somes of the cells. When incubated first with these sera and then with horse antihuman globulin conjugated with fluorescein, the euploid fibroblasts and the peripheral blood cells (Fig. 1) showed 46 discrete fluorescent chromosomes. When, after fluorescent staining, the same cell was restained with acetic acid–orcein and photographed in visible light, every chromosome staining with fluorescent antibody also stained with orcein (Fig. 2). Similar results were obtained with the Chinese hamster line (Figs. 3 and 4). The sera from the normal donors, one patient with lupus erythematosus, one patient with nephrosis, and from the three patients with Sjögren's syndrome did not lead to any chromosomal fluorescence. Easily detectable chromosomal fluorescence was obtained with one lupus erythematosus serum at a dilution of 1:320.

These investigations suggest that certain human sera react with mammalian chromosomes and that, moreover, the reaction is with the full chromosomal complement of the cell. It may be of interest to see whether sera which are more specific in their chromosomal reactions can be found. It may also be useful to try to absorb some of the antinuclear activity with nuclear fractions or with nuclei from one species before incubating the sera with chromosomes from another species.

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Possible Mode of Antidepressive Action of Imipramine

Abstract. Imipramine augmented and prolonged methamphetamine-induced increases in the rate of responding of rats working for "rewarding" hypothalamic and midbrain stimulation. In contrast, chlorpromazine antagonized the effects of methamphetamine on self-stimulation. These opposite psychopharmacological effects are consistent with the different clinical effects of these drugs and suggest a mechanism for the antidepressive action of imipramine.

Despite the similarity between their chemical structures, imipramine and chlorpromazine differ in certain of their clinical actions. As a rule, chlorpromazine calms agitated patients, while imipramine elates depressed ones (*1*). Pharmacological tests have provided no basis as yet for the qualitative difference in these clinical actions. Most often, the same pattern of results is seen with the two drugs in the laboratory (*2*).

As a basis for our experiments, we have tentatively taken the view that agitations and depressions result from abnormalities in motivational and reward processes—agitation from pathological overactivity of reward processes (*3*), and depression from underactivity. On this view, it may be supposed that drugs effective against agitation inhibit an excessive reward activity, and that drugs effective against depression enhance a deficient reward activity.

The brain system that subserves these functions of motivation and reward has been made accessible to experimental investigation by the development of precise methods both for stimulating deep in the brain and for measuring changes in behavior. The self-stimulation technique, in which an intact and unanesthetized animal is trained to obtain brief electrical stimulations of its own brain by performing an arbitrary response (such as pressing a lever), is the method of choice for these studies (*4*). In this paper, we report the effects, and particularly the interactions, of imipramine, chlorpromazine, and amphetamine on self-stimulation.

Adult male rats were implanted with permanent bipolar platinum electrodes in the posterior hypothalamus or midbrain tegmentum. After they recovered, they were trained to stimulate their brains electrically by pressing a lever. Each response delivered a 0.15-sec current train of moderately rewarding intensity (about 0.4 ma). The stimulating wave form was a square pulse of 0.2-msec duration presented at 100 pulses per second through a cathode follower output stage and a shielded, low-impedance isolation transformer to the electrodes (*5*). These stimulating conditions are relatively noninjurious and thus allow the self-stimulation base lines to be stable for many months. With properly placed electrodes, the training often requires only a few minutes.

After the rats became expert at self-stimulation, the stimulating current was lowered individually for each rat, to a level in the vicinity of the threshold for self-stimulation (0.1 to 0.25 ma). Drug tests were begun after many sessions under the minimal current conditions, after the response rates had stabilized at a low level. At least one week intervened between drug dosings. All doses reported are expressed in terms of the total salt.

Our first experiments compared the effects of chlorpromazine and imipramine. The results were somewhat disappointing, as both drugs were found to inhibit self-stimulation. Chlorpromazine was about ten times more potent as an inhibitor than imipramine. These results coincided with published pharmacological findings.

We then learned of work of Carlton (*6*) who found that imipramine augments the facilitating action of amphetamine on conditioned avoidance behavior. We knew from earlier studies that amphetamine is a highly active agent in the self-stimulating test; specifically, it lowers the threshold for electrical reinforcement, indicating a facilitating action on structures of the reward system (*7*). We therefore set about to determine the effects of imipramine and chlorpromazine on the amphetamine or methamphetamine) response in the self-stimulation test.

Figure 1 summarizes our main findings. Six weekly sessions of self-stimulation performance under various drug conditions are depicted for a rat implanted in a reward area of the midbrain tegmentum. The depressed base line rate of self-stimulation generated by the threshold current intensity is seen in Fig. 1*A*. A small dose of *d*-methamphetamine hydrochloride (0.25 mg/kg) produced a clear increase in rate beginning about 15 min after the injection (Fig. 1*B*). The methamphetamine dose was carefully selected to provide a moderate, but unequivocal, effect. Pretreatment with 3 mg/kg of

chlorpromazine hydrochloride antagonized the facilitative effect of the methamphetamine on self-stimulation (Fig. 1*C*). In contrast, pretreatment with 5 mg/kg of imipramine hydrochloride greatly augmented the increase in the self-stimulation rate induced by methamphetamine. This dose of imipramine has no apparent effect of its own on self-stimulation. Greater augmentation is seen to result from a 15 mg/kg dose than from a 5 mg/kg dose (compare Figs. 1*D* and 1*E*). The latency of the methamphetamine response also was decreased by imipramine pretreatment; the effect on latency may be seen to be dependent on the dose of imipramine. These effects are extremely dependable; we have obtained imipramine potentiation of amphetamine effects more than 100 times with about 30 different animals in various follow-up experiments (*8*). Also interesting was the finding that chlorpromazine will antagonize the augmenting effect of imipramine on methamphetamine (compare Figs. 1*E* and 1*F*).

The present findings make it clear that imipramine does in fact favorably influence the activity of the brain system associated with reward, as we expected an antidepressant would. However, it seems to act in some indirect augmenting capacity (since amphetamine is required for the action) rather than through direct stimulation. This accords with the suggestion of Sigg (*2*) that imipramine exerts a "sensitizing" influence on central adrenergic synapses. All our data can be viewed as supporting this notion in the light of two considerations: (i) the work of Vogt showing that structures of the reward system have an unusually rich distribution of norepinephrine, and (ii) the suggestions of Brodie and Shore that amphetamine acts centrally by mimicking norepinephrine, and chlorpromazine acts centrally by blocking norepinephrine (*9*). Whether imipramine sensitizes directly, as has been postulated for cocaine, or indirectly, by the blockade of inhibiting influences, for example, remains a problem for future research (*10*).

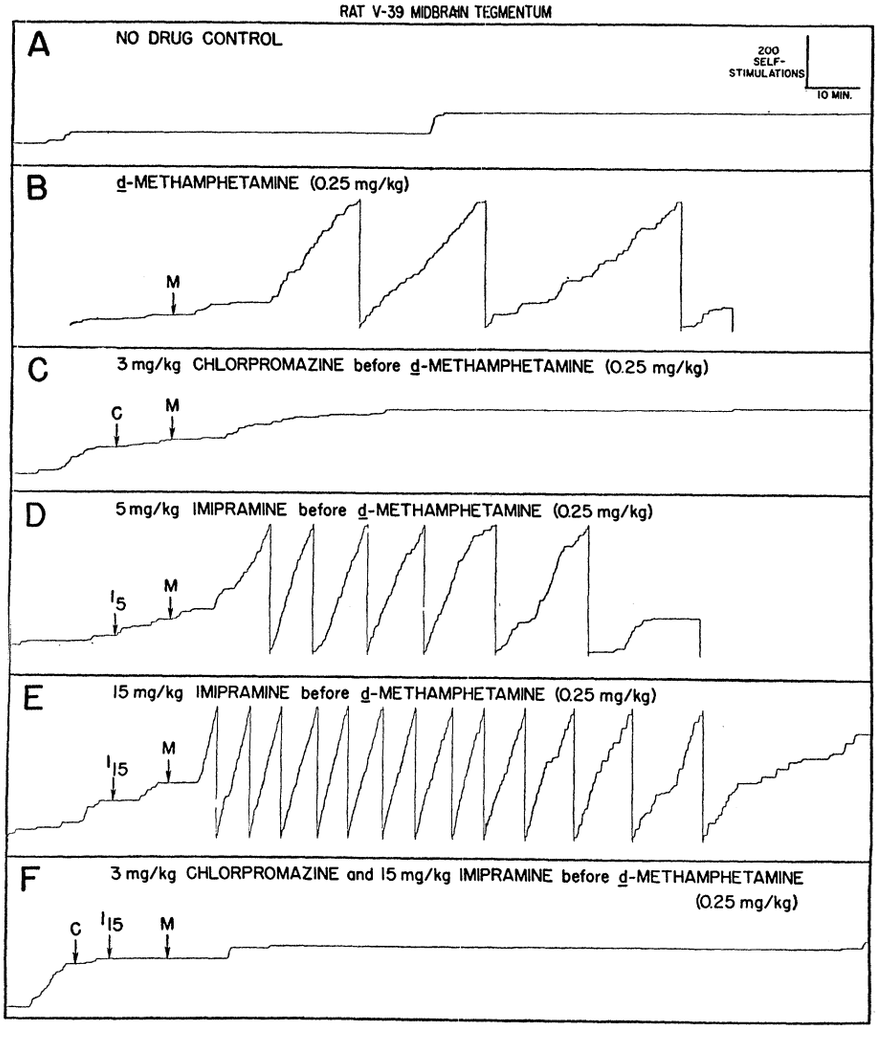

Fig. 1. Cumulative records of self-stimulation obtained from a representative rat with a midbrain electrode in six weekly experimental sessions. The different drug conditions are indicated. The arrows mark the time of the drug injections.

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8. The interactions between chlorpromazine and amphetamine are complex. In addition to its action of diminishing the magnitude of the amphetamine response, we have apparently paradoxical evidence that chlorpromazine also may prolong the duration of the amphetamine effect. Possibly this second action is related to the finding of W. R. Martin, J. L. Riehl, and K. R. Unna, *J. Pharmacol. Exptl. Therap.* **130**, 37 (1960), that chlorpromazine prolongs the pressor response to noradrenaline. This finding tempts one to view chlorpromazine and imipramine as belonging to the same psychopharmacological family. Both may be said to have an antidepressive action (seen as amphetamine potentiation or prolongation) at lower doses, and an ataractic action (seen as inhibition of self-stimulation) at higher doses. In imipramine, the antidepressive component is pronounced and well separated in dose from the ataractic component; in chlorpromazine, the ataractic component is pronounced and poorly separated, if at all, from the antidepressive component.
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