, microdialysis sample collection periods were conducted on four separate days and lasted for 7–8 h. One day of sampling was conducted prior to operant training conditions (basal collection) and three subsequent days of sample collection occurred between the periods of operant training/nontraining condition. During each microdialysis sample collection period, animals were placed in a sample collection bowl (BAS) and inserted with a 4-mm microdialysis probe (model number MD-2204; BAS). They were then allowed to rest for 3 h and then dialysate samples collections were made every 20 min (total volume of about 14 μl) and continued until three samples, occurring one after another, showed identical concentrations (generally took six to eight samples) when injected directly into a 5-μl loop of a LC-EC system fitted with a microbore column (model number MF-8912; BAS). The inserted microdialysis probes were connected via inlet tubing (inner diameter 0.12 mm, outer diameter 0.65 mm; BAS model number MF-5164) to a microdialysis syringe pump (CMA100; BAS) with a perfusion flow rate

of 0.7 μl/min. The probes were perfused with sterile artificial cerebrospinal fluid (aCSF; 120 mM NaCl, 20 mM $NaHCO_3$, 3 mM KCl, 1.2 mM $CaCl_2$, 1.0 mM $MgCl_2$, and 0.25 mM $NaHPO_4$) and were calibrated in vitro before each sampling period's insertion by using an in vitro probe recovery experiment (see next paragraph). The LC-EC mobile phase consisted of a filtered solution of 1 l of dH_2O with 25 mM NaH_2PO_4, 50 mM sodium citrate, 27 μM disodium–EDTA, 10 mM diethylamine–HCl, and 2.2 mM octanesulfonic acid sodium salt. A pH of 3.2 was obtained with H_3PO_4, and then 30 ml of methanol and 22 ml of dimethylacetamide were added (total volume of mobile phase was 1 l). The column and mobile phase were at ambient temperature and the flow rate was 0.1 ml/min. A LC4B (BAS) detector with glass carbon working electrode (3 mm) operated at 800 mV versus Ag/AgCl. The collected dialysate samples were stored in dry ice if they were not injected into the LC-EC system immediately. This happened on two occasions when samples from two animals (one nontrained control and one trained control) could not be injected on the HPLC immediately because it was not working correctly. These samples were frozen and later their concentrations were compared to concentrations of the same treatment groups during the same sample period. As no differences were found in the concentrations, these samples were included. Once the samples were completed, the microdialysis probes were removed, the dummy probes reinserted, and the animals returned to their home cage. The next day, the animal participated in operant training/nontraining conditions.

In vitro probe recovery was conducted the day prior to microdialysis sample collection periods to ensure that the probe was working correctly. Probe recovery was determined at room temperature by placing the dialysis probe in a standard solution containing (0.5 μM DA, 10 μM DOPAC, 5 μM HVA, 5 μM 5-HIAA) prepared in aCSF. Probe recovery samples continued until three continuous dialysate samples showed identical concentrations of the chemical compounds. Recovery is expressed as the ratio of the concentration of the studied compound in the dialysate sample and the concentration of the same compound in a standard solution of known concentrations. Analyte concentrations from in vivo dialysate samples were corrected using the in vitro recovery value for that probe.

2.6. HPLC assessment of in vitro tissue concentrations of DA and metabolites

For assessment of DA depletion due to neonatal 6-OHDA treatment and DA recovery following operant training conditions, cortical and striatal brain tissue concentrations of DOPAC, DA, and HVA were assessed by reverse phase liquid chromatography with electrochemical detection. Briefly, this assessment involved excising cortical and striatal tissue from experimental animals, weighing, and sonication [cortex at 1 g tissue/5 ml solution of 0.2 μM dihydroxybenzylamine (DHBA) as an internal standard and 0.1 M $HClO_4$, and striatum at 1 g tissue/10 ml solution of 1 μM DHBA and 0.1 M $HClO_4$). An ice water bath was used during sample sonication to inhibit sample degradation and samples were then centrifuged at a speed of 15,000 rpm for 30 min at 4 °C. The supernatant was filtered through a 0.2-mm Nylon acrodisc disk filter (Gelman Sciences, Ann Arbor, MI), aliquoted to sample vials and frozen at −70 °C. For analysis, thawed samples of approximately 40 μl were injected into a 20-μl loop of the LC-EC system fitted with a Biophase ODS column (BAS). Mobile phase consisted of 1 l of dH_2O with 3.5% acetonitrile and 96.5% 0.15 M monochloroacetate. The pH of this solution was adjusted to 3.0 with NaOH, and 0.86 mM sodium octyl sulfate was added. This solution was then filtered, degassed, and 14 ml of tetrahydrofuran was added for a total volume of 1 l. The column and mobile phase were at ambient temperature and flow rate was 1.6 ml/min. As before, a LC4B (BAS) detector with glass carbon working electrode (3 mm) operated at 800 mV versus Ag/AgCl. The sample injections were conducted until three continuous samples reflected similar concentrations.

3. Results

3.1. Behavioral performance

In the first training period during which the animals acquired the FR response contingency, the response rates of the 6-OHDA- and vehicle-treated animals of this study did not differ as shown in Fig. 1 and were similar to animals measured in our previous studies (Loupe et al., 1995; Tessel et al., 1995). Similarly, in the second and third training periods, the response rates on the center lever did not differ between the 6-OHDA and the control groups. During performance in the third training period, the 6-OHDA animals and the controls completed, on average, 97% and 82% of the trials per daily session for 3 days; however, the average percent error of the animals never improved above chance performance. As shown in Fig. 2, the vehicle animals displayed a response bias in lever selection during performance of the FRD task in the third training period. The bias occurred randomly either on the left or right lever, depending upon the animal.

3.2. Microdialysis collections of extracellular DA

Before the first training period and between each period of training, microdialysis samples of extracellular catecholamine concentrations were collected. Table 1 describes the basal values (before any training) of the extracellular catecholamine concentrations for the trained and untrained 6-OHDA- and vehicle-treated animals. There were no significant differences prior to participation in the training conditions

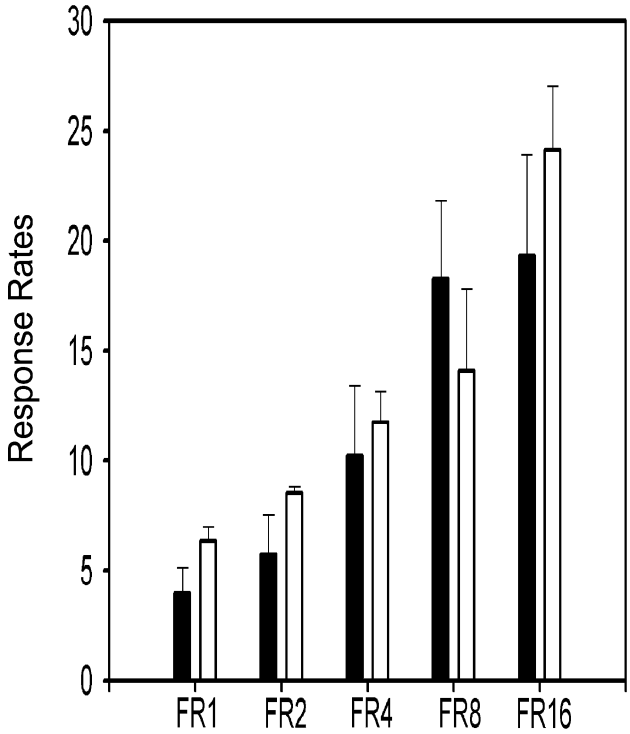

Fig. 1. Performance in fixed ratio schedules during the first training period. Solid bars (±S.E.M.) represent the group mean of the response rates (responses per minute) of the 6-OHDA-treated rats. Open bars (±S.E.M.) represent the mean response rates of the vehicle-treated rats. The number of lever presses required for food reward during each fixed ratio schedules is abbreviated as FR1, FR2, FR4, FR8, and FR16. There were no significant differences in the response rates of the 6-OHDA- and vehicle-treated animals.

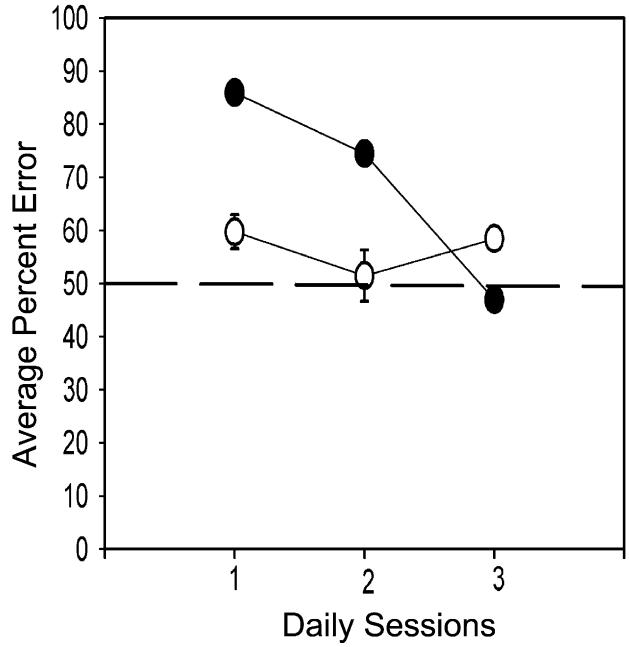

Fig. 2. Performance in the FRD task during the third training period. Open circles (±S.E.M.) and closed circles (±S.E.M.) represent the average percent error rate of the 6-OHDA- and vehicle-treated rats, respectively. Averaged percent error rate is the mean of the number of incorrect lever presses divided by total number of lever presses per animal per daily session. Approximately 180 trials occurred during each daily session for each animal.

in the basal extracellular concentrations of DA, DOPAC, or HVA between the trained and untrained 6-OHDA or between the trained and untrained vehicle animals (F values not reported). There were significant differences between 6-OHDA and vehicle animals in extracellular concentrations of DA, DOPAC, and HVA [$F(2,13)=8.244$, $P<.005$; $F(2,13)=4.63$, $P<.03$; $F(2,13)=4.47$, $P<.03$, respectively].

The data were analyzed for each individual animal in terms of the percent change from basal extracellular concentrations of DA, DOPAC, and HVA for the trained and untrained 6-OHDA and vehicle groups at each of the three posttraining sample times (shown in Fig. 3). The extracellular DA concentrations increased above basal concentrations in the trained 6-OHDA animals. Repeated measures ANOVA indicates that in the 6-OHDA animals, there was a significant change in DA concentration relative to basal after the third training period and a significant interaction between training and the repeated sampling [$F(1,8)=5.34$, $P<.05$; $F(3,24)=4.05$, $P<.018$]. The interaction between training and repeated sampling of DA concentration occurred because in the trained 6-OHDA animals, there was an increase in DA concentrations from basal values whereas in the 6-OHDA untrained animals, there was a decrease in DA concentrations. There were no significant percent changes in the concentrations of the DA metabolites, DOPAC and HVA, from basal values due to training experience or across repeated sampling in the 6-OHDA animals.

For the vehicle animals, there were significant percent decreases in the concentrations of DA, DOPAC, and HVA that were not affected by training experience [$F(3,15)=3.69$, $P<.036$; $F(3,15)=3.25$, $P<.05$; $F(3,15)=6.30$, $P<.006$, respectively]. Data from the vehicle animals and from the DA metabolites in the 6-OHDA animals are consistent with previous studies using microdialysis techniques that have shown catecholamine samples decrease slightly in concentration over time and repeated sampling (decreased by 20% in 23 days in Martin-Fardon et al., 1997) and are thought to be due to gliosis. However, despite this expected decrease in DA

Table 1
Basal catecholamine concentrations (μM)

Group	Dopamine	DOPAC	HVA
6-OHDA–trained ($n=7$)	0.03±0.01	3.51±0.74	1.04±0.45
6-OHDA–untrained ($n=8$)	0.03±0.01	1.82±1.04	0.76±0.61
Vehicle–trained ($n=5$)	0.12±0.04	8.57±2.32	3.87±1.20
Vehicle–untrained ($n=4$)	0.10±0.03	7.66±3.17	3.86±1.95

This table lists the mean±S.E.M. for the basal (pretraining) concentrations of DA and its metabolites. Statistical comparisons indicated significant differences in the concentrations of DA and its metabolites between the 6-OHDA- and vehicle-treated animals but not between the 6-OHDA trained and untrained groups.

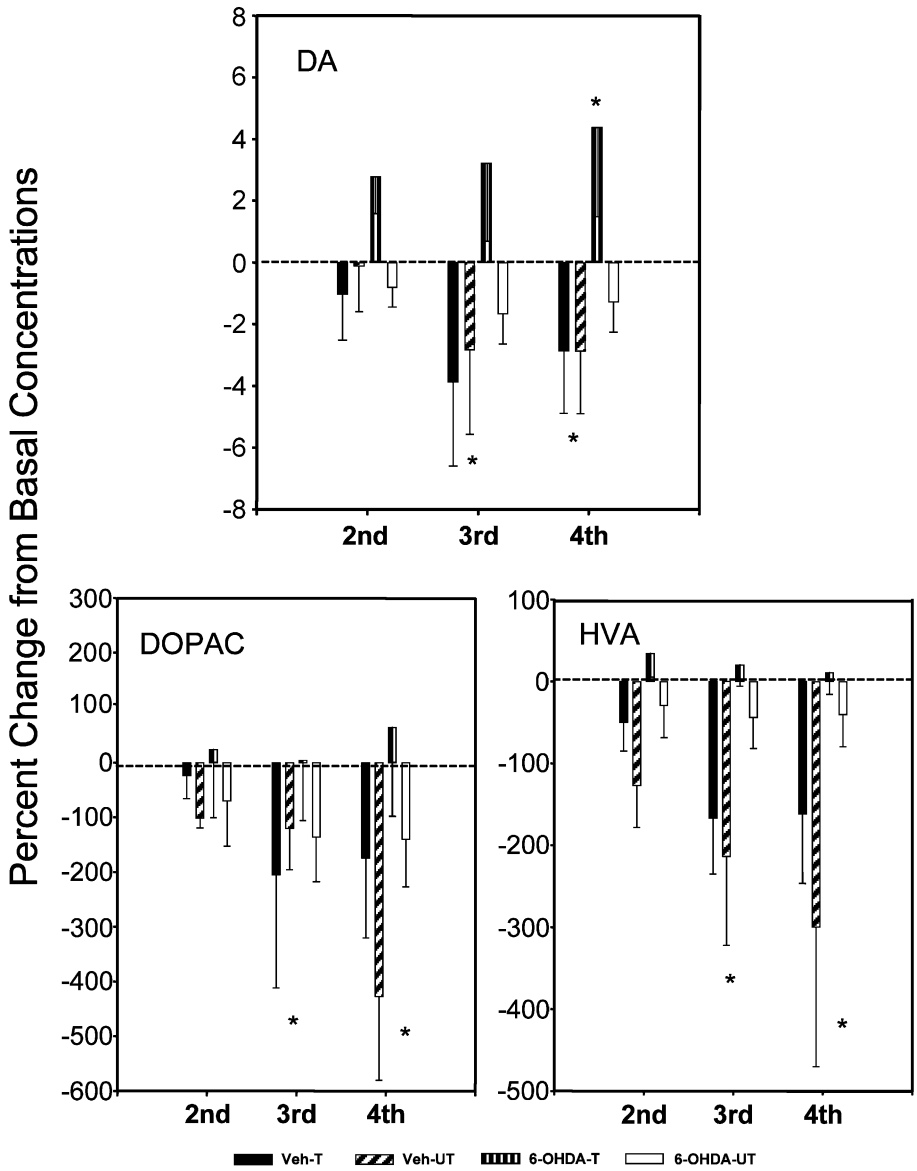

Fig. 3. Percent change from basal (pretraining) extracellular concentrations of DA, DOPAC, and HVA collected via microdialysis sampling between operant training sessions. Basal extracellular concentrations are listed in Table 1. Bars (black bar, vehicle–trained; diagonal striped bars, vehicle–untrained; vertical striped bars, 6-OHDA–trained; open bars, 6-OHDA–untrained) represent the group means (±S.E.M.) of the percent change from basal extracellular concentrations during the second, third, and fourth microdialysis sampling periods.

concentration over time, for the trained 6-OHDA animals, the levels of DA concentration increased after periods of operant training.

3.3. Cortical and striatal tissue concentrations

Fig. 4 illustrates the extracellular and tissue concentrations of DA (Panels a and b) and DOPAC (Panels c and d) for the 6-OHDA trained and untrained animals. As shown, there is a marked increase in the amount of DA content in both extracellular and tissue concentrations in the trained 6-OHDA animals as compared to the untrained 6-OHDA animals. There were significant differences in group means following the third training period in the fourth microdialysis sample of extracellular concentrations of DA and DOPAC, between the trained and untrained 6-OHDA [$F(1,12) = 12.45$, $P < .008$; $F(1,12) = 279$, $P < .000$, respectively]. Statistical analyses were not conducted on the tissue concentrations due to small sample sizes ($n = 2$ and $n = 3$ for 6-OHDA untrained and trained groups, respectively). Similar to findings by others in studies of untrained 6-OHDA-treated rats (Church et al., 1987), there was an increase in basal extracellular concentrations (25.37% and 31.14% of untrained control concentrations) relative to tissue content (11.89% and 3.83%) for both the trained and untrained 6-OHDA animals.

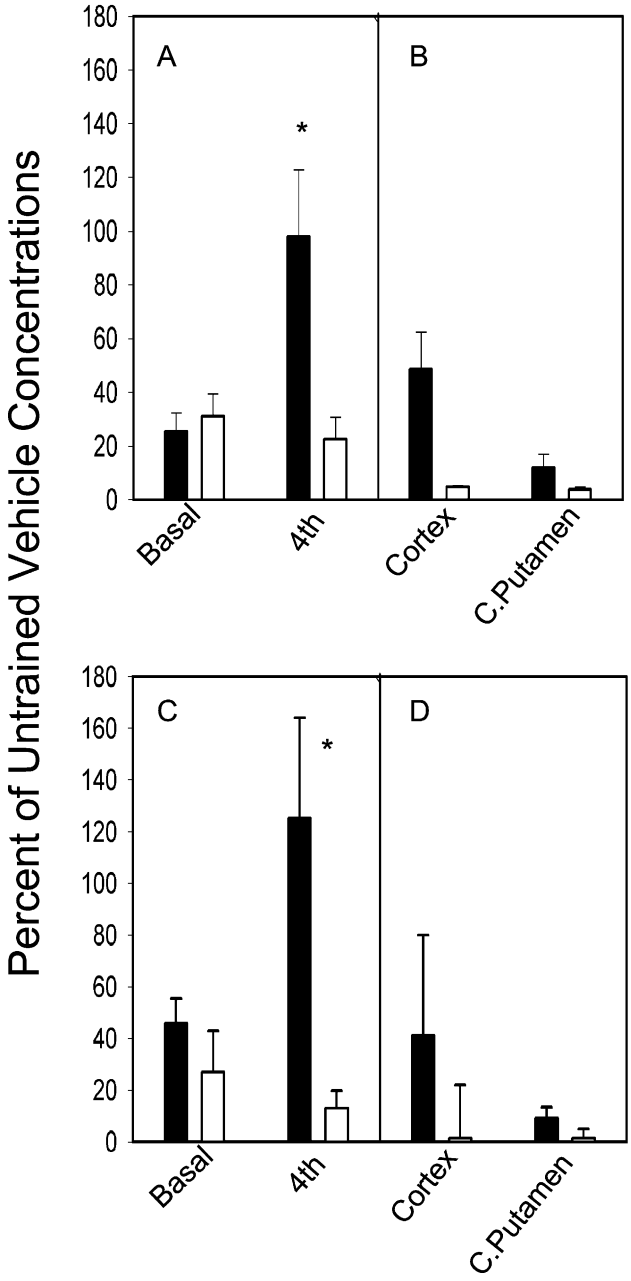

Fig. 4. Percent change from untrained vehicle-treated rats in in vivo extracellular striatal and postmortem striatal tissue concentrations of DA and DOPAC for the trained and untrained 6-OHDA-treated animals. For the untrained vehicle animals, the basal and fourth sample extracellular concentrations (in μM ± S.E.M.) for DA were 0.104 ± 0.033 and 0.07 ± 0.02 and for DOPAC were 7.66 ± 3.17 and 3.32 ± 1.64. For the untrained vehicle animals, the cortical and caudate–putamen tissue concentrations (in μg/mg tissue ± S.E.M.) for DA were 0.928 ± 0.004 and 15.95 ± 0.49 and for DOPAC were 0.600 ± 0.42 and 2.37 ± 0.20. Panels A and C represent the percent change from DA and DOPAC striatal concentrations of the untrained vehicle treated for the trained (solid bars) and untrained (open bars) 6-OHDA animals collected during the basal (pretraining) and fourth microdialysis sampling period. Panels B and D represent the group means (μg/mg tissue ± S.E.M.) of the percent change in DA and DOPAC, respectively, from the cortical and caudate–putamen tissue concentrations of the untrained vehicle animals for the trained (solid bars) and untrained (open bars) 6-OHDA-treated animals.

4. Discussion

Participation in the FRD task of the present study caused increases in extracellular DA in the 6-OHDA-treated animals, a DA depletion model of LND. These results suggest that the environmental conditions involved in operant training techniques of the FRD task caused an increase in the release of DA in the nigrostriatal pathway. Individuals with LND and other syndromes of mental retardation who exhibit aberrant behaviors such as stereotyped and SIBs have suppressed dopaminergic activity in their corpus striatum (Breese et al., 1984, 2001; Ernst et al., 1996; Harris et al., 1998; Jinnah, 1998; Wong et al., 1996). The 6-OHDA-lesioned animal also has reduced DA neurons and shows a behavioral sensitivity to repeated injections of DA agonists such as L-DOPA or apomorphine (Breese et al., 1984; Stodgell et al., 1998). It is possible that the resultant increase in extracellular concentrations of DA in the 6-OHDA-lesioned animals would cause an elevation of aberrant behaviors if given DA agonists. These animals were not given apomorphine or L-DOPA challenges; however, anecdotal evidence suggests a reduction in the behavioral responsiveness of the animals to environmental stimulants such as loud noises and unfamiliar activity. It is hypothesized that the massed presentation of training caused an increase in phasic firing of DA neurons such that sensitivity of the supersensitive postsynaptic neurons is reduced. Another study in our laboratory found that trained 6-OHDA-lesioned animals showed a reduced sensitivity to L-DOPA injections following participation in 90 sessions of FRD training as compared to untrained 6-OHDA-lesioned animals (unpublished observations). Increases in extracellular DA concentrations due to participation in operant training have been shown in studies of non-6-OHDA-treated animals (Salamone et al., 1994; Church et al., 1987). Factors that could lead to increased DA release include food consumption, motor response, and the lack of stimulus control during acquisition of a new stimulus response contingency. In the present study, the untrained 6-OHDA-lesioned and untrained vehicle animals were given the same amount of food "reinforcement" pellets as the trained animals, which did not lead to increased extracellular DA concentrations. One limitation of the present study was that the untrained 6-OHDA- and vehicle-treated animals were not given reinforcement pellets in the operant chamber but rather in their home cages. This was done to reduce excessive loss of animal subjects due to breakage of guide cannulas that was observed in preliminary data when the untrained animals were placed in operant chambers. The present study shows, similar to the above-cited studies, that mass food consumption alone does not cause the increases in DA release in the caudate–putamen. However, it does not control for other factors in operant training that may be involved such as placement in the operant chamber, motor performance, or noncontingent scheduled reinforcement.

In acquiring a new stimulus response contingency, there is a period of time during which the response is under less stimulus control and, therefore, the likelihood of an incorrect response and no reinforcement is highly probable. We presented the 6-OHDA-lesioned and vehicle animals with the FRD task, an operant task that had several training steps; both the 6-OHDA- and vehicle-treated animals never reached asymptotic performance levels (final percent error rates stayed around 50%) during the third training period. For the FRD task, it generally takes 12 daily 60-trial sessions (total of 720 trials) for our vehicle animals to show a reduction in error rates below 50%; therefore, it was not unexpected that the vehicle- and 6-OHDA lesioned animals performed at chance error rates during the third training period. It is also important to mention that not of all of the 6-OHDA-lesioned animals were able to complete each step during the training periods. Some of the animals were able to perform FR1–FR16 schedules of reinforcement but were unable to perform the response chain schedule during the second training period (they were not presented with the FRD task during the third training period). These animals successfully performed the increasing FR response requirements of the first training period, but when presented with a response chain schedule, such as pressing the center lever then pressing the left lever, they would not respond; lever-pressing responses became extinguished, and responses had to be reshaped to perform the FR1 schedule. For their second and third training periods, they were continuously being shaped to perform higher FR response requirements and, if possible, moved to a response chain schedule.

Therefore, microdialysis samples of the animals were collected after the animals had participated in a task they were learning and their responses were not under complete stimulus control. This differential between acquisition and performance phases of operant training is important given electrophysiological studies that suggest that DA firing from the substantia nigra increases during periods of learning but not during periods of performance of an acquired task. Schultz (1986), Suri and Schultz (1999), and Schultz et al. (1993) have found increases in DA neuron firing rates in A10, A8, and A9 areas of the basal ganglia during the learning of a complex task, the spatial delayed response. This increase in DA neuron firing occurred during learning of the operant task but there was no overall increase when the animals displayed established performance. It has been proposed that this increase in firing rate could influence postsynaptic structures including the caudate–putamen (Schultz et al., 1993). The fact that the firing increased during learning of the task suggests that these DA neurons may function during acquisition of new task contingencies and this may explain a mechanism by which learning could increase the amount of extracellular DA at the caudate–putamen.

The study by Schultz et al. evaluated recordings of impulse-derived elevations in DA concentrations in non 6-OHDA-treated primates. In the present study, we measured extracellular DA concentration in animals in a restful state between periods of intense operant training. We found significant increases in extracellular DA concentrations in the 6-OHDA-lesioned animals but only found nonsignificant trends in the non-6-OHDA-lesioned (vehicle) animals. This difference between the 6-OHDA-lesioned and vehicle animals has occurred in our previous studies evaluating increases in striatal DA tissue concentrations due to performance in the FRD task (Tessel et al., 1995; Stodgell et al., 1996). It is uncertain why the 6-OHDA animals, but not vehicle animals, showed significant increases in extracellular DA concentration due to learning the FRD task. It may be because the vehicle animals learned differently from the 6-OHDA-lesioned animals and that there were not enough learning sessions to cause a substantial increase in catecholamines. In the initial daily session of the third training period of FRD task, the vehicle animals had higher response rates due to a response bias that disappeared over the 3 days of training. For the vehicle animals, although they never performed better than chance error rates (50%) in the FRD task, the response bias was eliminated and in that sense, their performance improved. They moved from almost never reinforced state (90% error rate) to being reinforced half of the time (47% error rate). The 6-OHDA-lesioned animals that performed the FRD task consistently showed error rates at an average 50% error rate across all three daily sessions and the likelihood of reinforcement was weakly associated with stimulus responses. What this means in terms of changes in catecholamine release is unclear, but it does show a learning difference between the vehicle and the 6-OHDA-lesioned animals. Additionally, the 6-OHDA-lesioned animals that performed in the training steps successfully enough to be presented with the FRD task during the third training period were not necessarily the animals that showed the highest increases in extracellular DA. For instance, one rat that was unable to perform a response chain schedule (FR16 on center, FR1 on the left lever) presented during the second, and subsequently third, training period showed an increase of 128% of extracellular DA concentration as compared to nontrained vehicle animals during the fourth microdialysis sampling period. These results are consistent with the theory proposed by Suri and Schultz (1999) that the activation of DA neurons occurs following unpredictable, and not fully predictable, rewards.

The disparity in the increases in striatal DA release between the 6-OHDA and vehicle animals may also be the result of differences in striatal DA clearance processes. As the measures of the extracellular DA concentrations occurred the day following the learning trials, compensatory processes in the vehicle animals may have been effective in removing extracellular DA concentrations. Studies in adult 6-OHDA-lesioned animals have shown decreases in DA autoinhibition and increased DA synthesis (Uretsky and Iversen, 1970; Synder et al., 1990; Zigmond and Stricker, 1984). There was a relative increase of DA efflux and overflow in animals lesioned as adults with 6-OHDA as compared to control animals (Synder et al., 1990). This

increase in DA efflux was attributed to a marked decrease in DA uptake sites in the 6-OHDA animals as indicated by a decrease in the DOPAC/DA ratio. They also found that if a DA synthesis inhibitor, 3-iodotyrosine, was added to striatal slices from 6-OHDA rats, there was a marked decrease in DA outflow. A future study to determine the processes involved in the increases in DA release in the 6-OHDA animals might be to treat the vehicle animals with DA reuptake blockers and presynaptic D_2 autoreceptor antagonists and evaluate whether operant training leads to an increase in extracellular DA concentrations.

Another mechanism by which extracellular DA concentrations may be increased in the 6-OHDA-lesioned animals may be due to the expression of neurotropic factors such as glial-derived neurotropic factor (GDNF), fibroblast growth factor (bFGF), or neurotropin-3 (NT-3) in the spared dopaminergic cells. A recent study found that GDNF induced increased tyrosine hydroxylase (TH) immunostaining in fibers and nerve endings of host striatal cells while NT-3 improved the survival of grafted fetal dopaminergic cells (Espejo et al., 2000). In animals, lesioned with 6-OHDA as adults, treatment with GDNF increases neurons expressing TH and TH activity when given either before or after intranigral or intrastriatal lesions (Bowenkamp et al., 1995; Lapchak et al., 1997; Sauer et al., 1995). This increase in TH activity could lead to increased DA production, thus enabling DA release.

Participation in the FRD task of the present study caused increases in extracellular DA in the neonatal 6-OHDA-treated animals. Previous work by our laboratory and others indicate that the application of DA agonists in animals with early depletion of striatal DA leads to an increase in stereotyped and SIB analogous to that displayed by individuals with Lesch–Nyhan and other syndromes of mental retardation. The results of the present study show that the application of operant training procedures in a rigorous manner may increase extracellular DA in such a manner as to potentially increase or decrease the likelihood of stereotyped and SIBs. This may explain, in part, why behavior therapy techniques employed to reduce the severity of stereotyped and SIBs in persons with mental retardation may be effective in some individuals and may exacerbate symptoms in others.

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