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Increased Lever Pressing for Amphetamine after Pimozide in Rats: Implications for a Dopamine Theory of Reward

Abstract. *Low and high doses of a dopamine blocking agent had effects on lever pressing for intravenous amphetamine reward which resembled the effects of reward reduction and reward termination, respectively. Noradrenaline blockade had no such effects. A role in central mediation of reward perception is suggested for dopamine but not for noradrenaline.*

Research on the nature of central reward mechanisms generally utilizes the intracranial self-stimulation paradigm (*1, 2*) and currently focuses on the question of which of several catecholamine pathways are primarily involved in reward mediation (*3–5*). Noradrenaline and dopamine blocking agents have both been shown to reduce the rate of lever pressing for stimulation (*4, 5*). Similar response decrements occur when the reward value (current intensity) of stimulation is reduced, and consequently both noradrenaline (*2, 6*) and dopamine (*5*) have been proposed as neurotransmitters in a central reward mechanism. There is controversy, however, over this interpretation since response decrements might alternatively be due to arousal deficits associated with noradrenergic blockade (*5, 7*). Increased distractability, sedation, or other nonspecific drug consequences might also be argued to account for response decrements with noradrenaline blockade, and a motor deficit might account for response decrements with dopamine blockade. The usual self-stimulation data do not resolve this question, since they do not dissociate reward deficits from these other, nonspecific, types of deficit.

Rats will also lever press for intravenous injections of amphetamine (*8*), and amphetamine, like rewarding brain stimulation, seems to activate catecholamine mechanisms (*9*). Many response characteristics of intracranial self-stimulation are also typical of amphetamine self-administration (*8*); these common characteristics suggest that a common reward mechanism mediates the two behaviors. Human subjective report supports this suggestion: A patient with a history of amphetamine abuse who was subsequently given septal brain stimulation likened his responses to stimulation with the "pleasurable states he had sought and experienced through the use of amphetamines" (*10*, p. 26).

The most obvious differences between lever pressing for amphetamine and lever pressing for intracranial stimulation is a difference in rate; this difference and its underlying causes allow amphetamine self-administration to be used to dissociate true reward deficits from secondary deficits. The lever-press rate for intravenous amphetamine depends primarily on the duration of effectiveness of each injection and therefore varies inversely with the injection dose (*11*). Thus, in the case of amphetamine self-administration, when the amount of reward (dose) per injection is reduced, the lever-press rate increases. Since any nonspecific interference with the animal would decrease lever pressing, the self-administration paradigm permits clear dissociation of true reward deficits from nonspecific response disruption. The fact that the catecholamine synthesis–blocking agent α-methyl-*p*-tyrosine causes increased lever pressing for intravenous amphetamine (*12*) indicates that one of the catecholamines does, in fact, play a primary role in the reward function. We now report that it is dopamine, not noradrenaline, that plays this role. Increased rates of amphetamine self-administration (followed by extinction when high doses are used) are seen after treatment with the dopamine blocking agent pimozide, whereas decreased rates are seen with the α- and β-noradrenaline blocking agents phentolamine and *l*-propranolol.

Each of 22 adult male Sprague-Dawley rats was prepared with a permanent jugular catheter that passed subcutaneously to an exit anchored to the skull (*13*). The infusion tubing was interrupted by a feed-through swivel, so that the animal could move freely in a test box containing two levers: one lever activated a syringe pump that delivered 0.25 mg of *d*-amphetamine sulfate per kilogram of body weight with each lever press; the other caused the same relay noise but led to no injection.

The animals were trained to lever press in one or two overnight sessions in the test box. Lever pressing on the control lever was seen at first but was not sustained. Once self-administration was initiated, animals continued pressing at their characteristic rates unless they were treated with a drug, or unless amphetamine injections were ceased.

At the beginning of each test the animals were given 2 to 4 hours to settle into regular response patterns (*8*). The effects of catecholamine blocking agents were assessed over 10 hours after this stabilization period. The animals were taken from the test box, given a preassigned drug injection, and replaced in the box. Each animal was

tested under as many drug conditions as possible, but due to deaths and catheter damage not all animals were tested in every condition. The drugs and injection doses tested were pimozide (0.0625, 0.125, 0.25, and 0.5 mg/kg; $N = 6, 7, 9$, and 10), phentolamine HCl (2.5, 5, and 10 mg/kg; $N = 6, 8$, and 6), and *l*-propranolol HCl (2.5, 5, and 10 mg/kg; $N = 6, 5$, and 4). A minimum of 24 hours of rest was given between test sessions.

Each dose of pimozide caused an increased rate of lever pressing (Figs. 1 and 2). With the higher doses the latency of rate acceleration was shorter, and with the highest dose complete cessation of responding followed the period of increased rate in each of the ten animals tested. The extinction seen with the high dose was complete, in that the animals did not respond again during the session. Subsequent tests revealed that responding could be reinitiated by priming, but not until at least 12 hours after injection of pimozide. Responding reinitiated at such time was at the high rates typical of treatment with low doses of pimozide. Characteristic stereotypic behavior was seen during all phases of the testing, except during the periods of nonresponding after high doses of pimozide.

Both phentolamine and *l*-propranolol tended to depress lever pressing. The amount of depression was dose-dependent in the case of the α-noradrenergic blocking agent phentolamine, but not in the case of the β-noradrenergic blocking agent *l*-propranolol (Fig. 2). Priming injections inhibited self-administration of amphetamine in the phentolamine and propranolol conditions, indicating that the rats were drug-satiated during the period of inhibited responding.

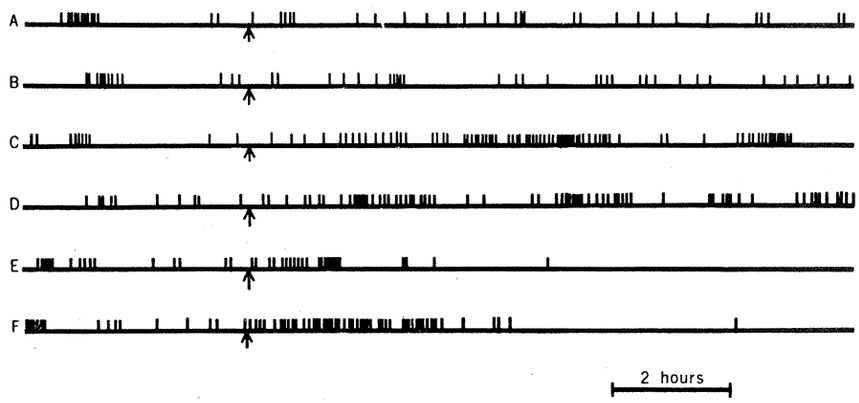

Fig. 1. Lever-pressing data from a representative animal after various manipulations. Each vertical line indicates a lever press; arrows mark the time of experimental manipulation. The manipulations were injections of (A) saline (intraperitoneal), and (per kilogram of body weight) (B) 0.0625 mg of pimozide, (C) 0.125 mg of pimozide, (D) 0.25 mg of pimozide, (E) 0.5 mg of pimozide, and (F) substitution of nonrewarding intravenous saline injections in place of rewarding intravenous amphetamine.

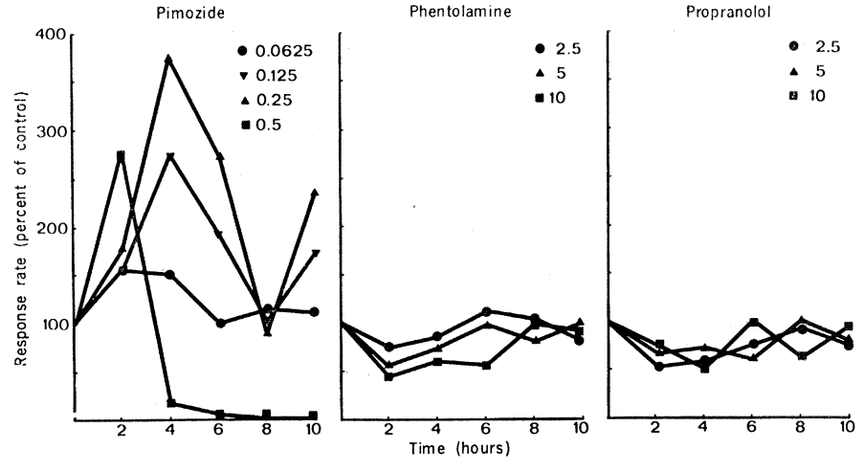

Fig. 2. Median lever pressing for intravenous amphetamine after intraperitoneal injections of pimozide, phentolamine, and *l*-propranolol, expressed in milligrams per kilogram of body weight.

The increased responding seen after pimozide was unique and unexpected. It was unexpected in that pimozide inhibits other forms of behavior (*14*) and might be expected to cause motor deficits that would interfere with stable response patterns. It was unique in the sense that most drugs, including stimulants, depressants, antidepressants, and hypnotics, depress stimulant self-administration (*15*). Other pharmacologically induced increases in stimulant self-administration have been reported only after the administration of other drugs that have antagonizing actions on catecholamine transmission (*15, 16*). The only nonpharmacological manipulations that cause increased responding in this paradigm are reward reduction or reward termination, and response records of our animals after high doses of pimozide were similar to those seen (*13*) when injections of saline were substituted for injections of amphetamine (Fig. 1). It would appear that dopamine blockade reduces or eliminates the rewarding properties of amphetamine and thus makes it necessary for the animal to take more drug than usual in order to reach the same level of drug satiety (*17*). This suggestion is consistent with human ratings of the euphoric effects of amphetamine; the euphoric effect is decreased by pimozide (*18*). Therefore, we offer the hypothesis that normal functioning of a dopaminergic mechanism is essential for the perception of the rewarding consequences of amphetamine and intracranial electrical stimulation. The same mechanism may also be involved in the perception of the reward properties of naturally occurring reinforcers.

The fact that noradrenaline blockade produced a very different pattern of altered responding than does reward reduction or reward termination raises serious problems for the hypothesis that noradrenaline is the primary neurotransmitter in a reward system (*2, 6*). The present data do not clarify the role of noradrenaline, but they do indicate that noradrenaline and dopamine play quite different roles. The effects of noradrenaline blockade could be explained by a noradrenergic involvement in any of a number of behavioral support mechanisms.

ROBERT A. YOKEL, ROY A. WISE
Department of Psychology, Center for Research on Drug Dependence, Sir George Williams University, Montreal, Quebec, Canada H3G 1M8

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Myogenic Defect in Acetylcholinesterase Regulation in Muscular Dystrophy of the Chicken

Abstract. *To determine whether inherited muscular dystrophy of the chicken is neurogenic or myogenic in origin, limb buds from homozygous normal and dystrophic chick embryos were exchanged prior to muscle differentiation and innervation. Biceps muscles of hatched chicks, in which muscle of the donor was innervated by nerves of the host, were analyzed for embryonic properties of muscle acetylcholinesterase and for fiber diameter, two distinctive markers for expression of the dystrophic gene. The results indicate that muscular dystrophy of the chicken is caused by an initial biochemical lesion in the limb and its muscle rather than in its innervating nerve.*

Recently there has been much interest in whether inherited muscle abnormalities, particularly the muscular dystrophies, are myogenic or neurogenic in origin (*1*). Inherited muscular dystrophy of the chicken is one of the models that has been studied to determine the nature of the primary biochemical defect and its cellular site of expression. This disorder is a progressive abnormality involving a single codominant gene, and affecting mainly fast twitch, glycolytic muscle fibers (*2, 3*). Many of the properties that are altered in dystrophic chick muscle are known to be regulated by neural activity. A good example is the enzyme acetylcholinesterase (AChE); embryonic properties of muscle AChE that disappear after hatching in normal muscle are maintained in dystrophic chick muscles and return with denervation but not tenotomy of normal chick muscles (*3*). This report presents evidence that nerves of genetically dystrophic chickens are capable of regulating muscle AChE and that dystrophic muscle cells lack the ability to respond normally to their nerves (*4*).

The experimental approach used was limb bud transplantation in which primordial limb regions were exchanged at an early embryonic age between genetically different embryos, producing muscles of one genotype innervated by nerves of another genotype (*5*). In the experiments reported here, right wing limb buds were removed from stage-19 to stage-20 embryos (3½ days of incubation) and replaced by limb buds of the same or different genotype. Normal limb buds were grafted onto normal hosts and dystrophic hosts; dystrophic limb buds were grafted onto normal hosts. Only birds with morphologically normal, healthy wings were used for analysis. Transplants were done before the motor nerve axons had reached the primordial limb tissue (*6*) so that muscles of the transplanted wings became innervated by neurons of the host and subject to the host's systemic regulation. Chicks were killed 5 to 14 weeks after hatching, and biceps muscles of the donor and host limbs were examined for AChE activity and muscle fiber diameter. In the 15 birds analyzed, AChE-positive motor end plates and spindle fibers were seen in all transplant muscles, no fiber degeneration was observed, and chicks could voluntarily contract muscles in their transplanted wings.

The strain of dystrophic chickens used in this study exhibits pronounced muscle fiber hypertrophy in afflicted muscles, making this parameter a useful marker for expression of the dystrophic gene (*7*). The AChE properties studied differ greatly between normal and dystrophic chick muscle. Adult dystrophic muscle maintains high levels, extrajunctional localization, and small molecular weight isozymes of AChE characteristic of embryonic muscles; normal muscle has low levels, no extrajunctional localization, and only a single high molecular weight isozyme of AChE (*2*).

The act of transplantation did not affect AChE activity or fiber diameters of either normal or dystrophic muscles. Both parameters were unchanged when normal muscles were transplanted to normal hosts (Table 1). When transplants were made between genetically different embryos, the transplanted muscles retained the properties of their origins and did not take on the characteristics of their hosts. Dystrophic muscle in a normal host had high levels of AChE activity and large muscle fiber diameters, and normal muscle in a dystrophic host had low AChE and normal muscle fiber diameters.

The cytochemical distributions of AChE and the number of AChE isozymes in transplanted muscles confirmed that normal and dystrophic muscle transplants retained the AChE properties characteristic of their genotypes (Fig. 1). In the birds studied, dystrophic muscle, whether of host or transplant origin, always had high extrajunctional AChE activity, and em-