Because the depolarizing responses are increased in amplitude by depolarizing current injections and are usually associated with an increase in input resistance, they are clearly not generated by an increase in sodium conductance. Similar depolarizations have been observed in sympathetic ganglion cells (*12*) and in responses of cerebral cortical neurons to acetylcholine (*13*) and have been attributed to inactivation of resting conductance to potassium ion. This mechanism could also be responsible for the depolarizations we have observed.

The locus coeruleus has been reported to have depressant effects in most studies in vivo (*1*) and in a recent study of cocultured explants of the hippocampus with the locus coeruleus region (*14*). However, in studies of spinal neurons in vivo, both excitatory (*15*) and depressant (*16*) responses have been observed. Iontophoretic application of noradrenaline has also been found to give either mainly excitatory (*17*) or depressant (*18*) responses in spinal neurons. Recently, it was reported that noradrenaline applied to facial motoneurons evokes depolarizations (*19*) similar to those we have found in the cultured spinal neurons. Further data from the same laboratory indicate that neurons of the lateral geniculate nucleus are facilitated by both noradrenaline and by locus coeruleus stimulation (*20*).

Particularly because of the advantages of dissociated neurons in culture for electrophysiological studies, this coeruleo-spinal system promises to be a valuable preparation for the further investigation of noradrenergic actions and mechanisms (*21*).

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Response Artifact in the Measurement of Neuroleptic-Induced Anhedonia

Abstract. *Systemic administration of the neuroleptic drug α-flupenthixol attenuated lever-pressing behavior in rats responding for rewarding brain stimulation. The magnitude of this attenuation was dose-dependent and resembled the effects of reward reduction and termination. However, when the operant response requirements of the same rats were changed to nose poking, identical drug treatments produced relatively little attenuation in performance. These data do not support the belief that neuroleptics produce a general state of anhedonia. Rather, the apparent suppression of reinforced behaviors depends at least in part on the kinetic requirements of the response.*

The term "anhedonia" has been used to describe a state in which the reward value of usually reinforcing stimuli is blocked (*1*). Recent reports suggest that such a state can be produced by the administration of antipsychotic neuroleptic drugs (*2*, *2a*). Animals treated with such drugs stop responding for food or brain stimulation in a manner that resembles the behavioral effects of reward termination. Since many neuroleptic drugs block central dopamine receptors (*3*), these observations lend support to the concept of a central dopamine reward system mediating the behavioral consequences of positive reinforcement.

Other investigations, however, have demonstrated that the pattern of responding observed during neuroleptic administration is not equivalent to that seen when reward is withheld (that is, during extinction) (*4*). Many have therefore argued that neuroleptics produce their behavioral effects by interfering with the animal's ability to maintain responding and not with reward per se. We now report that doses of a neuroleptic which produce anhedonic-like effects when rats press a lever for reinforcement have relatively little effect when the same rats are tested with nose poking as the operant response. Our results suggest that even when the dose is high, positive reinforcing events maintain their reward value. It would seem, therefore, that the suppression of reinforced behaviors observed during drug treatment is at least in part a result of the type of response employed in the experimental paradigm.

Adult male Wistar rats were stereotaxically implanted with a bipolar stimulating electrode aimed at the lateral hypothalamus (*5*). Following surgery, the animals were trained (through shaping) to press a lever for 300-msec trains of rewarding 60-Hz sine-wave intracranial stimulation on a continuous reinforcement schedule. The self-stimulation apparatus consisted of four identical Plexi-

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glas cubicles equipped with a self-stimulation lever on one wall and two 1.5-cm-diameter holes located side by side (7 cm apart) on the opposite wall.

Training consisted of daily sessions during which self-stimulation rate-intensity functions were computed for ascending 5-μA adjustments in current (*6*). Once rate-intensity functions had stabilized for lever pressing, access to the levers was blocked and the rats were shaped to poke their noses through the holes for intracranial stimulation. During the trials that involved lever pressing, delivery of brain stimulation was produced only by the depression of the lever. During the trials that involved nose poking, one of the two holes was randomly activated such that a poke through the "positive" hole interrupted a small photocell beam and resulted in the delivery of rewarding stimulation. Nose pokes through the "negative" hole were counted but did not result in any reinforcement. Rate-intensity functions were calculated daily in a manner identical to that used during the sessions involving lever pressing.

On test days each rat was treated 2.5 hours before testing with saline or one of three doses of the neuroleptic α-flupenthixol (0.1, 0.2, or 0.4 mg/kg) (*7*). The effects of each dose were tested on nose poking for brain stimulation and then on lever pressing (*8*).

The drug differentially affected rates of nose poking and lever pressing for rewarding brain stimulation (Fig. 1). Rats in the lever-pressing response situation demonstrated dose-dependent reductions in response rates, with the highest dose virtually eliminating all responding (Fig. 1A). The same doses did not affect rates of nose poking nearly as much (Fig. 1B). Even at the highest dose, rats continued nose poking at rates nearly 60 percent of those measured when the animals were not drugged (Fig. 1C) (*9*). It might be argued that the ineffectiveness of α-flupenthixol to further attenuate nose poking indicates that less reward is required to maintain nose poking than lever pressing in the first place. Hence, even nose poking would eventually be eliminated simply by increasing the dose. We have found this not to be the case. Even an extremely high dose of α-flupenthixol (0.8 mg/kg, which produced visible cataleptic effects and completely abolished lever pressing for rewarding brain stimulation) did not reduce nose poking below 50 percent of normal rates (Fig. 1B). Furthermore, this failure to block responding could not reflect stereotyped behavior or some other inability to cease responding, since these animals still made over 98 percent of their responses on the positive hole and immediately stopped responding when the current was terminated.

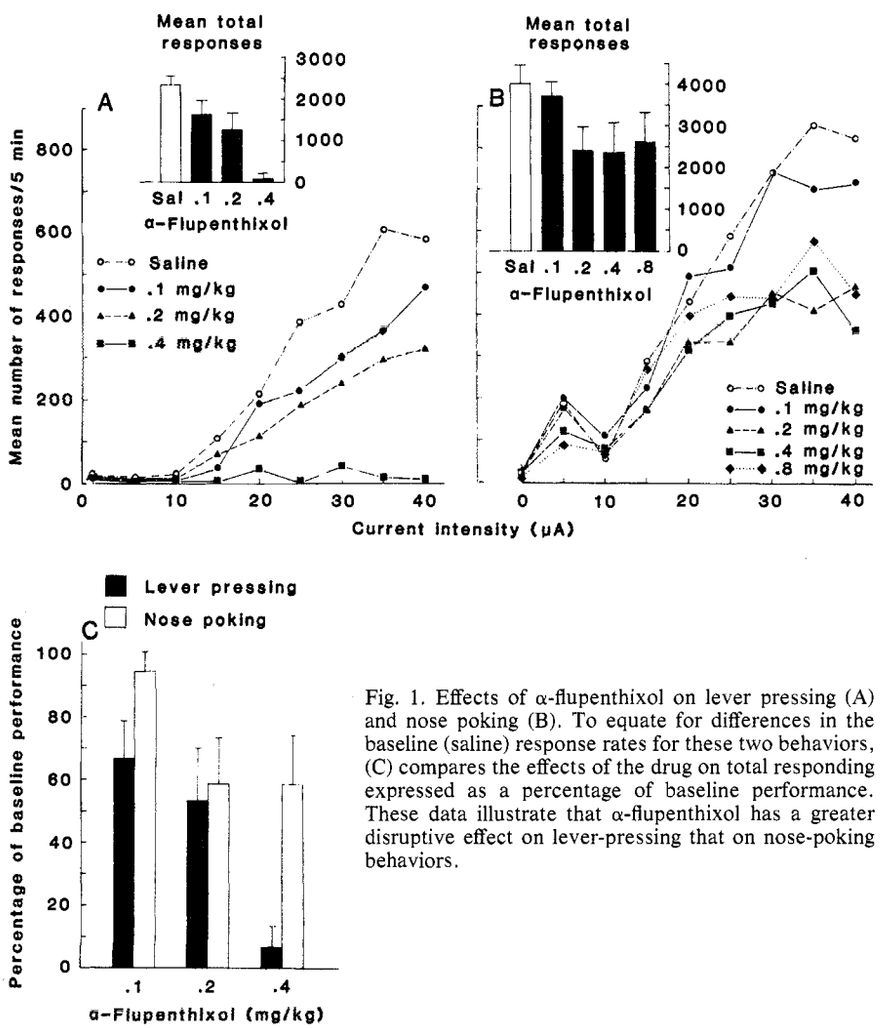

Fig. 1. Effects of α-flupenthixol on lever pressing (A) and nose poking (B). To equate for differences in the baseline (saline) response rates for these two behaviors, (C) compares the effects of the drug on total responding expressed as a percentage of baseline performance. These data illustrate that α-flupenthixol has a greater disruptive effect on lever-pressing that on nose-poking behaviors.

Of course, it may be that even at extremely high doses of neuroleptic enough dopamine receptors are left functional to maintain nose poking. However, if this were the case, then increasing the dose of α-flupenthixol should have decreased the remaining number of functional dopamine receptors and thereby produced a greater behavioral impairment. Instead, doses of 0.2, 0.4, and 0.8 mg/kg produced essentially the same depression in rates of nose poking (Fig. 1B). The fact that doses larger than 0.2 mg/kg did not produce dose-dependent attenuation in rates of nose poking may suggest that any dopamine contribution to the reward value of the stimulation is blocked at these higher doses. It seems unlikely, therefore, that dopamine neurons represent a "critical link" (*2*) in the neural circuitry mediating reward. This does not mean that dopamine is not involved in the mediation of reward. Indeed, many studies suggest just such an involvement (*10*). However, it is clear that any estimate of the extent of that involvement is confounded by the nature of the response employed in the test situation. Thus, in the present study, one might erroneously conclude from the data on lever pressing that high doses of neuroleptic actually block the rewarding properties of the stimulation—that is, produce anhedonia. However, it is obvious from the data on nose poking that the hedonic properties of brain stimulation can still be demonstrated even at the highest drug dose.

Another hypothesis that might explain these results assumes that there are different neural substrates mediating the rewarding properties that result from different self-stimulation behaviors. White and his colleagues (*11*), for example, have provided lesion, electrophysiological, and pharmacological data that support the hypothesis that different neural systems mediate the reward produced by different behaviors. Others have also emphasized the importance of the response leading to brain stimulation (*12*). In the present context, one might pro-

pose that the reward that supports lever pressing is mediated by a dopamine system while nose poking is supported by the activity of some other nondopaminergic substrate. This would account for the differential effects of α-flupenthixol on the two responses. No matter which hypothesis one adopts to explain these results, it remains the case that α-flupenthixol does not produce anhedonia.

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6. Each 5-μA current adjustment was maintained for 5 minutes, followed by a 15-second intertrial period during which no stimulation was available. A new trial was signaled by turning on a masking noise delivered through speakers inside each test chamber. For sessions involving nose poking, the positive hole was alternated after each 5-minute trial. This procedure was maintained throughout testing. Currents ranged in intensity from 0 to 40 μA.
7. α-Flupenthixol (1.0 ml per kilogram of body weight) was dissolved in 0.9 percent sodium chloride solution and injected intraperitoneally. The ability of α-flupenthixol to inhibit binding of [^{3}H]haloperiodol and [^{3}H]spiroperidol and its average clinical daily dose are virtually identical to that of pimozide, the neuroleptic used in most of the previous studies [I. Creese, D. R. Burt, S. H. Snyder, *Science* **192**, 481 (1976); J. E. Leysen, W. Gommeren, P. M. Laduran, *Biochem. Pharmacol.* **27**, 3077 (1978)].
8. To reduce the possibility that tolerance would develop after repeated injections of the drug, the test days were separated by 1 week. Self-stimulation rates were measured 1 and 2 days before each test day to ensure that performance had returned to normal levels. Self-stimulation thresholds on the day before testing remained stable over the entire experiment for nose poking and lever pressing. Differences observed on test days cannot, therefore, be attributed to shifts in baseline values over the course of the study.
9. A two-factor analysis of variance with repeated measures on both factors was computed for the arc sine–transformed data plotted in Fig. 1C. Response rates in both response conditions were reduced by the administration of α-flupenthixol [$F(2, 14) = 11.71$, $P < .005$)]. In addition, lever pressing was attenuated more than nose poking during the three drug trials [$F(1, 7) = 5.60$, $P < .05$)]. This difference in performance was consistent over all three doses of α-flupenthixol as revealed by the lack of a reliable dose × response interaction [$F(2, 14) = 2.12$, not significant].
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Bee Venom Enhances Guanylate Cyclase Activity

Abstract. *Bee venom and phospholipase A2 extracted from bee venom enhanced guanylate cyclase (E.C. 4.6.1.2) activity two- to threefold in rat liver, lung, heart, kidney, ileum, and cerebellum. Dose-response relationships revealed that bee venom at concentrations as low as 1 microgram per milliliter and phospholipase A2 at 1 microunit per milliliter caused a maximal enhancement of guanylate cyclase.*

Each year in the United States, nearly twice as many people die from hymenopterous insect bites (including bees, wasps, hornets, and yellow jackets) as from poisonous snake bites (*1*). The majority of these deaths appear to be due to severe systemic anaphylactic reactions, characterized by respiratory distress often followed by vascular collapse or shock. Bee venom is one of the best characterized of hymenopterous venoms and contains 0.1 to 1.5 percent histamine, two enzymes (phospholipase A_2 and hyaluronidase) and a series of toxic polypeptides (the hemolyzing mellitin, the neurotoxic apamin, and a mast cell degranulating peptide) (*2*). Mellitin, which makes up 50 percent of the dry weight of bee venom, is thought to be the main toxin of bee venom although practically all the effects of bee venom have been ascribed to its phospholipase activity (*2*). Mellitin, a known membrane-active peptide, has been shown to stimulate the activity of phospholipase A_2 (*3*) and heart microsomal guanylate cyclase (E.C. 4.6.1.2) activity (*4*). Since mellitin's effects may be mediated via activating phospholipase A_2, which in turn might activate guanylate cyclase activity, experiments were performed to examine whether phospholipase A_2, isolated from bee venom of *Apis mellifera* (honey bee), and the total honey bee venom itself have any influence on guanylate cyclase activity. With respect to possibility of bee venom affecting guanylate cyclase activity there is the recent demonstration (*5*) that α-toxins of the poisonous snakes krait (*Bungarus multicinctus*) and cobra (*Naja naja siamenis*) enhance the soluble form of guanylate cyclase in rat lung, spleen, and kidney. Both the total bee venom and the honey bee phospholipase A_2 enhanced guanylate cyclase activity in rat liver, lung, heart, kidney, ileum, and cerebellum suggesting that bee venom's mechanism of action is similar to that of the α-toxins of poisonous snakes.

Tissues used in these experiments were from Sprague-Dawley rats; they were homogenized and processed (*6*) to obtain the supernatant and particulate cell extracts after centrifugation at 37,000*g*. Guanylate cyclase was assayed (*6*) with the use of a reaction mixture consisting of 20 m*M* tris-HCl, *p*H 7.6; 4 m*M* $MnCl_2$; 2.67 m*M* cyclic guanosine monophosphate (GMP) (used to minimize destruction of ^{32}P-labeled cyclic GMP); a guanosine triphosphate (GTP) regenerating system (5 m*M* creatine phosphate and 11.25 units of creatine phosphokinase, E.C. 2.7.3.2); 100 μg of bovine serum albumin; 20 m*M* caffeine; and 1.2 m*M* (α-^{32}P)-labeled GTP, approximately 5×10^5 count/min. The enzyme preparations had 0.1 to 0.4 mg of protein. The cyclic [^{32}P]GMP formed was isolated by sequential chromatography on Dowex-50-H^+ and alumina (*6*). Reactions were conducted at 37°C. One unit of phospholipase A_2 is the amount that will hydrolyze 1.0 μmole of L-α-phosphatidylcholine to lysophosphatidyl choline and fatty acid per minute at *p*H 8.5 at 37°C. Sources of all other reagents have been reported (*6*). Each assay was conducted in triplicate, and each value in Table 1 and Fig. 1 was the mean ± the standard error of the mean in three separate experiments with three animals for each experiment each day ($N = 9$).

Honey bee venom and phospholipase A_2 extracted from honey bee venom enhanced soluble guanylate cyclase activity in various tissues (Table 1). Thus, phospholipase A_2 (1 μU/ml) and the bee venom (1 μg/ml) itself enhanced guanylate cyclase activity two- to threefold in rat liver, kidney, lung, heart, ileum, and cerebellum. Both bee venom and phospholipase A_2 increased guanylate cyclase activity more in the ileum and lung

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