Pimozide Blocks Establishment but not Expression of Amphetamine-Produced Environment-Specific Conditioning

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mand? The decrease of cerebral metabolic rate accompanying increments in brain size (slope = −0.13) (*13*) is too small to suggest a constancy in overall energy demands by larger brains. Mammals differ, however, in the relative amount of energy used by the brain. Primate brains, as represented by *Macaca mulatta* and *Homo sapiens*, use a relatively higher proportion of their body metabolism (9 and 20 percent, respectively) (*22*) than do the nonprimate brains of rat, cat, and dog (4 to 6 percent) (*13*). These proportions correlate significantly with the species-specific deviations of both adjusted and unadjusted relative brain size (r = .986 and .98; respectively, $P < .01$)—that is, the proportion of available energy directed toward the brain accounts for much of the observed deviations in relative brain size. A major primate adaptation appears to have been the allocation of a larger proportion of the body's energy supply for the brain. An analysis of the brain's energetics is necessary for a better understanding of the relation of brain to body.

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Pimozide Blocks Establishment But Not Expression of Amphetamine-Produced Environment-Specific Conditioning

Abstract. *Animals with a history of receiving daily injections of +-amphetamine in a specific environment showed a placebo effect (enhanced activity) when injected with saline and placed there; control animals with similar but dissociated drug histories and experience with the test chamber failed to show the effect. The dopamine receptor blocker pimozide antagonized the establishment of conditioning. However, the same dose of pimozide, when given to previously conditioned animals on the placebo test day, failed to antagonize the expression of conditioned activity. Thus, during conditioning dopaminergic neurons mediated a change that subsequently influenced behavior even when dopaminergic systems were blocked. Although schizophrenia may be related to hyperfunctioning of dopamine, neuroleptic drugs, which block dopamine receptors on their first administration, do not have therapeutic effects for a number of days. The results of the pimozide experiments may resolve this paradox.*

Chronic abuse of psychomotor stimulant drugs such as +-amphetamine and cocaine can lead to schizophrenia-like behavior in humans (*1*). Because the stimulant effects are mediated by dopaminergic neurons in the brain (*2*), dopaminergic hyperfunctioning has been suggested as a cause of schizophrenia (*3*). A number of animal studies have shown that these stimulant effects can become conditioned to environmental stimuli associated with the drug state (*4*). We now show that although a dopamine antagonist blocks the establishment of this effect, once conditioning has occurred the same drug fails to block its expression. This finding raises the possibility that during conditioning, dopaminergic neurons mediate a change that can subsequently influence behavior even when dopaminergic systems are blocked.

Experimentally naïve male Wistar rats (250 to 300 g) were housed individually in a climatically controlled colony room kept on a 12-hour light-dark cycle. Food and water were freely available.

Experiments were conducted at the same time each day seven days a week.

The general conditioning procedure was always the same. Each day each rat was removed from its home cage, given an injection, and placed in the observation chamber for 30 minutes (*5*). Then the rat was returned to its home cage and given a second injection. While in the observation chamber the rat was scored at 5, 10, 20, and 30 minutes according to a 9-point activity rating scale ranging from asleep (1), through normal alert activity (4), up to stereotyped activity (8) and dyskinetic movements (9) (*6*); ratings were always made by two independent observers, one of whom was unaware of the treatment conditions (*7*).

The purpose of experiment 1 was to demonstrate environment-specific conditioned activity, with +-amphetamine the unconditioned stimulus. One group of 12 rats received +-amphetamine sulfate (2.5 mg/kg injected intraperitoneally) before being placed in the observation box and saline on being returned to their home cages; the other group received saline in the observation box and amphetamine in the home cage.

Tests for environment-specific conditioned activity occurred on days 6, 12, and 24 with regular conditioning sessions on the intervening days. On the test days, both groups received saline before being placed in the observation box. In spite of their identical drug histories, the animals previously treated with amphetamine in the observation box were more active than those treated in the home cage on the three test days (Fig. 1).

Pilot studies conducted prior to experiment 2 showed that the dopamine receptor blocker, pimozide (*8*), at a dose of 0.4 mg/kg, almost completely antagonized the stimulant effects of the amphetamine treatment. In experiment 2, this dose of pimozide was administered to all animals each conditioning day, 4 hours before placement in the observation box; otherwise, the animals were treated like those in experiment 1.

The test session in experiment 2 occurred on day 11 after 10 conditioning sessions. No pimozide injections were given on the test day. The activity ratings of the two groups did not differ at any observation time in the test session (Fig. 1). As pimozide almost totally antagonized the unconditioned stimulant effects of amphetamine in the experimental group, it is not surprising that little conditioning was observed.

The purpose of experiment 3 was to test the effects of pimozide on the expression of an established environment-specific conditioned drug effect. Two groups of 12 rats each were conditioned as in experiment 1. Test sessions occurred on days 11, 17, 23, and 29, with both groups receiving saline injections immediately before being placed in the observation chamber. Additionally, on test days 11 and 29, both groups received an injection of pimozide (0.4 mg/kg) 4 hours before testing.

On test days 17 and 23, a significant conditioning effect was observed (Fig. 1), replicating the results of experiment 1. On test days 11 and 29, in spite of prior treatment with pimozide, significant conditioning still occurred. The unconditioned effect of amphetamine (day 5, experiment 1 and day 10, experiment 3), although larger and longer lasting than the conditioned effect, was almost totally antagonized by this dose of pimozide (day 10, experiment 2). Yet, the conditioned effect was not blocked.

Dopaminergic neurons have been suggested to mediate the behavioral effects of reinforcement (*9*). Bindra has suggested that the reinforcement event increases the incentive motivational (response-eliciting) properties of neutral environmental stimuli associated with it (*10*). In the context of our experiments, environment-specific conditioned activity occurred because stimuli repeatedly present when dopaminergic neurotransmission was enhanced by amphetamine became conditioned incentive stimuli.

When viewed in this way, the finding that pimozide blocks the establishment of conditioned activity but not its expression can be seen to be consistent with reports of the effects of this drug on food-reinforced operant responding. Thus, untrained animals under the influence of pimozide (1.0 mg/kg) failed to learn to press a lever for food (*11*), whereas previously trained animals receiving the same dose continued to respond for a time (*12*).

These observations support the conclusion that dopaminergic neurons play a role in learning; namely, they mediate incentive learning associated with the

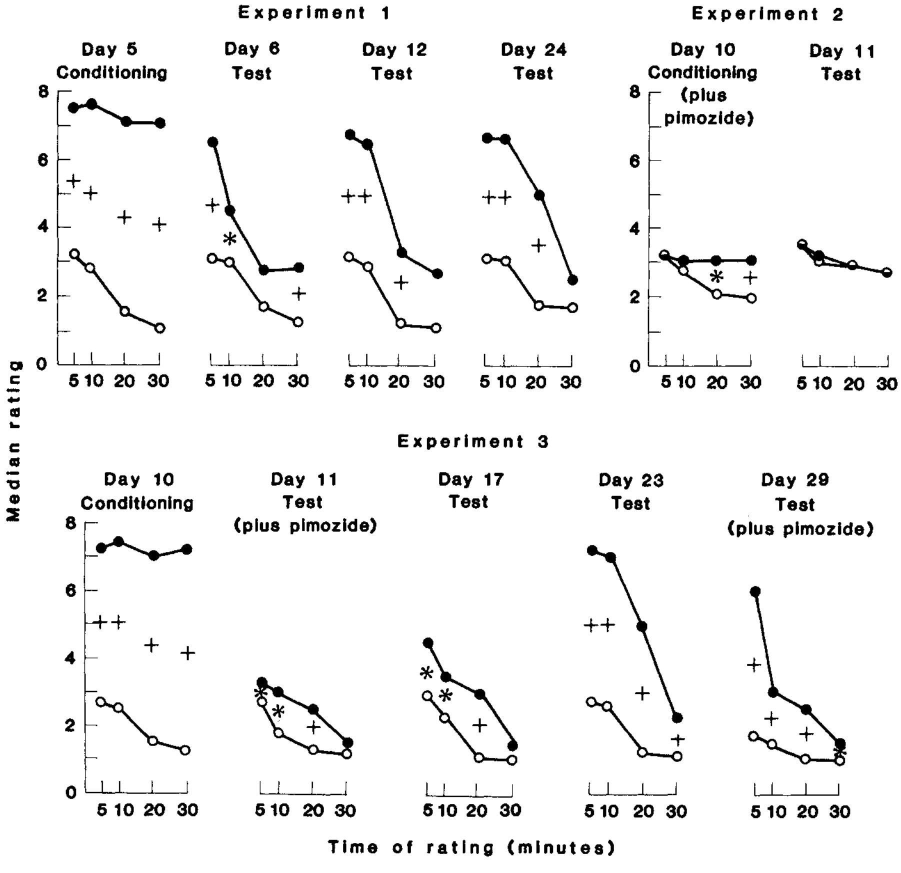

Fig. 1. Median activity rating for the experimental (●) and control (○) groups. In experiment 1, the experimental group received amphetamine and the control group saline before conditioning session 5. Both groups received saline before test sessions. In experiment 2, both groups received pimozide (0.4 mg/kg) 4 hours before conditioning session 10, and the experimental group received amphetamine and the control group saline immediately before that session. Both groups were injected with saline before the test on day 11. In experiment 3, the experimental and control groups received amphetamine and saline, respectively, before conditioning session 10. Both groups received saline before test sessions; however, both groups were also injected with pimozide (0.4 mg/kg) 4 hours before test sessions on days 11 and 29. Statistical comparisons were made with Mann-Whitney *U* tests: $*P < .05$; $\dagger P < .01$.

presentation of biologically significant (reinforcing) stimuli. Under the influence of drugs that elevate dopaminergic neurotransmission, incentive learning leads to the enhanced ability of drug-associated environmental stimuli to elicit responses. The symptoms of psychosis seen in individuals who chronically abuse psychomotor stimulants may be caused by excessive incentive learning. Overactivity in dopaminergic systems has been proposed as the underlying etiology of schizophrenia (*3*); it follows that this disorder also may result from excessive incentive learning. The strength of this conclusion regarding incentive learning depends on the fate of the dopaminergic overactivity hypothesis of schizophrenia; one alternative, for example, is the hypothesis that schizophrenia results from reduced sensitivity of dopaminergic receptors (*13*). Although some data may make this alternative attractive (*14*), recent postmortem studies of receptor binding in the brains of schizophrenics who were not receiving medication at the time of death continue to support the hyperfunctioning hypothesis (*15*).

Of particular relevance to the possibility that the symptoms of schizophrenia are produced by dopamine-mediated incentive learning is the observation that although dopamine receptor blockers impair the establishment of incentive learning, they do not prevent its expression. The therapeutic effects of antipsychotic drugs (known to block dopamine receptors immediately) are not seen until at least a week after the initiation of treatment (*16*). This delay may occur because these drugs initially fail to prevent the expression of aberrant incentive learning that occurred prior to the initiation of treatment. As is the case with incentive learning produced by food reinforcement, which weakens over repeated trials when dopamine receptors are blocked (*9*), aberrant incentive learning that has occurred in psychotic patients as a result of dopamine hyperfunctioning may weaken over the course of neuroleptic therapy and thus result in improvement over time.

Although other explanations are possible—for example, as yet unknown biochemical effects of long-term treatment with neuroleptics—our findings may explain the long-standing observation that the therapeutic action of antipsychotic drugs is delayed.

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High Fetal Estrogen Concentrations: Correlation with Increased Adult Sexual Activity and Decreased Aggression in Male Mice

Abstract. *In the house mouse* (Mus musculus), *fetuses may develop in utero next to siblings of the same or opposite sex. The amniotic fluid of the female fetuses contains higher concentrations of estradiol than that of male fetuses. Male fetuses that developed in utero between female fetuses had higher concentrations of estradiol in their amniotic fluid than males that were located between other male fetuses during intrauterine development. They were also more sexually active as adults, less aggressive, and had smaller seminal vesicles than males that had developed between other male fetuses in utero. These findings raise the possibility that during fetal life circulating estrogens may interact with circulating androgens both in regulating the development of sex differences between males and females and in producing variation in phenotype among males and among females.*

Sexual differentiation begins during early fetal life in mammals. If the gonads are removed surgically before the onset of sexual differentiation, mammals develop into phenotypic females regardless of their genetic sex (*1*). During sexual differentiation in males, therefore, masculine traits are induced (masculinization) and, in some species, feminine traits are suppressed (defeminization) (*2*). Androgens, primarily testosterone or its metabolites, are secreted at a high rate by the testes of males during fetal life (*3*) and are thought to induce most of the prenatal changes in morphology, physiology, and behavior potential.

In the house mouse (*Mus musculus*), fetuses may develop in utero next to (and possibly be influenced by the hormonal secretions of) siblings of the same or opposite sex. Offspring from known intrauterine positions can be obtained by time-mating female mice and delivering the offspring by cesarean section shortly before normal parturition. Intrauterine position influences morphology, physiology, and behavior in female mice and rats (*4*). For consistency, the classification scheme that has previously been used to identify female fetuses from known intrauterine positions is also used for males (*5*). Males that develop between two other male fetuses are referred to as 2M males, males that develop between a male and a female fetus are referred to as 1M males, and males that develop between two female fetuses are referred to as 0M males. In the experiments described here we used CF-1 mice to test whether the intrauterine proximity of a male fetus to other male or female fetuses is correlated with its adult phenotype.

Male CF-1 mice were castrated within 1 hour of cesarean delivery and injected with hormones in adulthood. The objectives were (i) to eliminate possible differences between 0M and 2M males in the concentrations of gonadal hormones that they would have been exposed to during postnatal life and (ii) to assess the sensitivity of the neural substrates mediating reproductive behaviors to the activating effects of a known amount of hormone in adulthood. Differences between 0M and 2M males could thus be related to prena-