taneous contractions of smooth muscle cells in the testicular capsule—contractions that in man represent approximately a 40 percent shortening of the capsule (*1*).

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Dopaminergic and Noradrenergic Substrates of Positive Reinforcement: Differential Effects of d- and l-Amphetamine

Abstract. *Intracranial self-stimulation was elicited from electrodes located in either the lateral hypothalamus or substantia nigra of the rat. Facilitatory effects of* d- *and* l-*isomers of amphetamine on self-stimulation were assessed. The* d-*isomer was seven to ten times more effective than the* l-*isomer at the hypothalamic placement, whereas the two isomers were equipotent for substantia nigra electrodes. These data support the hypothesis that both dopaminergic and noradrenergic systems subserve positive reinforcement.*

Catecholaminergic involvement in the phenomenon of intracranial self-stimulation (ICS) is supported by neuroanatomical (*1–3*), neurochemical (*4, 5*), histochemical (*6*), and pharmacological (*7–9*) data. With respect to the pharmacological evidence, amphetamine increases the release and prevents the uptake of catecholamines in adrenergic neurons (*10–12*), while having pronounced facilitatory effects on ICS (*7–9*).

Although ICS may be obtained from a variety of subcortical sites (*13, 14*), the highest rates are obtained in the medial forebrain bundle (MFB) region of the lateral hypothalamus (LH), an area that coincides with a major rostral projection of noradrenergic fibers (*15*). Utilizing animal preparations with electrodes in the MFB, Stein and his co-workers have amassed evidence in support of their proposal that ICS is subserved by noradrenergic neurons (*5, 9*). However, the exclusive role of noradrenergic neurons has recently been challenged by neuroanatomical and histochemical evidence of a possible dopaminergic involvement in ICS. Stimulation in the region of the substantia nigra (SN) will maintain ICS (*2, 14*) and yet the fibers of passage and cell bodies in this region are almost exclusively dopaminergic rather than noradrenergic (*15*).

It has recently been demonstrated that *d*- and *l*-amphetamine have differential effects on the uptake of dopamine and noradrenaline in the brain, the levo isomer being approximately ten times less potent in blocking catecholamine uptake into noradrenergic neurons, but being equally efficient in blocking uptake into striatal dopaminergic neurons (*16, 17*). Because *d*-amphetamine was shown to be ten times more potent in enhancing locomotor activity but only twice as potent in producing compulsive gnawing behavior, it was suggested that the former behavior was mediated by brain noradrenaline while the latter had dopaminergic substrates.

In view of these findings, our experiments were undertaken to determine if the noradrenergic and the dopaminergic substrates of ICS are pharmacologically identifiable. Specifically it was hypothesized that electrodes placed in a noradrenergic pathway would be approximately ten times more responsive to the rate increasing effects of *d*-amphetamine than of *l*-amphetamine, whereas dopaminergic placements would be approximately equally responsive to both isomers.

Two groups of ten male Wistar rats had bipolar electrodes implanted in either the MFB as it courses through the LH, or in the SN, in accordance with standard stereotaxic procedures (*18*). Those animals that did not learn to deliver electrical stimulation (0.2-second train of 5 to 100 μa, 60 hz sine wave a-c) to their brains by pressing a lever in a Skinner box, were rejected after seven daily 30-minute training sessions. Subjects displaying high rates of lever pressing during this period were given additional testing to establish the lowest current intensities that would maintain low but consistent response rates, that is, response threshold (*19*). Five days of responding at the response threshold ensured stable response rates at the newly established current intensities.

Six rats with LH electrodes displayed stable response rates at low current intensities and were subsequently injected with different doses of *d*- and *l*-amphetamine to assess the effects of these two isomers on ICS. To facilitate direct comparison of the "drug" test with the "no-drug" control test, a daily schedule of two 15-minute test sessions separated by a 20- to 30-minute intertest period was incorporated. Two "no-drug" days preceded each "drug" day, and the previously established current intensities were used throughout this phase of the experiment. On a "drug" day, intraperitoneal injections of either *d*-amphetamine (0.10, 0.25, 0.50, or 1.0 mg/kg) or *l*-amphetamine (0.50, 1.0, or 2.5 mg/kg) were randomly administered to all animals immediately after the first 15-minute test session. The animals were then placed in their home cages, and 20 minutes later they were returned to the test chamber so that we could assess the effects of the drug on the response rate for reinforcing brain stimulation.

Five of the ten animals with electrodes aimed at the dopaminergic fibers of the SN displayed stable ICS behavior when tested exactly as described above. The testing procedure used to assess the effects of *d*- and *l*-amphetamine on self-stimulation at these placements was

also identical to that employed with the LH electrodes, although the drug doses differed. Both *d*- and *l*-amphetamine at a dose of 0.5 mg/kg produced similar facilitatory effects on self-stimualtion in the SN, and therefore the same dosages of *d*- and *l*-amphetamine (0.25, 0.50, and 1.0 mg/kg) were used in this part of the experiment. On completion of the behavioral experiments the animals were killed, and the electrode placements were verified histologically.

The results are summarized in Fig. 1 (*20*). The *d*- and *l*-isomers of amphetamine produced a significant increase in ICS in both areas of the brain, the magnitude of which was dose-dependent ($P < .05$). With regard to the hypothalamic noradrenergic electrode placement, *d*-amphetamine produced a moderate increase in responding at 0.1 mg/kg and a sharp increase in responding from 0.25 to 1.0 mg/kg. In contrast, it is evident from the slope of the dose-response curve that *l*-amphetamine was considerably less effective in enhancing ICS. *d*-Amphetamine was significantly ($P < .05$) more effective than *l*-amphetamine in increasing ICS at both 0.5 and 1.0 mg/kg. Although the results do not permit an exact evaluation of the potency of the *d*-isomer with respect to *l*-amphetamine, the data suggest that it was between 7 and 10 to 1. Thus, at 0.25 mg/kg *d*-amphetamine produced an increase similar to that produced by *l*-amphetamine at 2.5 mg/kg; and at 0.1 mg/kg the *d*-isomer produced an increase greater than that produced by 0.5 mg/kg but less than that produced by *l*-isomer at 1.0 mg/kg. Considerably different results were obtained from electrodes located in the SN. Here there was a close correspondence between the rate-increasing effects of the two isomers and, in contrast to the hypothalamic placements, at no dosage were the effects of the two isomers significantly different.

The rate-increasing effects of *d*-amphetamine were significantly greater in the noradrenergic than in the dopaminergic system ($P < .01$, Fig. 1). With regard to its behavioral effects it has been suggested that the ability of amphetamine to increase the synaptic release of catecholamines is probably more important than are its uptake inhibiting properties (*21*). It is therefore significant that Farnebo (*12*) has shown that *d*-amphetamine produces a quantitatively greater increase in the spontaneous release of catecholamines from noradrenergic than from dopaminergic neurons in the animals that received prior treatment with reserpine and nialamide. Our data are consistent with these findings if it is assumed that the reinforcement produced by stimulation of the dopaminergic and noradrenergic systems is qualitatively similar. They furthermore suggest that *d*- and *l*-amphetamine may differ in their ability to increase the synaptic release of noradrenaline from noradrenergic neurons, but have a similar action on spontaneous release from dopaminergic neurons. This latter suggestion has received partial support from the experiments of Carr and Moore (*10*), but further experiments will be required to determine its overall validity. In any event, our data are consistent with previous reports showing that behaviors thought to be mediated by brain norepinephrine or dopamine are differentially responsive to the two isomers of amphetamine (*17, 22*).

Based on biochemical and histochemical evidence (*23*) it has been suggested that both dopaminergic and noradrenergic systems in the brain will support ICS (*2, 3*). The differential facilitatory effects of *d*- and *l*-amphetamine at sites in the MFB and the SN provide pharmacological support for this hypothesis. They furthermore suggest that it may be possible to identify any given electrode which will support ICS as having either dopaminergic or noradrenergic substrates, or both. In view of these considerations, the "noradrenergic theory" of ICS should be qualified (*5, 9*).

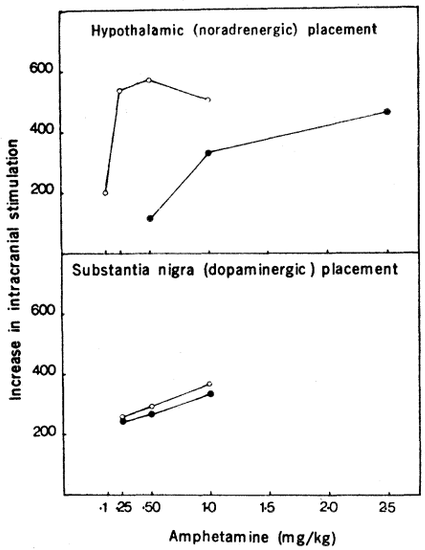

Fig. 1. Increase in intracranial self-stimulation (ICS) above control levels from two electrode placements in the brain, produced by different doses of *d*- (○—○) and *l*-amphetamine (●—●). Control levels were obtained for each animal by determining the difference between two daily 15-minute test sessions on each of the 6 or 7 days before the drug was given. The abscissa represents the drug induced increase in ICS relative to the average change observed on control days.

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19. Values of "response thresholds" were determined by first raising the current intensity in 5-μa steps every 5 minutes, from an initial value of 0 to 100 μa. A more precise estimate was then obtained on the next 2 days by raising the current intensity in 2-μa steps throughout a 20-μa range already established on the first day of threshold determination.
20. The results were analyzed statistically by analysis of variance and by the Wilcoxon matched-pairs signed-ranks test. S. Siegel, *Nonparametric Statistics for the Behavioral Sciences* (McGraw-Hill, New York, 1956), pp. 75–83.
21. *d*-Amphetamine produces an increase in the spontaneous release of catecholamines at doses which are ten times lower than those required to inhibit catecholamine uptake and these lower doses are in accord with the central stimulant properties of low doses of amphetamine (*12*). Furthermore, drugs that are potent inhibitors of catecholamine uptake

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A Neuronal Inhibition Mediated Electrically

Abstract. *When the goldfish Mauthner cell fires an impulse there is a simultaneous hyperpolarization of adjacent medullary neurons as far as 200 micrometers from its soma. This hyperpolarization is due to an inward transmembrane flow of some of the current generated by the Mauthner cell spike and is sufficiently large to block spikes evoked both directly or transsynaptically.*

"Electrical inhibition" can be mediated in a number of vertebrate and invertebrate systems where neuronal elements are electrotonically coupled (*1*), but since the coupling is often weak, its excitatory effects generally predominate. In addition, the extracellular currents generated by nerve cell impulses can also decrease the excitability of adjacent neurons (*2*); again, however, the effects of such ephaptic currents have generally been found to be facilitatory (*3*). The clearest case of a significant electrical inhibition described so far, that of the Mauthner cell, has been attributed to a failure of impulse propagation in the neurons mediating this effect (*4*). We report here a presumably related case where the extracellular currents set up by the action potential of a single neuron can impose an inhibitory hyperpolarization on the membrane of neighboring cells.

Experiments were performed on goldfish (14 to 22 cm in length) paralyzed with Flaxedil (1 μg per gram of body weight) and perfused through the mouth with dechlorinated tap water. The methods used for exposure of the medulla and for electrical stimulation of the eighth nerve and spinal cord were similar to those described elsewhere (*4*, *5*). Intracellular recordings from the Mauthner cell (M-cell) and from adjacent neurons located as far as 200 μm from its soma were obtained with single- or double-barreled micropipettes filled with 0.6*M* K_2SO_4 or 3*M* KCl. A Howland pump (*6*) was used to inject current intracellularly.

Antidromic invasion of the M-cell produced by a spinal cord stimulation results in a characteristic all-or-none extracellular negativity which is followed by a later positivity, the well-known (*4*) "extrinsic hyperpolarizing potential" (Fig. 1A). The negativity is as large as 20 to 40 mv in the axon cap and falls off steeply in amplitude as the recording electrode is moved away from the maximum focus. Surprisingly, we have found when recording from cells located in regions where the extracellular field is no more than a few millivolts that there is a corresponding but significantly larger intracellular negativity, as illustrated in Fig. 1B. Subtraction of the extracellular field (Fig. $1B_2$) from the intracellular potential (Fig. $1B_1$) indicates a net membrane hyperpolarization of 6.4 mv. Usually lower values were found (Fig. 1C); in 93 cells the average was 2.01 mv, with a range of 0.7 to 6.4 mv. In all the investigated neurons it had the same threshold, all-or-none character (Fig. 1, B and C), latency, and peak time (Fig. 1, A and B) as the M-cell field. Since the M-cell has the fastest conducting axon in the goldfish spinal cord (*5*) this hyperpolarization cannot be mediated through chemical synapses (*7*); it is therefore necessarily brought about by an inward transmembrane flow of the current generated by the M-cell spike, and it may be termed a "passive hyperpolarizing potential" (PHP).

If this hypothesis is correct, the PHP should decrease membrane excitability and should be relatively independent of membrane potential. Both characteristics were demonstrated. As shown in Fig. 1, D and E, when adequately timed the PHP did block the generation of spikes either directly evoked by depolarizing current pulses (Fig. 1D) or synaptically induced by stimulation of the ipsilateral eighth nerve (Fig. 1E). The latter effect could already be observed extracellularly prior to penetration of the neurons. Finally, when the PHP was made to interact with a subthreshold excitatory postsynaptic potential (EPSP) the two summated algebraically. The insensitivity of the PHP to changes in membrane potential produced by applied transmembrane currents is illustrated in Fig. 2: the size and time course of the PHP evoked during large hyperpolarizing

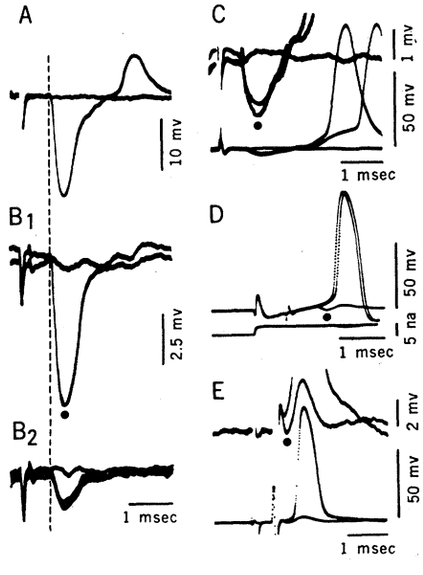

Fig. 1. Evidence that impulses evoked in the M-cell can generate inhibitory PHP's in adjacent neurons. (A to C) The spinal cord was stimulated at strengths straddling the threshold for M-cell antidromic activation; several sweeps are superimposed on each record. (A) Antidromic field potential extracellularly recorded in the axon cap. (B_1) Intracellular recording obtained during the same experiment as in (A) from a neuron located 75 μm caudal to the axon cap; the spinal stimulus evoked a PHP. (B_2) Field potential recorded outside that cell. The vertical dashed line indicates that the potentials in (A) and (B) had the same latency. (C) Another example of an intracellular PHP, followed in this case by an EPSP which fired the cell. The records in (D) and (E) are from two different neurons; in each record, sweeps were superimposed without and with spinal stimulation. (D) A spike evoked by a depolarizing current pulse (lower trace) was blocked by an adequately paired PHP. (E) A spike synaptically evoked by stimulation of the ipsilateral eighth nerve was similarly blocked; its failure unmasked a now subthreshold EPSP [the upper and lower traces in (C) and (E) are recordings of high a-c and low d-c gain, respectively]. In all records positivity is upward, and the PHP is indicated by a filled circle.