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Neuroadaptations in the dopaminergic system after active self-administration but not after passive administration of methamphetamine

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Abstract

Methamphetamine is a strong and long-lasting stimulant that can be easily synthesized and is effective when taken either orally, intravenously, or smoked as 'ice'. Due to it's escalating abuse, a clear need exists for laboratory procedures to evaluate motivational components of methamphetamine abuse and their underlying neurobiological mechanisms. In the present experiment, we utilized a 'yoked' procedure in which rats were run simultaneously in groups of three, with two rats serving as yoked controls which received an injection of either 0.1 mg/kg methamphetamine or saline which was not contingent on responding each time a response-contingent injection of 0.1 mg/kg methamphetamine was self-administered by the third paired rat. Rats that had actively self-administered methamphetamine for 5 weeks and were then withdrawn from methamphetamine for 24 h showed marked decreases in somatodendritic dopamine D_2 autoreceptors levels in the ventral tegmental area (34%) and medial (31%) and dorsal (21%) part of the substantia nigra zona compacta with a corresponding down-regulation of dopamine D_1 receptors in the shell of the nucleus accumbens (15%), as measured by in vitro quantitative autoradiography. Since the decreases in levels of dopamine D_1 and D_2 receptors which occurred in rats self-administering methamphetamine did not occur in littermates that received either yoked injections of methamphetamine or saline, these changes likely reflect motivational states that were present when methamphetamine injection depended on active drug self-administration behavior.

Keywords: Methamphetamine; Self-administration; Neuroadaptation; Dopamine; Dopamine transporter; Dopamine D_1 receptor; Dopamine D_2 receptor

1. Introduction

Although the popularity of methamphetamine as a drug of abuse first emerged in the 1940s, it was in the late 1980s that a smokable form of methamphetamine with the street names 'ice', 'crystal' or 'glass' spread eastward from Hawaii and California and became increasingly popular, not only because of its strong and long-lasting stimulant properties but also because of its increasing availability, relatively inexpensive cost and multiple routes of administration (i.e., intravenous injection, 'snorting', ingestion and smoking) (Cho, 1990). Methamphetamine abuse has rapidly become a significant health problem since its long-term use may result in serious psychiatric (Sato et al., 1983; Iwanami et al., 1994), neurologic (Weiss et al., 1970; Yu et al., 1983; Rothrock et al., 1988), cardiovascular (Hong et al., 1991; Perez-Reyes et al., 1991) and gastrointestinal changes (Pecha et al., 1996). Also, there is the possibility that human abusers of this drug may be at risk for methamphetamine-induced dopamine neurotoxicity (Woolverton et al., 1989; Wilson et al., 1996; Villemagne et al., 1998).

The reinforcing and psychomotor stimulant effects of amphetamines have been attributed to activation of

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dopaminergic transmission, particularly in the nucleus accumbens (Hoebel et al., 1983; Carboni et al., 1989; Koob and Le Moal, 1997; Nestler and Aghajanian, 1997; Munzar et al., 1999). A substantial body of literature has established amphetamine-like stimulants as potent indirect agonists at dopaminergic synapses and this agonist activity arises from the combined ability of the drugs to release dopamine not only from presynaptic nerve terminals but also at the level of the cell body (Raiteri et al., 1975; Fischer and Cho, 1979; Cheramy et al., 1981; Butcher et al., 1988; Parker and Cubeddu, 1988), to block dopamine uptake (Harris and Baldessarini, 1973; Parker and Cubeddu, 1988) and, at high doses, to inhibit dopamine degradation by monoamine oxidase (Miller et al., 1980).

Due to it's escalating abuse, a clear need exists for laboratory procedures to evaluate motivational components of methamphetamine abuse and their underlying neurobiological mechanisms. One approach is the use of a 'yoked' self-administration procedure in which rats are run simultaneously in groups of three, with two rats serving as yoked controls that receive an injection of either drug or placebo which is not dependent on responding each time a response-dependent injection of drug is self-administered by a third rat. Using such a procedure, Di Ciano et al. (1996) found that dopamine oxidation currents in the nucleus accumbens were significantly greater when rats self-administered D-amphetamine, as compared to rats receiving the identical dose and pattern of yoked infusions. Similarly, Hemby et al. (1997) found that response-dependent administration of cocaine resulted in greater increases in the nucleus accumbens extracellular concentrations of dopamine than observed with response-independent administration, as estimated by in vivo microdialysis.

In the present experiment we used a yoked self-administration procedure to assess behavioral, as distinct from pharmacological, factors in determining neuroadaptations in the dopaminergic system of rats withdrawn for 24 h from chronic methamphetamine self-administration. This was done by first developing a set of conditions under which methamphetamine would be consistently self-administered over time at dose levels low enough to preclude neurotoxic effects. Subsequently, rats were studied in groups of three, with only one rat actively self-administering methamphetamine while the other two received yoked injections of either methamphetamine or saline.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (Charles River, Wilmington, MA) weighing approximately 300 g at the start of the experiment were individually housed in a temperature- and humidity-controlled environment under a 12-h light/dark cycle (lights on at 7:00 a.m.). In contrast to many previous investigations, rats had no operant history and food and water were available ad libitum in the home cage. Rats were trained and tested between 12:00 and 5:00 p.m. Animals used in this study were maintained in facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC) and all experimentation was conducted in accordance with the guidelines of the Institutional Care and Use Committee of the National Institute on Drug Abuse, NIH, and the Guide for Care and Use of Laboratory Animals, National Research Council Academy Press, Washington, D.C., 1996.

2.2. Drugs

S(+)-Methamphetamine hydrochloride was obtained from Research Biochemicals Int. (Natick, MA) and dissolved in sterile physiological saline. Injection speed was adjusted daily according to the weight of each rat in order to provide injections of 0.03, 0.056, 0.1, 0.3 or 0.56 mg/kg in a volume of 250 μl/kg over a 2-s period.

2.3. Surgery

Catheters were implanted into the right jugular vein under Equithesin (pentobarbital 9.72 mg/ml, chloral hydrate 44.4 mg/ml, propylene glycol 44%) anesthesia (3 ml/kg, i.p.). A small incision was made to the right of the midline of the neck and the external jugular vein was isolated and opened. A silastic catheter was inserted into the vein and anchored into the neck muscles by sutures. The other end of the catheter was threaded subcutaneously around the animal's back to exit the skin through a small opening near the midscapular region. A stylet cap was inserted into the distal end of the catheter protruding from the animal's back to prevent its clogging and maintain a closed system. Under the same anesthesia, a nylon bolt was fixed to the skull with dental cement anchored by stainless steel screws. The nylon screw thread served as a tether to protect the catheter from being pulled out while the rat was in the self-administration chamber. A minimum of 10 days of recovery was allowed before initiation of the experiments. On each day following surgery, catheters were flushed with a 0.1 ml saline solution containing heparin (1.25 units/ml) and gentamicin (0.16 mg/kg) to maintain their patency. Catheter patency was tested periodically, or whenever an animal displayed behavior outside baseline parameters, with the ultrashort-acting barbiturate anesthetic methohexital (10 mg/kg, i.v.) for loss of consciousness within 5 s. In addition, the patency of all catheters was verified at the end of the experiment.

2.4. Apparatus

Self-administration sessions were conducted in twenty standard operant chambers (Coulbourn Instruments, Allentown, PA) equipped with two nose-poke operanda. Nose-poke responses in one of the holes (defined as 'active')

resulted in drug delivery to the animal when schedule requirements were met, whereas nose pokes in the other hole (defined as 'inactive') were recorded but not reinforced. Each nose poke produced a brief feedback tone. A house light was on during drug availability but was turned off during the entire infusion and time-out periods. The injector system contained a fluid swivel (Alice King Chatham, Hawthorne, CA) mounted on top of each chamber. One end of the swivel was connected via polyethylene tubing encased in a protective stainless steel spring tether to the animal's catheter while the other end of the swivel was connected via polyethylene tubing to the infusion pump (Harvard Apparatus, Model 22, South Natick, MA). The operant chambers were enclosed in ventilated, sound-attenuating cubicles and controlled by an IBM compatible computer using the MED Associates MED-PC software package.

2.5. Acquisition and maintenance of methamphetamine self-administration at different doses

Rats were allowed to acquire self-administration of methamphetamine at one of three doses: 0.1, 0.3 or 0.56 mg/kg/injection. Sessions were conducted Monday to Friday and were 2 h in duration. At the beginning of each session, a priming injection (0.1, 0.3 or 0.56 mg/kg/injection) was automatically delivered. Once responding was initiated, the number of responses required to produce each injection was gradually increased over a two-week period to a final value of FR-5 (every fifth response produced an injection). Following each injection there was a 30-s time-out period during which responding was recorded but had no programmed consequences. After approximately six weeks, saline was substituted for methamphetamine for 8 days followed by testing of methamphetamine injection doses ranging from 0.03 to 0.3 mg/kg. Each dose of methamphetamine was tested for up to 8 sessions until responding did not vary more than 15% over the last 3 sessions.

2.6. Yoked self-administration procedure: contingent *vs.* noncontingent methamphetamine administration

After establishing parameters for acquisition and maintenance of methamphetamine self-administration (see above), 15 additional naive rats were run simultaneously in groups of three, with two rats serving as yoked controls which received an injection of either 0.1 mg/kg methamphetamine or saline which was not contingent on responding each time a response-contingent injection of 0.1 mg/kg methamphetamine was self-administered by the third paired rat. Unlike self-administering rats, nose-poke responses by the yoked rats were recorded but had no programmed consequences. After 5 weeks, rats self-administering methamphetamine and their littermates receiving yoked injections of methamphetamine or saline were sacrificed 24 h after the last session and densities of dopamine uptake sites and dopamine D_1 and D_2 receptors were measured in different brain regions.

2.7. Quantitative autoradiography

The brains were quickly removed and frozen by immersion in isopentane (−35°C) for 30 s and stored at −70°C until sections of 20 μm were cut on a cryostat (−20°C) and thaw-mounted on gelatin-chromate coated slides. The slides were desiccated and stored at −70°C until used for autoradiography.

2.7.1. Dopamine transporter assay

Sections were equilibrated at room temperature and incubated for 60 min at 25°C with 0.07 nM [^{125}I]RTI-121 ([^{125}I]-3β-[4-(trimethylstannyl]phenyl]-tropan-2β-carboxylic acid isopropyl ester) (2200 Ci/mmol) (DuPont NEN, Boston, MA) in a binding buffer consisting of 137 mM NaCl, 2.7 mM KCl, 10.14 mM Na_2HPO_4, 1.76 mM KH_2PO_4 and 10 mM NaI. After incubation, sections were washed twice (for 20 min each time) in ice-cold buffer, dipped in deionized ice-cold water and then dried with a stream of cool dry air. Nonspecific binding was defined by the addition of 10 μM GBR-12909 (1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-[3-phenylpropyl] piperazine dihydrochloride) (Research Biochemicals Int.). The dry labeled slides and plastic standards (^{125}I-labeled microscales, Amersham, Arlington Heights, IL) were apposed to radiosensitive films (Hyperfilm-βmax, Amersham) in light-tight cassettes for 2 days at 4°C.

2.7.2. Dopamine D_1 receptor assay

Sections were equilibrated at room temperature and preincubated in 50 mM Tris–HCl buffer (pH 7.4) containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 1 mM $MgCl_2$ at 25°C for 15 min to remove endogenous catecholamines. Sections were then incubated for 60 min at 25°C in buffer containing 4 nM [^{3}H]SCH 23390 (*N*-methyl-^{3}H-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride) (81.4 Ci/mmol) (DuPont NEN, Boston, MA) and 1 μM mianserin (Research Biochemicals Int.) to block 5-HT_2 receptors. After incubation, sections were washed twice (for 5 min each time) in ice-cold buffer, dipped in deionized ice-cold water and dried with a stream of cool dry air. Nonspecific binding was defined by the addition of 10 μM unlabeled *R*(+) SCH 23390 (*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride) (Research Biochemicals Int.). The dry labeled slides and plastic tritium standards (^{3}H-labeled microscales, Amersham) were apposed to tritium-sensitive films (Hyperfilm-^{3}H, Amersham) in light-tight cassettes for 7 days at room temperature.

2.7.3. Dopamine D_2 receptor assay

Sections were equilibrated at room temperature and preincubated in 50 mM Tris–HCl buffer (pH 7.4) contain-

ing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 1 mM $MgCl_2$ at 25°C for 15 min to remove endogenous catecholamines. Sections were then incubated for 30 min at 25°C in buffer containing 0.1 nM [^{125}I]Iodosulpride (2000 Ci/mmol) (Amersham) and 5 nM PD 128,907 (*S*(+)-(4a*R*, 10b*R*)-3,4,4a,10b-Tetrahydro-4-propyl-2*H*,5*H*-[1]benzopyrano-[4,3-*b*]-1,4-oxazin-9-ol hydrochloride) (Research Biochemicals Int.) to block D_3 receptors. After incubation, sections were washed twice (for 5 min each time) in ice-cold buffer, dipped in deionized ice-cold water and dried with a stream of cool dry air. Nonspecific binding was defined by the addition of 1 μM unlabeled domperidone (Research Biochemicals Int.). The dry labeled slides and plastic standards (^{125}I-labeled microscales, Amersham) were apposed to radiosensitive films (Hyperfilm-βmax, Amersham) in light-tight cassettes for 4 days at 4°C.

2.7.4. Densitometry

Films were developed in Kodak GBX developer and fixative according to manufacturer's instructions and autoradiograms were analyzed using a Macintosh computer-based image analysis system (National Institutes of Health, Image 1.58 software program). Quantitation of brain images was performed using standard curves generated from the ^{125}I- and ^{3}H-labeled microscales (Amersham). Anatomical regions were defined according to Paxinos and Watson (1986).

2.8. Immunohistochemistry

Slides were brought to room temperature and fixed in 4% paraformaldehyde (in 0.1 M phosphate buffer) for 10 min. Sections were then washed 4×15 min in 50 mM Tris-buffered saline (TBS; pH 7.6) before being incubated in 1% H_2O_2 and 100% methanol for 10 min. After washing with TBS (4×5 min), the slide-mounted sections were incubated in 3% normal goat serum and 0.2% Triton X-100 in TBS for 1 h, and then exposed to a rabbit polyclonal antibody (diluted 1:10000 in TBS containing 1% normal goat serum) specific for tyrosine hydroxylase (Eugene Tech International, Ridgefield Park, NJ) for 18 h at room temperature. After incubation with the primary antibody, sections were washed for 3×5 min in 50 mM TBS before being incubated with biotinylated goat anti-rabbit secondary IgG (diluted 1:200 in TBS; Vector Laboratories, Burlingame, CA) for 1.5 h at room temperature. Following washes in TBS (4×15 min), the sections were incubated with ABC reagents (Vector Laboratories) for 2 h. Tyrosine-hydroxylase-immunopositive neurons were visualized by 3,3′-diaminobenzidine, nickel and H_2O_2 using a commercially available kit (DAB substrate kit, Vector Laboratories). Negative controls were run using normal goat serum instead of the primary antibody. Tyrosine-hydroxylase-immunoreactive neurons in the substantia nigra zona compacta and ventral tegmental area were counted manually on 4 slices per each animal ($n = 5$ rats/group) using a Zeiss microscope at a magnification of $\times 400$ with a superimposed grid. The person counting cells was blind to animal treatment. Cell size and relative density of the tyrosine-hydroxylase-positive neurons in the substantia nigra zona compacta and ventral tegmental area were assessed on 10 tyrosine-hydroxylase-positive cells per each animal ($n = 5$ rats/group) using the microscope at a magnification of $\times 630$ and a Macintosh computer-based image analysis system (Image 1.58, NIH). Only tyrosine-hydroxylase-positive neurons with a complete nucleus in the section were assessed and outlined with the screen cursor driven by a hand-held mouse. This allowed for the measurement of neuron size and relative density of tyrosine-hydroxylase-staining. Relative density of tyrosine-hydroxylase-immunoreactive fibres was also measured in four representative areas of the striatum and two representative areas of the nucleus accumbens (500 μm × 500 μm) per each rat using the microscope at a magnification of $\times 400$ and Image 1.58, NIH. All sections were processed at the same time under the same conditions.

2.9. Statistical analysis

For self-administration, data are presented as the group mean, and bars show the standard error of the mean. Data were analyzed using multifactorial analysis of variance (ANOVA) for repeated measures and post-hoc Student's *t*-test comparisons were performed to locate differences between groups means. For quantitative autoradiography, data are presented as mean ± S.E.M. percentage of change compared to the yoked-methamphetamine group. The error bars represent mean ± S.E.M. of individual values expressed as a percent of the corresponding mean value for the yoked-methamphetamine group. Since our autoradiography data do not appear to originate from normally distributed populations with equal variances and sample sizes are small ($n = 5$) the significance of the difference between means was determined with a Mann–Whitney *U*-test. For immunohistochemistry, the values for number or size of tyrosine-hydroxylase-positive neurons in the substantia nigra zona compacta and ventral tegmental area are presented as the group mean, and bars show the standard error of the mean. The significance of the difference between means was determined with a Student's *t*-test.

3. Results

3.1. Acquisition and maintenance of methamphetamine self-administration

For the data from the 0.1 mg/kg/injection dosage group (data not shown), a two-factor ANOVA for repeated measures revealed significant effects between active and inactive hole responding [$F(1,461) = 448.05$, $P < 0.001$] over the 33 sessions [$F(32,461) = 5.36$, $P < 0.001$]. In

addition, there was an overall significant interaction between nose-poke responding and sessions [$F(32,461) = 6.24$, $P < 0.001$]. Post-hoc analysis revealed that a significant preference for the active hole occurred on sessions 22–33 ($P < 0.01$). When saline was substituted for methamphetamine (sessions 34–39), the number of active nose-pokes decreased progressively over sessions. A one-factor ANOVA for repeated measures indicated significant differences on sessions 36, 37, 38 and 39 of extinction [$F(5,35) = 4.64$, $P < 0.01$]. Substitution of saline for methamphetamine did not produce any significant change in the number of inactive nose-pokes [$F(5,35) = 1.19$, n.s.].

For the data from the 0.3 mg/kg/injection dosage group (data not shown), a two-factor ANOVA for repeated measures revealed significant effects between active and inactive hole responding [$F(1,461) = 1314.9$, $P < 0.001$] over the 33 sessions [$F(32,461) = 7.8$, $P < 0.001$]. In addition, a significant interaction between nose-poke responding and sessions was found [$F(32,461) = 10.73$, $P < 0.001$]. Post-hoc analysis revealed that a significant preference for the active hole occurred on sessions 7–33 ($P < 0.01$). Substitution of saline for methamphetamine (sessions 34–41) did not produce any significant change in the number of active [$F(7,47) = 0.98$, n.s.] and inactive nose-pokes [$F(7,47) = 0.74$, n.s.]. Following the extinction test, animals were given access to methamphetamine at the dose of 0.1 mg/kg/injection (sessions 42–52). A two-factor ANOVA for repeated measures indicated a significant effect between active and inactive hole responding [$F(1,153) = 93.83$, $P < 0.001$]. The effect of sessions [$F(10,153) = 0.46$, n.s.] as well as the interaction between nose-poke responding and sessions [$F(10,153) = 0.5$, n.s.] failed to reach significance. Post-hoc analysis revealed that a significant preference for the active hole occurred on sessions 42, 43, 46 and 48–52. When saline was substituted for methamphetamine again (sessions 53–64), a one-factor ANOVA for repeated measures did not reveal any significant changes in the number of active [$F(11,83) = 0.72$, n.s.] and inactive nose-pokes [$F(11,83) = 0.83$, n.s.].

For the data from the 0.56 mg/kg/injection dosage group (data not shown), a two-factor ANOVA for repeated measures did not reveal significant effects between active and inactive hole responding [$F(1,461) = 2.29$, n.s.] over the 33 sessions [$F(32,461) = 1.08$, n.s.]. In addition, no significant interaction between nose-poke responding and sessions was found [$F(32,461) = 1.13$, n.s.]. Substitution of saline for methamphetamine (sessions 34–41) did not produce any significant change in the number of active [$F(7,47) = 0.6$, n.s.] and inactive nose-pokes [$F(7,47) = 0.36$, n.s.]. Following the extinction test, animals were given access to methamphetamine at the dose of 0.1 mg/kg/injection (sessions 42–52). A two-factor ANOVA for repeated measures did not reveal a significant effect between active and inactive hole responding [$F(1,153) = 0.4$, n.s.]. The effect of sessions [$F(10,153) = 0.21$, n.s.] as well as the interaction between nose-poke responding and sessions [$F(10,153) = 0.44$, n.s.] failed to reach significance. When saline was substituted for methamphetamine again (sessions 53–64), a one-factor ANOVA for repeated measures did not reveal any significant changes in the number of active [$F(11,83) = 0.65$, n.s.] and inactive nose-pokes [$F(11,83) = 0.71$, n.s.].

3.2. Dose-response curves for methamphetamine self-administration

Fig. 1 shows the dose-response functions for methamphetamine self-administration maintained under the final FR-5 (fixed-ratio 5) schedule of reinforcement. With the 0.1 mg/kg/injection training-dose regimen (left panel), the dose-effect curve for methamphetamine had an inverted-U shape and maximal responding in the active hole occurred at the 0.1 mg/kg/injection dose [$F(4,77) = 9.28$, $P < 0.001$]. Individual mean comparisons with 0.1 mg/kg/injection-dose data revealed significant decreases in responding for methamphetamine at 0.3 and 0.03 mg/kg/injection and for saline ($P < 0.01$). No statistically significant difference was observed in the number of inactive nose-pokes (data not shown).

With the 0.3 mg/kg/injection training-dose regimen (right panel), a one-factor ANOVA for repeated measures on the number of active-hole responses revealed a significant effect of dose [$F(4,90) = 4$, $P < 0.01$]. Maximal responding occurred at an injection dose of 0.1 mg/kg dose and a higher dose of 0.3 mg/kg/injection methamphetamine did not maintain responding above saline extinction levels. Post-hoc comparisons with the 0.1 mg/kg/injection-dose data revealed significant decreases in responding for methamphetamine at 0.3, 0.056 and 0.03 mg/kg/injection and for saline ($P < 0.01$). No statistically significant difference was observed in the number of inactive nose-pokes (data not shown).

3.3. Yoked self-administration procedure: contingent vs. noncontingent methamphetamine administration

Fig. 2 shows the average number of active and inactive hole responses for rats actively self-administering methamphetamine and rats receiving yoked injections of either methamphetamine or saline. The number of responses required to produce each injection was increased over days, reaching a final value of 5 (fixed-ratio 5 schedule of drug injection; FR-5) by the 13th session of training.

For the data from the methamphetamine self-administration group (left panel), a two-factor ANOVA for repeated measures revealed significant effects between active and inactive hole responding [$F(1,249) = 331.55$, $P < 0.001$] over the 25 sessions [$F(24,249) = 4.93$, $P < 0.001$]. In addition, there was an overall significant interaction between nose-poke responding and sessions [$F(24,249) =$

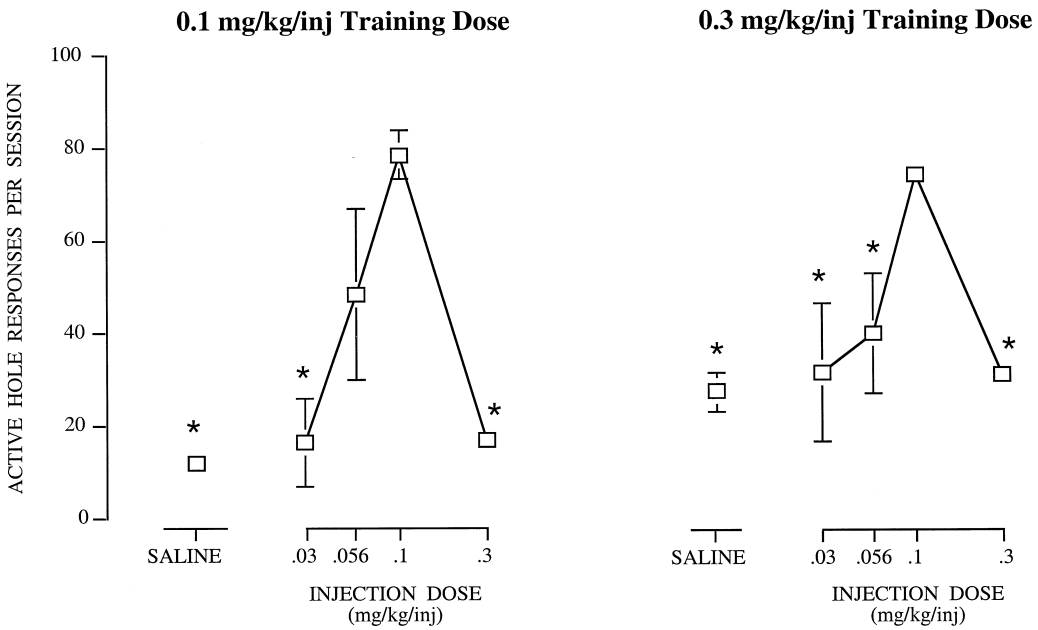

Fig. 1. Dose-response curves for methamphetamine self-administration under the FR-5, time-out 30-s schedule of reinforcement. Each point represents the mean (±S.E.M.) number of nose-poke responses in the active hole over the last three sessions of testing at a particular dose of methamphetamine or saline. Each dose of methamphetamine was tested for up to 8 sessions until responding did not vary more than 15% over the last 3 sessions. Asterisks (*) denote significant differences ($P < 0.01$) in the number of active hole responses from the 0.1 mg/kg/injection unit dose. Sample size was $n = 7$ in both the 0.1 mg/kg/injection training group and the 0.3 mg/kg/injection training group.

6.01, $P < 0.001$]. Post-hoc analysis revealed that a significant preference for the active hole occurred on sessions 8–25 ($P < 0.01$).

For the data from the yoked-methamphetamine group (middle panel), a two-factor ANOVA for repeated measures indicated a significant effect of sessions [$F(24,249) = 2.75$, $P < 0.001$]. The effect between active and inactive hole responding [$F(1,249) = 2.12$, n.s.] as well as the interaction between nose-poke responding and sessions [$F(24,249) = 0.29$, n.s.] failed to reach significance.

For the data from the yoked-saline group (right panel), a two-factor ANOVA for repeated measures indicated a

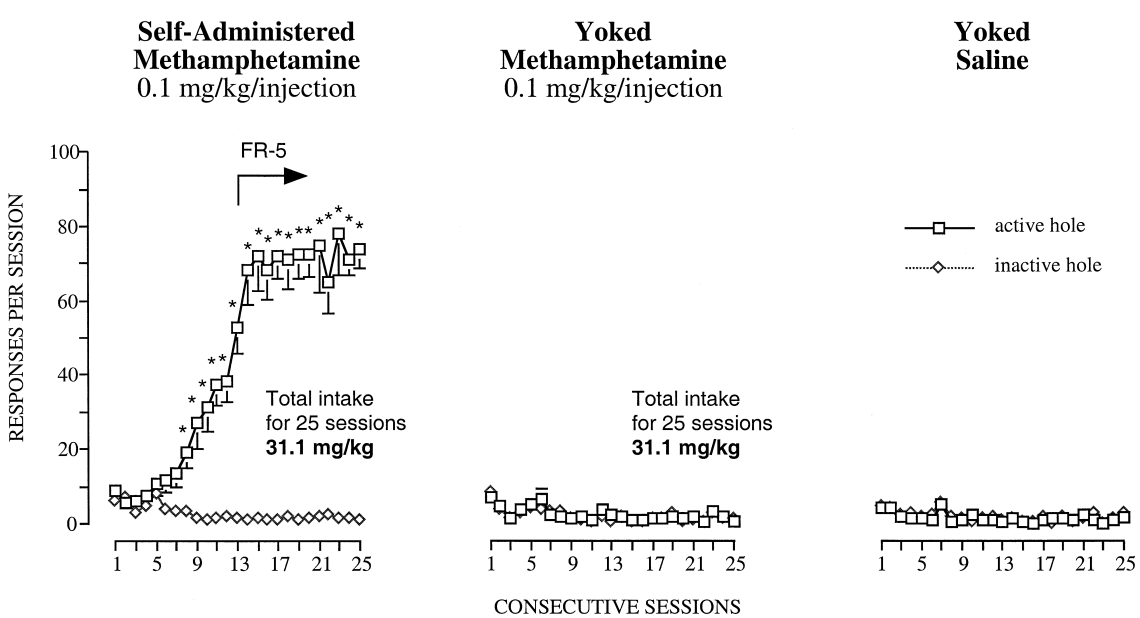

Fig. 2. The mean number (±S.E.M.) of responses in the active and inactive holes for rats that were allowed to acquire self-administration of methamphetamine at a dose of 0.1 mg/kg/injection ($n = 5$) and their littermates that received yoked infusions of methamphetamine ($n = 5$) or saline ($n = 5$) during each of the daily 2 h sessions. The arrow indicates the period when methamphetamine self-administration was maintained under the final FR-5 schedule of reinforcement. Asterisks (*) denote significant differences ($P < 0.01$) between active and inactive nose-pokes.

significant effect of sessions [$F(24,249) = 2.75$, $P < 0.001$]. The effect between active and inactive hole responding [$F(1,249) = 0.4$, n.s.] as well as the interaction between nose-poke responding and sessions [$F(24,249) = 0.39$, n.s.] failed to reach significance.

3.4. Neuroadaptations to methamphetamine self-administration: contingent vs. noncontingent infusions of drug

Fig. 3 shows the effect of withdrawal from chronic methamphetamine self-administration for 24 h on dopamine transporters and dopamine D_1 and D_2 receptors in various brain regions. Representative autoradiograms of [^{3}H] SCH-23390-labeled dopamine D_1 receptors and [^{121}I]Iodosulpride-labeled dopamine D_2 receptors are shown in Fig. 4. Only actively self-administered methamphetamine produced a decrease in somatodendritic dopamine D_2 autoreceptor levels in the ventral tegmental area (34%) ($P < 0.01$) and medial (31%) ($P < 0.01$) and dorsal (21%) ($P < 0.01$) part of the substantia nigra zona compacta with a corresponding down-regulation of dopamine D_1 receptors in the shell of the nucleus accumbens (15%) ($P < 0.05$). No changes in dopamine transporter or dopamine D_1 and D_2

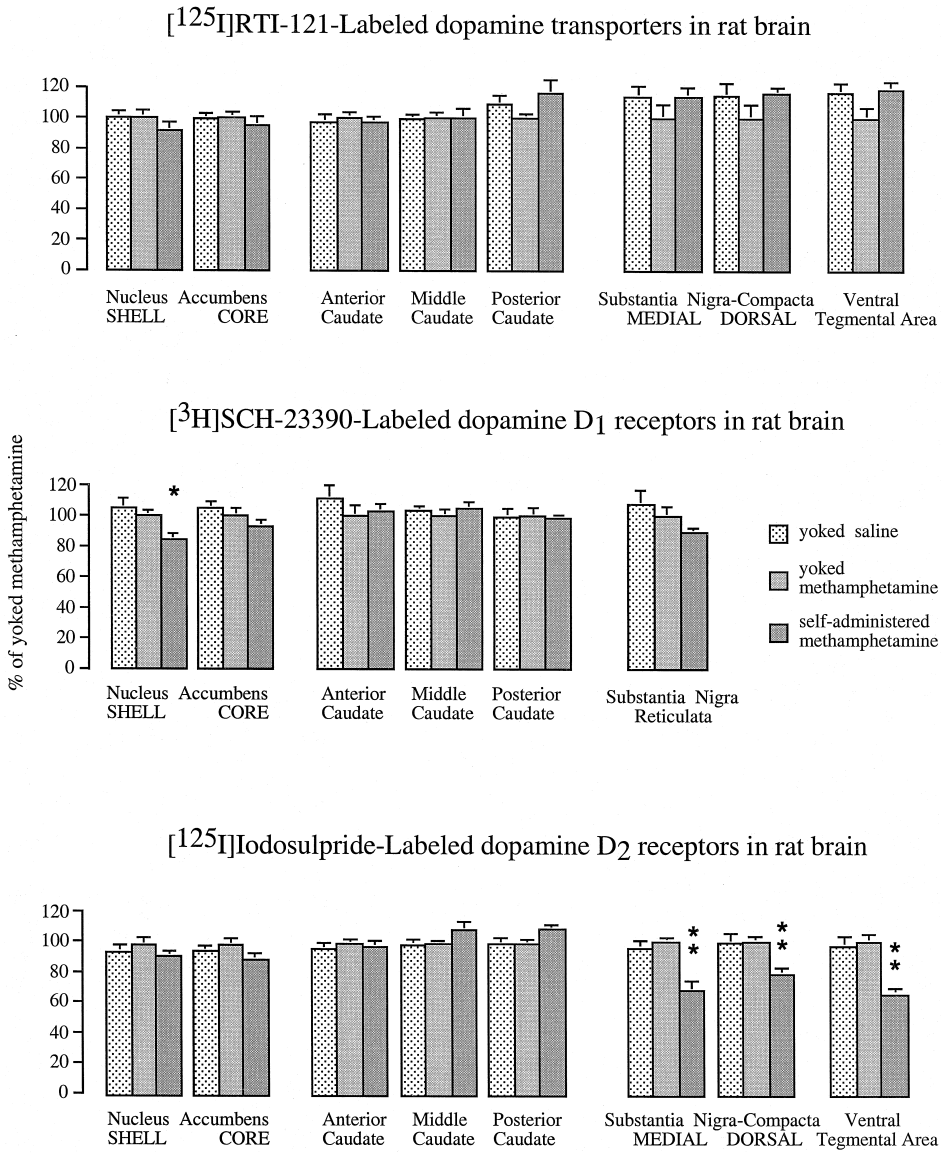

Fig. 3. Effects of withdrawal from chronic methamphetamine self-administration for 24 h on dopamine transporters, dopamine D_1 receptors and dopamine D_2 receptors in various brain regions. Data are presented as mean (±S.E.M.) percentage of change compared to the yoked-methamphetamine group. Sample sizes are as follows: yoked-saline group ($n = 5$), yoked-methamphetamine group ($n = 5$), methamphetamine self-administration group ($n = 5$). The significance of the difference between means determined with a Mann–Whitney U-test were $^*P < 0.05$ and $^{**}P < 0.01$. The self-administration group was compared with the yoked-methamphetamine group and the latter with the yoked-saline group.

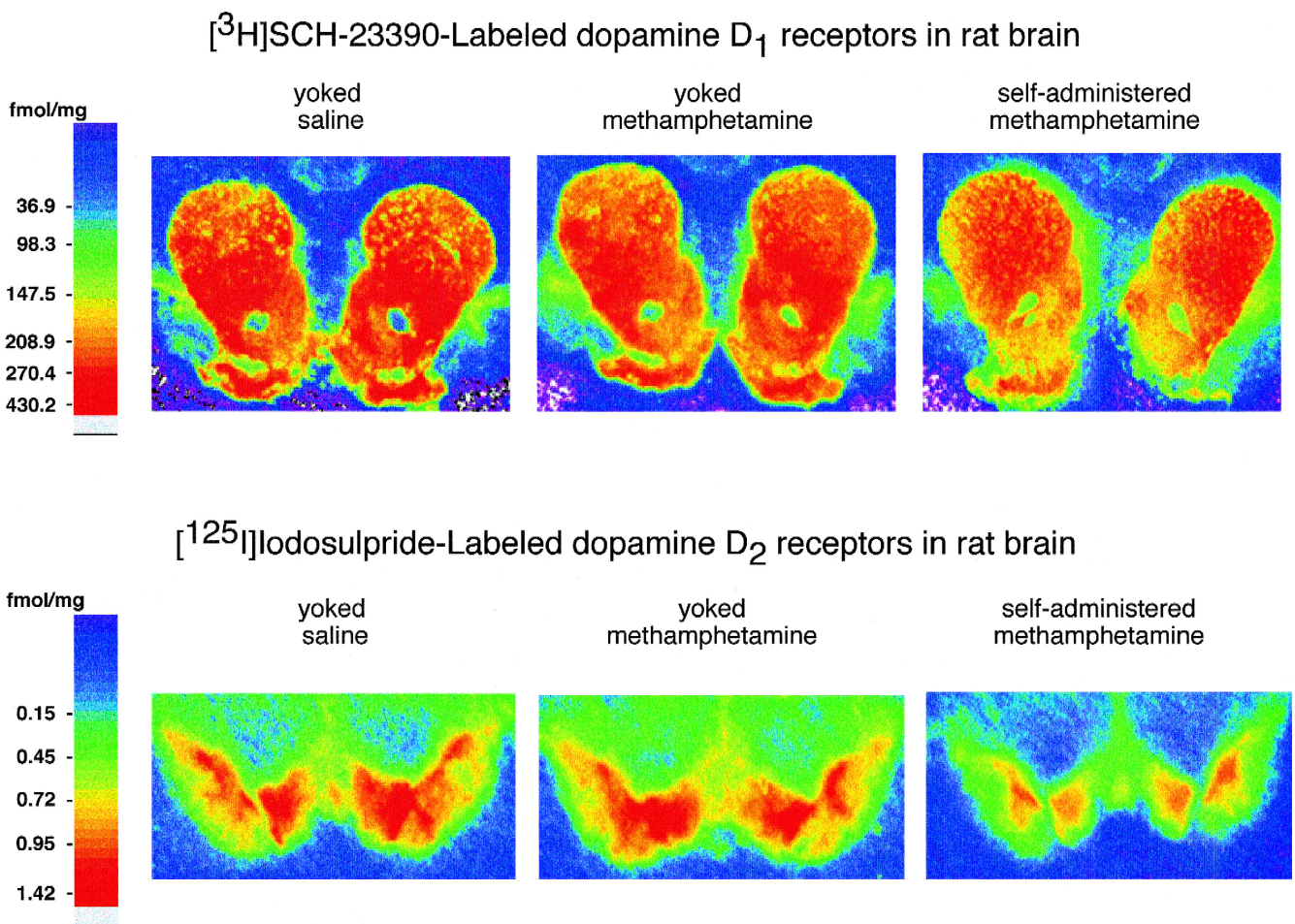

Fig. 4. Representative autoradiograms of striatal [^{3}H]SCH-23390-labeled dopamine D_1 receptors and [^{121}I]Iodosulpride-labeled dopamine D_2 receptors in the substantia nigra zona compacta and ventral tegmental area of rats actively self-administering methamphetamine and rats receiving yoked injections of either methamphetamine or saline. Quantitative data are presented in Fig. 3.

receptor density were detected in the dorsomedial, dorsolateral, ventrolateral and ventromedial subdivisions of the three rostrocaudal levels of the striatum examined in rats self-administering methamphetamine compared with littermates receiving yoked infusions of either methamphetamine or saline (data not shown). The mean [^{125}I]RTI-121 binding in the shell and core of the nucleus accumbens, anterior, middle and posterior caudate, medial and dorsal part of the substantia nigra zona compacta and ventral tegmental area regions of the yoked-methamphetamine group were 1.84 ± 0.1, 2.82 ± 0.11, 3.63 ± 0.14, 3.55 ± 0.13, 2.38 ± 0.13, 1.68 ± 0.12, 1.38 ± 0.15, and 1.9 ± 0.15 fmol/mg tissue, respectively. The mean [^{3}H]SCH-23390 binding in the shell and core of the nucleus accumbens, anterior, middle and posterior caudate and substantia nigra zona reticulata regions of the yoked-methamphetamine group were 234.9 ± 8.9, 275.9 ± 13.8, 274.2 ± 16.1, 266.9 ± 14.9, 189.6 ± 11.2, and 168.1 ± 10.8 fmol/mg tissue, respectively. The mean [^{125}I]Iodosulpride binding in the shell and core of the nucleus accumbens, anterior, middle and posterior caudate, medial and dorsal part of the substantia nigra zona compacta and ventral tegmental area regions of the yoked-methamphetamine group were 2.39 ± 0.14, 2.9 ± 0.11, 3.7 ± 0.11, 3.53 ± 0.1, 2.64 ± 0.1, 1.22 ± 0.1, 0.98 ± 0.12, and 1.18 ± 0.13 fmol/mg tissue, respectively.

3.5. Tyrosine hydroxylase immunohistochemistry in ventral tegmental area and substantia nigra zona compacta

Fig. 5 shows representative photomicrographs of tyrosine-hydroxylase-immunoreactive neurons in the ventral tegmental area and substantia nigra zona compacta of rats actively self-administering methamphetamine and rats receiving yoked injections of either methamphetamine or saline. The quantitative data are presented in Table 1. No changes in cell number or size of tyrosine-hydroxylase-immunoreactive dopamine neurons in the substantia nigra zona compacta and ventral tegmental area were detected in rats self-administering methamphetamine compared with littermates receiving yoked infusions of either metham-

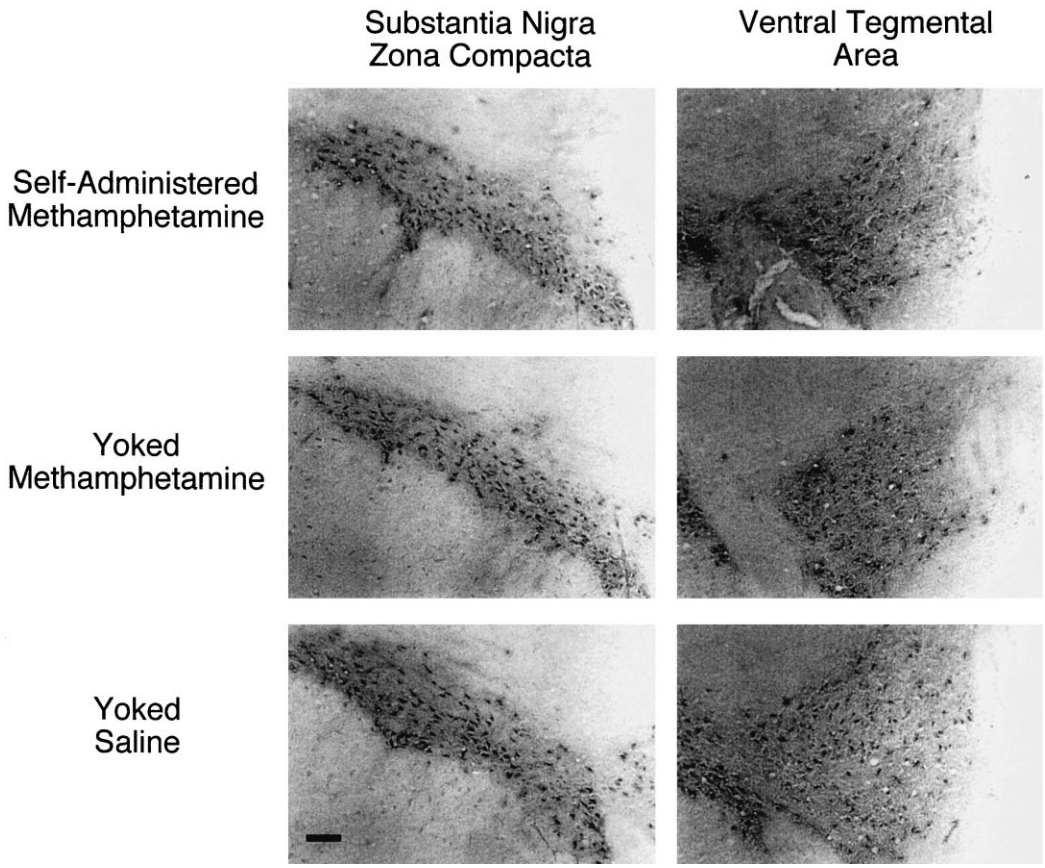

Fig. 5. Representative photomicrographs of tyrosine-hydroxylase-immunoreactive neurons in the ventral tegmental area and substantia nigra zona compacta of rats actively self-administering methamphetamine and rats receiving yoked injections of either methamphetamine or saline. The quantitative data are presented in Table 1. Scale Bar = 100 μm.

phetamine or saline. Relative densities of accumbens and striatal tyrosine-hydroxylase-immunoreactive fibres were not significantly different in the three groups of rats (data not shown).

Table 1
Effects of contingent vs. noncontingent infusions of methamphetamine on the number and size of tyrosine hydroxylase-immunoreactive cells in the substantia nigra zona compacta and ventral tegmental area

	Substantia nigra zona compacta	Ventral tegmental area
Cell number		
Self-administered methamphetamine	104.33 ± 7.14	79.23 ± 7.45
Yoked methamphetamine	101.45 ± 5.89	83.56 ± 5.23
Yoked saline	106.34 ± 6.54	86.24 ± 6.87
Cell size (μm^2)		
Self-administered methamphetamine	189.54 ± 6.16	170.75 ± 6.49
Yoked methamphetamine	191.15 ± 6.03	172.90 ± 6.42
Yoked saline	183.63 ± 4.95	170.21 ± 5.47

For cell number, the values represent means ± S.E.M. for 20 slices from five rats per each group. For cell size, the values represent mean ± S.E.M. for 50 neurons from five rats per each group. The significance of the difference between means was determined with a Student's *t*-test. The self-administration group was compared with the yoked-methamphetamine group and the latter with the yoked-saline group.

4. Discussion

4.1. Reinforcing effects of methamphetamine

The present data confirm earlier reports that methamphetamine can serve as a positive reinforcer in rats via the intravenous route of administration (Pickens et al., 1967; Yokel and Pickens, 1973; Munzar et al., 1999). With both the 0.1 and 0.3 mg/kg/injection training-dose regimen, self-administration of methamphetamine was acquired, as measured by selective responding in the active vs. the inactive hole, and subsequently maintained by rats. Following the initial acquisition period with training doses of 0.1 and 0.3 mg/kg/injection, rats showed stability in their daily intake and there were no indications of any further changes in sensitivity (tolerance or sensitization) to methamphetamine's reinforcing effects as would be indicated by an increase or a decrease in the rate of self-administration. It was interesting that rats failed to acquire self-administration of methamphetamine with the high 0.56 mg/kg/injection training-dose regimen. This did not appear to be due to a masking effect resulting from response-rate suppressant effects of this high dose of

methamphetamine, since rats did not increase their responding in the active hole when injection dose of methamphetamine was reduced to 0.1 mg/kg.

Event records of fixed-ratio methamphetamine self-administration showed variations in intake within a session; methamphetamine self-administration was characterized by a burst of 5–8 injections taken within the first 30 min of the session followed by alternating irregular periods of drug self-administration and abstinence during the remainder of the 2-h session. A similar pattern of responding was found by Munzar et al. (1999) using the same testing conditions as the present experiment but with a 0.06 mg/kg/injection training dose of methamphetamine. This pattern of responding within sessions can be accounted for by the long duration of action of methamphetamine and may reflect appearance of other direct behavioral effects of methamphetamine, such as stereotypy, that would follow ingestion of large amounts of drug. Indeed, at the higher training dose of 0.3 mg/kg/injection in the present experiment, with a daily average intake of 2 mg/kg methamphetamine, hyperactivity with stereotypical behaviors, including mild sniffing, licking and head bobbing, was observed at the end of each session. However, in rats with 0.1 mg/kg/injection (present experiment) or 0.06 mg/kg/injection (Munzar et al., 1999) training doses of methamphetamine, average daily intakes were lower (1.4 and 1.21 mg/kg, respectively) and the alterations in behavioral activities described above were less profound.

Nose-poke responding was a function of contingent methamphetamine injection because (a) at an optimal injection dose, methamphetamine injections maintained higher rates of responding than saline injections, (b) there was selective responding in an active vs. an inactive hole, (c) responding was sensitive to changes in methamphetamine dose, and finally (d) with the pairs of subjects, the rate of responding of the animal actually self-administering methamphetamine was significantly higher than that of a paired animal which passively received an injection whenever the first animal self-administered methamphetamine. Although the high injection dose of 0.3 mg/kg methamphetamine did not maintain self-administration behavior above saline extinction levels, it did appear to be acting as an effective reinforcer, as evidenced by the rapid development of selective responding in the active hole and the increase in responding when dose per injection was lowered.

Thus, methamphetamine served as a positive reinforcer of self-administration behavior with maximal responding occurring at an injection dose of 0.1 mg/kg under the present experimental conditions.

4.2. Neuroadaptations to methamphetamine self-administration: contingent vs. noncontingent infusions of drug

To investigate the neurobiological correlates of actively self-administered methamphetamine, in contrast to methamphetamine administered passively at the same dose and frequency, we utilized a 'yoked' procedure in which paired animals simultaneously received a drug or placebo injection whenever active drug-seeking responses by one member of the group resulted in a self-administered injection. Only actively self-administered methamphetamine produced a decrease in somatodendritic dopamine D_2 autoreceptor levels in the ventral tegmental area (34%) and medial (31%) and dorsal (21%) part of the substantia nigra zona compacta with a corresponding down-regulation of dopamine D_1 receptors in the shell of the nucleus accumbens (15%). Since yoked control rats received identical doses of methamphetamine at identical times, we conclude that all neuroadaptations in the dopaminergic system were related to the active process of reinforced responding and not to the chronic administration of methamphetamine per se. These results are consistent with findings from previous in vivo chronoamperometry studies demonstrating that dopamine oxidation currents in the nucleus accumbens were significantly greater when rats self-administered D-amphetamine, as compared to rats receiving the identical dose and pattern of yoked infusions (Di Ciano et al., 1996). Similarly, response-dependent administration of cocaine resulted in greater increases in the nucleus accumbens extracellular concentrations of dopamine than observed with response-independent administration, as estimated by in vivo microdialysis (Hemby et al., 1997).

The results of the present experiments also demonstrate that downregulated somatodendritic dopamine D_2 autoreceptors may be crucial for the reinforcing effects of methamphetamine. These changes may be due to functional hyperactivity of mesostriatal neurons, since dopamine released from cell bodies and/or dendrites in the ventral tegmental area and substantia nigra zona compacta exerts a negative control on mesolimbic and nigrostriatal dopaminergic pathways by activating somatodendritic dopamine D_2 autoreceptors (Kalivas and Duffy, 1991; Santiago and Westerink, 1991). It has long been accepted that chronic treatment with psychostimulants can produce increases in drug-induced dopamine release, not only at the nerve terminals in the nucleus accumbens and striatum (Carboni et al., 1989; Robinson and Camp, 1990; Kuczenski et al., 1991; Kalivas and Duffy, 1993; Yokoo et al., 1994), but also at dendrites in the ventral tegmental area and substantia nigra (Paden et al., 1976; Cheramy et al., 1981; Bradberry and Roth, 1989; Robertson et al., 1991; Timmerman and Abercrombie, 1996). Data from electrophysiological studies strongly suggest that both amphetamine and its derivative 3,4-methylenedioxymethamphetamine (MDMA) can stimulate somatodendritic dopamine release from dopamine cells in the ventral tegmental area and substantia nigra zona compacta (as they do from dopamine terminals in the nucleus accumbens) which could, by acting on the impulse-regulating somatodendritic dopamine D_2 autoreceptors, inhibit the firing of the cells (Wang, 1981; Kelland et al., 1989; Wolf et al.,

1993; Gifford et al., 1996). In the early stages of withdrawal from chronic amphetamine or cocaine exposure, a transient increase in basal activity of ventral–tegmental-area dopamine neurons is accompanied by a subsensitivity of somatodendritic dopamine D_2 autoreceptors (Kamata and Rebec, 1984; White and Wang, 1984; Henry et al., 1989; Ackerman and White, 1990; Wolf et al., 1993). Our results in rats withdrawn from chronic passive exposure to methamphetamine for 24 h indicate that this subsensitivity does not involve a down-regulation of dopamine D_2 autoreceptors. However, in rats actively self-administering methamphetamine, downregulated dopamine D_2 autoreceptors may result from overstimulation of the ventral tegmental area and substantia nigra zona compacta dopamine D_2 autoreceptors related to reinforcement processes and not simply to chronic methamphetamine exposure itself.

In addition to this local feedback mechanism, long-loop neuronal feedback pathways can also come into play. Several studies have demonstrated anatomical and functional differences in the core and shell regions of the nucleus accumbens with respect to dopamine neurotransmission (Heimer et al., 1991; Jones et al., 1996). There also appear to be marked differences in response to administration of amphetamines in different regions of the nucleus accumbens. For example, acute intravenous administration of low doses of amphetamine or cocaine to rats by Pontieri et al. (1995) preferentially increased extracellular dopamine in the shell, whereas at higher doses there were no differences in the concentration of dopamine in dialysates from the shell and core regions. Given that accumbens dopamine D_1 receptors are preferentially expressed by GABA-ergic neurons projecting to the ventral tegmental area (Yim and Mogenson, 1980; Heimer et al., 1991; Le Moine and Bloch, 1995; Lu et al., 1998), decreases in dopamine D_1 receptor binding density in the shell of the nucleus accumbens may produce an increase in dopamine cell firing by attenuating the dopamine D_1 receptor-mediated feedback inhibition of ventral–tegmental-area dopamine neurons.

To exclude the possibility that neuroadaptations in the dopaminergic system may represent a loss of dopaminergic neurons in the cell body and terminal regions as a result of methamphetamine-induced neurotoxicity, we investigated the effects of the 5-week exposure to methamphetamine on dopamine transporters and tyrosine-hydroxylase-immunoreactive neurons in the ventral tegmental area and substantia nigra zona compacta. Densities of dopamine transporters in the cell body and terminal regions were not significantly different in rats self-administering methamphetamine compared with littermates receiving yoked infusions of either methamphetamine or saline. No changes in cell number and size of tyrosine-hydroxylase-immunoreactive dopamine neurons in the substantia nigra zona compacta and ventral tegmental area, as well as in relative density of accumbens and striatal tyrosine-hydroxylase-immunoreactive fibres, were observed in the three groups of rats. This suggests that neuroadaptations in the dopaminergic system were due not to neurotoxicity but rather to functional hyperactivity of mesostriatal neurons.

In conclusion, there were clear neurobiological consequences in animals actively self-administering methamphetamine that were not present in paired littermates passively receiving identical amounts of methamphetamine under identical circumstances at the same time and frequency. Also, limiting daily methamphetamine intake through the use of relatively short sessions, timeout periods, and a requirement of multiple responses to produce an injection, likely prevented development of neurotoxicity which might have masked the observed neuroadaptations. Thus, the neurobiological changes we observed cannot be solely due to direct pharmacological actions of methamphetamine. Instead, they likely reflect motivational states that were present when methamphetamine injection depended on active drug-seeking behavior. Since neuroadaptations differ in animals exposed to actively self-administered methamphetamine vs. passively administered methamphetamine at exactly the same time and dose and circumstances, the use of active self-administration procedures may be critical to elucidation of neurobiological mechanisms underlying abuse of methamphetamine or other drugs.

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