INTRACEREBRAL SELF-ADMINISTRATION OF AMPHETAMINE BY RHESUS MONKEYS

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Four rhesus monkeys received intracranial implantation of cannulae aimed at the orbitofrontal cortex. Electrical intracranial self-stimulation was obtained readily from insulated electrodes placed temporarily into the orbitofrontal cortex via the outer guide tubes. Subsequently, each subject acquired a panel press operant response to deliver 0.05 µl of p-amphetamine (10^-6 M) into the orbitofrontal cortex via an inner cannula. The rate of panel pressing increased over several daily test sessions and extinguished after substitution of the vehicle solution. One animal responded readily on a fixed ratio-10 schedule. Control injections into the nucleus accumbens and lateral ventricles failed to maintain self-administration behavior.

Two complementary techniques are used extensively to study the neurochemical substrates of reward. One approach examines the effects of drugs on intracranial self-stimulation (ICS). This behavior is facilitated by dopamine (DA) receptor agonists such as amphetamine and inhibited by neuroleptics that serve as DA antagonists [4]. Recent data confirm that the rate-increasing effect of amphetamine on ICS is due to a selective influence on the ascending DA pathways [12]. A second technique involves i.v. self-administration of drugs with known neuropharmacological properties. Psychomotor stimulants (i.e. amphetamine and cocaine) and opiates are among the many compounds self-administered by a variety of species ranging from rat to monkey [18]. A large body of pharmacological data suggests an important role for DA in mediating the rewarding effects of amphetamine and cocaine [19]. These effects, in turn, may be related to the block of DA reuptake by both compounds [2, 17]. Consistent with this hypothesis is the blockade of cocaine self-administration by neurotoxic lesions of the mesocorticolimbic DA pathway at the level of the nucleus accumbens [16].

As an alternative to the procedures described above, Olds et al. have employed...
intracerebral microinjections of drugs in conjunction with behavioral tests for positive reinforcement [10]. The initial study screened a variety of drugs but the results were not conclusive. However, subsequent studies have reported intracerebral self-injection of opiates into the lateral hypothalamus by rats [11]. Recently, microinjection of noradrenaline has been used successfully to reinforce choice behavior in a T-maze [3]. Intracerebral microinjection of opiates into the ventral tegmental area also provided positive reinforcement as indicated by a conditioned reinforcement paradigm [13]. The following experiments summarize recent attempts to extend the use of microinjection procedures to study the neurochemistry of reward in primates.

Four male rhesus monkeys (Macaca mulatta) were anesthetized with pentobarbital sodium prior to stereotaxic surgery. Two animals (Rhesus 1 and 2) had a 22-gauge stainless steel tube implanted unilaterally into the orbitofrontal cortex. The orbitofrontal cortex of the rhesus monkey contains axon terminals of the mesocorticolimbic DA neurons and supports high rates of ICS by this species [15]. Two other subjects (Rhesus 3 and 4) had eight 22-gauge guide cannulas implanted bilaterally into the orbitofrontal cortex, head of the caudate nucleus, nucleus accumbens and lateral ventricle. Each cannula was kept patent by insertion of a metal stylet. Between behavioral tests, the animals were housed individually in a climatically controlled colony room. All testing was conducted in a quiet room with the monkeys sitting in a primate chair.

The initial tests were conducted with Rhesus 1 and 2. A length of tubing insulated except at the tip was passed through the guide cannula into the orbitofrontal cortex, permitting it to serve as a stimulating electrode when connected to a Grass S44 stimulator. Brain-stimulation consisted of a 0.5 sec train of 0.5 msec square wave pulses (100 Hz) at an intensity of 0.2–2.0 mA. The animals were trained to self-stimulate by touching the end of a metal bar located 20 cm in front of the chair at shoulder height. ICS behavior was acquired during the first 5 min of the test and responding stabilized at rates in excess of 50/min. Subsequent testing for intracerebral self-administration of amphetamine at the same loci employed this operant response to activate an infusion pump calibrated to inject 0.05 μl/sec of fluid into the brain. Prior to the behavioral test, the stylet was replaced by a sterile 30-gauge inner cannula adjusted to extend 1.0 mm beyond the tip of the guide cannula. The inner cannula was connected by tubing to a microsyringe that was depressed by the infusion pump.

On the first day of the self-injection regimen for Rhesus 1, the syringe contained sterile saline (0.9%) and 23 operant responses were recorded in a 30 min test session (i.e. 0.76 responses/min). The following day the syringe contained a solution of D-amphetamine sulfate in a concentration of $10^{-8}$ M and a shaping procedure was employed in an effort to induce spontaneous intracerebral self-administration. Neither this nor a higher concentration ($10^{-7}$ M) produced an increase in operant responding during 4 daily test sessions. Furthermore, no observable physiological or
behavioral changes accompanied experimenter-administered injections of either dose of amphetamine. However, marked behavioral changes were observed during the first test with D-amphetamine at a concentration of $10^{-6}$ M, pH = 5.4. These effects included an initial period of activity, some vocalization and piloerection followed by a period of quiescence and staring. In vitro, D-amphetamine caused an activation of striatal synaptosomal DA synthesis with an ED50 of $10^{-6}$ M [6]. This concentration of amphetamine was used in all subsequent tests for intracerebral self-administration. After the initial observations with D-amphetamine ($10^{-6}$ M), an effort was made to condition self-injection by shaping the bar touching response throughout the remainder of the 60 min trial. On trial 2, this behavior was shaped again during the first half of the trial and spontaneous responses were recorded in the last 30 min. No free injections were administered during the third session; the injection pump was activated after a 15 min control period and a record of operant responding was obtained on a cumulative recorder. Rhesus 1 displayed clear evidence of intracerebral self-administration of D-amphetamine at this dose and increased responding from 42/45 min to 230/45 min between the third and the sixth daily test session. The operant rate of responding by Rhesus 2 during the 30 min test with saline (0.9%) was 14/30 min (i.e. 0.46 responses/min). This subject increased the response rate for D-amphetamine ($10^{-6}$ M) to 154/45 min by the sixth test session.

As a control for the possibility that the increased responding may reflect an increase in motor activity, Rhesus 1 and 2 were trained next on a discrimination task. A white plastic triangle was attached to the bar that activated the infusion pump and the neutral bar was indicated by a black square. The position of the two bars was varied over the next 5 test sessions. Both subjects displayed good discrimination over the 5 sessions and pressed significantly more on the positive lever ($F (1,1) = 486.4, P<0.031$). Individual scores on each lever across the 5 sessions were: Rhesus 1, positive $\bar{X} = 90 \pm 11.9/60$ min, neutral $\bar{X} = 37 \pm 7.8/60$ min; Rhesus 2, positive $\bar{X} = 63 \pm 6.5/60$ min, neutral $\bar{X} = 5.4 \pm 1.9/60$ min.

The demonstration of intracerebral self-administration of D-amphetamine ($10^{-6}$ M) into ICS sites in the orbitofrontal cortex was replicated with Rhesus 3 and 4 using a different operant response. The touch bar was replaced by a panel (6.5 $\times$ 6.5 cm) the surface of which was placed vertically at arm's length from the subject. Depression of the panel activated the infusion pump and at the same time turned on an overhead light (25 W) for 5 sec. Rhesus 3 attained a spontaneous self-administration rate of 351/60 min by the fifth test session. Rhesus 4 acquired the panel pressing response on the second test session and recorded 395/60 min. Rhesus 1 also was trained with the panel press response after extinction of the touch response. As shown in Fig. 1, responding for this animal increased from an operant rate of 9/30 min to 247/60 min after 6 daily sessions with intracerebral injections of D-amphetamine. This animal also responded readily on fixed ratio (FR) schedules FR-2, FR-5, FR-10, but not FR-20. Each schedule was maintained for 3 consecutive
Fig. 1. Cumulative records of panel presses by Rhesus 1 for intracerebral microinjection of $\text{D}$-amphetamine sulphate ($10^{-6}$ M; 1/20 µl) into the orbitofrontal cortex. A: acquisition of response over 6 daily 1-h test sessions with amphetamine available on a continuous reinforcement schedule, as compared to 30 min control with NaCl (0.9%) microinjection. A light stimulus (25 W; 5 sec) also accompanied each panel press response. B: records from 60 min sessions with amphetamine available on fixed-ratio (FR) schedules, FR-10 and FR-20.

days and mean response rate and number of infusions were as follows: FR-2, $\bar{X} = 274/60$ min, 137 infusions; FR-5, $\bar{X} = 529/60$ min, 106 infusions; FR-10, $\bar{X} = 569/60$ min, 57 infusions.

A further test for the reinforcing effect of amphetamine microinjection involved extinction of the self-administration behavior with Rhesus 1, 3 and 4. During the first test of extinction the animals received a microinjection of saline (0.9%) following each panel press when the light cue was absent. Under these conditions two animals had reduced their panel pressing to operant levels (< 10 responses/30 min) during the last 30 min of the first 1-h test session ($\bar{X} = 14.3 \pm 14.3/30$ min). Rhesus 1 extinguished responding during the first 30 min of the second test session. All animals re-established intracerebral self-administration when D-amphetamine was available on subsequent sessions. When extinction trials were conducted with saline and the light cue, two animals were still responding strongly at the end of the first extinction trial ($\bar{X} = 46.3 \pm 28.7/30$ min). Rhesus 1 reached criteria (< 10 responses/30 min) during the second extinction trial (4/30 min) and Rhesus 4 on the third trial (6/30 min).

The multiple cannulae placements of Rhesus 3 and 4 allowed tests of intracerebral self-administration of D-amphetamine ($10^{-6}$ M) at different brain loci. In addition to self-administration into the orbitofrontal cortex, obtained in all 4 subjects, reliable self-administration was obtained in the caudate nucleus of Rhesus 3. However, intracerebral self-administration could not be elicited from the
accumbens placements in either animal despite 4–7 days of shaping. Similarly, injections of D-amphetamine into the lateral ventricle failed to maintain self-administration behavior. Histological analysis of the brains of the 4 subjects confirmed accurate placements in the orbitofrontal cortex 1–2 mm above the base of the brain. Rhesus 3 and 4 had cannulae terminating in the ventral aspect of the head of the caudate nucleus 2–3 mm above the internal capsule, in the medial region of the nucleus accumbens, and the lateral ventricles.

The present data indicate that the rhesus monkey will self-administer small quantities of D-amphetamine directly into the same region of the orbitofrontal cortex that will sustain electrical self-stimulation behavior [15]. Four lines of evidence suggest that this behavior reflects a genuine reinforcement effect and these are: (1) the generation of learning curves over successive trials; (2) the ability to discriminate visually an operant which activates the infusion pump; (3) the extinction of this behavior; and (4) an indication of conditioned reinforcement from the resistance to extinction in the presence of a light cue paired previously with the drug injection.

Previous studies by Olds reported reinforcing effects of intrahypothalamic infusion of iproniazide phosphate [9]; however, this effect was subsequently attributed to extreme pH values [10]. While the pH values of the amphetamine solutions employed in the present study were acidic, their value was very similar to physiological saline that serves as a control solution. As very little operant behavior accompanied infusion of NaCl, it is unlikely that the responding for D-amphetamine solution simply reflected an activating effect of intracranial infusion of an acidic solution.

The region of the orbitofrontal cortex that supports both ICS and amphetamine self-administration contains significant concentrations of DA [7]; as does the caudate nucleus which also was positive for intracerebral self-administration in one animal. Neurochemical studies have shown increased synthesis and release of DA from striatal [6] and cortical tissue [1], with significant effects produced by amphetamine concentrations of $10^{-6}$ and $10^{-5}$ M. In vivo studies with monkeys have reported a 15–20-fold increase in the level of DA release from the caudate after intracranial infusion of D-amphetamine [5]. These neurochemical data lend support to the hypothesis that the rewarding effects of psychomotor stimulants could be due to a direct action of the drugs on DA neurons. Several lines of evidence point to an important role for frontal cortical DA neurons in ICS behavior of primates [8, 14], thereby raising the possibility that rewarding and euphoric effects of psychomotor stimulants are mediated in part by an action on this region of the primate brain.

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