unwanted side effects. Although the type and cause of side effects are diverse, we will consider a simple model in the hope that it will provide some insight into the advantages of two-drug therapy for more complex cases. We consider the case (*4*) in which drugs exhibit their side effects by binding to side-effect sites whose dissociation constants (K_S) are proportional to the dissociation constants for their desired effects (K_D):

$$K_S = R\,K_D \quad (3)$$

The parameter R is a measure of how much higher the affinity of the drug is for its desired receptor than for its side-effect receptor. In general, large R corresponds to large therapeutic index.

In our model we assume that the side effects of the two drugs are based on equilibrium binding, are additive, and are not synergistic. This corresponds to a case, for example, in which (i) the desired effect is to block sodium channels, which have two equivalent binding sites, and (ii) the side effects are caused by interactions of both drugs with the same receptor of a different channel.

With the above assumptions, the fraction of states related to the side effects that have a drug bound, F_{S2}, can be found from Eq. 1 by eliminating the cross-terms D_1D_2/K_1K_2 and by substituting RK for each K. The result is

$$F_{S2} = \frac{(D_1/K_1) + (D_2/K_2)}{R + (D_1/K_1) + (D_2/K_2)} \quad (4)$$

Since we want to obtain a desired value of F_2 with a minimum value of F_{S2}, regardless of dosage, we combine Eqs. 1 and 4 to obtain F_2 as a function of F_{S2} and R. In general, F_2 is also a function of the relative dosages of the two drugs. It can be shown that in order to maximize the ratio of desired effect to side effect, the two drugs should be administered in proportion to their dissociation constants. For this mixture, F_2 can be expressed as a function of F_{S2} and R only:

$$F_2 = \frac{(1/4R^2 - R)F_{S2}^2 + R\,F_{S2}}{(1/4R^2 - R + 1)F_{S2}^2 + (R - 2)F_{S2} + 1} \quad (5)$$

To obtain the comparable equation for a single drug, we can substitute RK for K in Eq. 5 to obtain the side-effect relation for one drug:

$$F_{S1} = (D_1/K_1)/(R + D_1/K_1) \quad (6)$$

We can combine Eqs. 2 and 6 to obtain F_1 as a function of F_{S1} and R for a single drug:

$$F_1 = (R\,F_{S1})/[(R - 1)F_{S1} + 1] \quad (7)$$

The two-drug side effect–response curve represented by Eq. 5 is compared with the one-drug side effect–response curve of Eq. 7 in Fig. 1A for several values of R. The reduction in the side effects of two-drug therapy is strongly dependent on both R and the fraction of states with at least one drug bound. This can be seen more explicitly in Fig. 1B, which shows the ratio of two-drug side effects to one-drug side effects corresponding to a given degree of binding and a given value of R. Figure 1B shows that side effects measured in molecular terms (such as fraction of channels blocked) can be reduced by as much as a factor of 2. However, Fig. 1B also shows that in order to obtain even a 20 percent reduction in side effects, it is necessary to block about half of the channels. It is not known what fraction of sites are blocked by drugs, but the large safety factor in number of available channels in axons suggests that blocking half of the available channels is a possible role for therapeutically useful drugs. The significance of a 20 percent reduction in side effects is difficult to evaluate, but there are two reasons why such a potential advantage should not be ignored. One is that this advantage can be obtained with no loss in efficacy. The other is that even a modest reduction in the number of side-effect channels blocked may provide a large physiological improvement.

Figure 1B only applies to cases in which side effects are additive. If the side effects are not only additive, but also synergistic (in the same way that the desired effects are synergistic), benefits will be smaller than indicated in Fig. 1B. On the other hand, if the side effects are neither synergistic nor additive, the benefits of two-drug therapy will be larger than indicated in Fig. 1B.

GERALD EHRENSTEIN
LI-YEN MAE HUANG
Laboratory of Biophysics, National Institute of Neurological and Communicative Disorders and Stroke, Bethesda, Maryland 20205

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Intracranial Self-Stimulation in 3-Day-Old Rat Pups

Abstract. *Three-day-old rat pups with electrodes directed at the medial forebrain bundle at the level of the lateral hypothalamic area were trained to push a paddle to receive electrical brain stimulation. Pups receiving stimulation that was contingent on lifting the paddle responded more frequently than did control pups and also learned a two-choice spatial discrimination task that was rewarded with brain stimulation. The experiments indicate that a neural substrate in the area of the medial forebrain bundle is involved in the central mediation of reinforcement in the rat pup.*

Although their neural development is not mature (*1*), during the first week of life rat pups can eat and drink independently (*2*), and they have been successfully trained in classical (*3*) and instrumental (*4*) conditioning experiments. One-day-old rat pups have learned an operant task to obtain oral injections of milk (*5*). Thus, under certain testing conditions, altricial rat pups exhibit motivated behaviors that are adultlike in complexity.

This behavioral complexity suggests that the central nervous system of the newborn rat contains elements for the representation of relationships among classes of stimuli, responses, and consequences. The central mediation of adult affective behavior has been investigated by electrical self-stimulation of the brain (*6*). We have developed methods that allow exploration of the infant brain by electrical self-stimulation and report that 3-day-old rat pups raise a paddle and solve a spatial discrimination task to receive electrical stimulation to the medial forebrain bundle.

Three-day-old Sprague-Dawley rat pups from our colony were removed from their litter, weighed (*7*), and anesthetized in ice. An ice bath, which was attached as a surgical stage to a modified Stoelting-Stellar stereotaxic device, maintained anesthesia throughout the electrode implantation. The pup's head was immobilized by gently securing it between concave clamps that fit snugly over the ears. To minimize the duration of anesthetization, a littermate served as a model for the construction of an acrylic crown around the electrode unit, which consisted of a monopolar electrode and

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skull ground embedded in the dried acrylic. The crown was fastened to the skull of the experimental pup with a cyanoacrylic cement (Zipbond, Tescom Corporation). Electrodes were placed in the medial forebrain bundle at the level of the lateral hypothalamus (*8*). Pups were anesthetized for less than 10 minutes and, upon removal from the cold, recovered rapidly.

After surgery, subjects were placed with littermates in a heated (30°C), humid environment to recuperate for 3 to 4 hours. Pups were then pretested (*9*) to ascertain the current threshold for use in the operant task. Pups received 60 cycles per second for 500 msec of direct-current stimulation trains. Response thresholds varied from 40 to 60 μA.

We adapted the testing situation of Johanson and Hall (*5*) to exploit the pup's propensity to probe upward along a surface. Clear plastic drinking cups (8 ounces) served as experimental chambers. Felt-covered response paddles extended into the chambers and were mounted 4 cm from the floor. A small patch of felt leading toward the paddle directed the pup's movement. In the first two experiments the chambers contained one response paddle. Lifting the paddle, which required a force of 5 g, closed a microswitch and activated the stimulator, causing threshold current to pass into the brain at the electrode tip for 500 msec.

First we examined the pups' ability to increase their rate of responding to obtain brain stimulation by comparing the response rates of pups that received stimulation with those of identically treated controls over 18 hours. In all cases ($N = 4$) the experimental pups probed into the response paddle more frequently than did controls (mean 18-hour totals: experimental, 161.7 ± 24.3 responses; control, 94.7 ± 12.3 responses; $t(7) = 2.442$, $P < .05$). During the first 10 hours of testing there was no statistically significant difference in response rates between the two groups (mean responses per hour: experimental, 4.3 ± 0.09; control, 4.9 ± 1.5). After 10 hours, the response rates of the stimulated pups more than tripled, but the response rates of control rats did not change (mean responses per hour: experimental, 14.9 ± 2.3; control, 5.4 ± 0.5; $t(7) = 3.848$, $P < .01$).

Although the pups receiving brain stimulation may have increased their rate of responding to obtain the reinforcement of the stimulation, brain stimulation also activates pups (*9*), and the differences in response rates may only have reflected an increase in activity. To test this possibility, we repeated the experiment with a new group of animals and used yoked rather than nonstimulated controls. Experimental pups ($N = 6$) again received a 500-msec pulse train of brain stimulation for probing into the response paddle. The littermates used as yoked controls were placed in a chamber with a response paddle but received brain stimulation only when the paired experimental pup made a response. To further discount activation as an explanation of response differences, the pup that was the more robust responder during the pretest (*9*) served as the yoked control. Pups were tested for 18 hours.

Experimental pups responded more than the yoked controls (mean responses, 298.5 and 137, respectively; matched pair, $t(5) = 3.216$, $P < .05$). The cumulative response curve for the most representative experimental pup and its yoked control (Fig. 1A) indicates that at the beginning of the test period experimental and control pups probed the response paddle at the same rate; at the end of the test period, however, the response rate of experimental pups increased greatly, while that of yoked pups did not change although they received the identical pattern of brain stimulation (*10*). During the last 2 hours of the test, experimental pups emitted a mean of 43 more responses than did the yoked pups. Indeed, the response rates of five of the six experimental pups more than tripled during the last 2 hours as compared with rates from the first 2 hours of the test.

These experiments demonstrate that the probability of pups emitting a response is increased when that response results in brain stimulation and that the increase is contingent on the reinforcing rather than the activating properties of the stimulation. Thus 3-day-old rat pups can learn a relation between a response and the reinforcing effects of brain stimulation. We next evaluated the ability of pups to learn a spatial discrimination to receive brain stimulation. A new group of pups was tested in an operant chamber with two paddles on opposite sides of the chamber. A probe into only one of the paddles resulted in brain stimulation. Pups were given 18 hours to learn to discriminate between the paddles.

In all six cases (*11*) pups responded much more frequently to the paddle that produced stimulation than to the unrewarded paddle (mean responses, 304.8

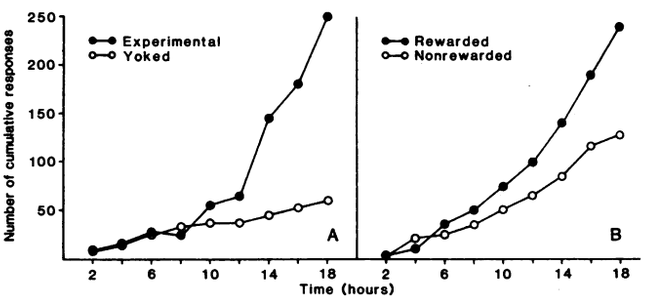

Fig. 1. Cumulative responses at 2-hour intervals (A) for a representative experimental pup and its yoked control and (B) to rewarded and nonrewarded paddles for a representative pup.

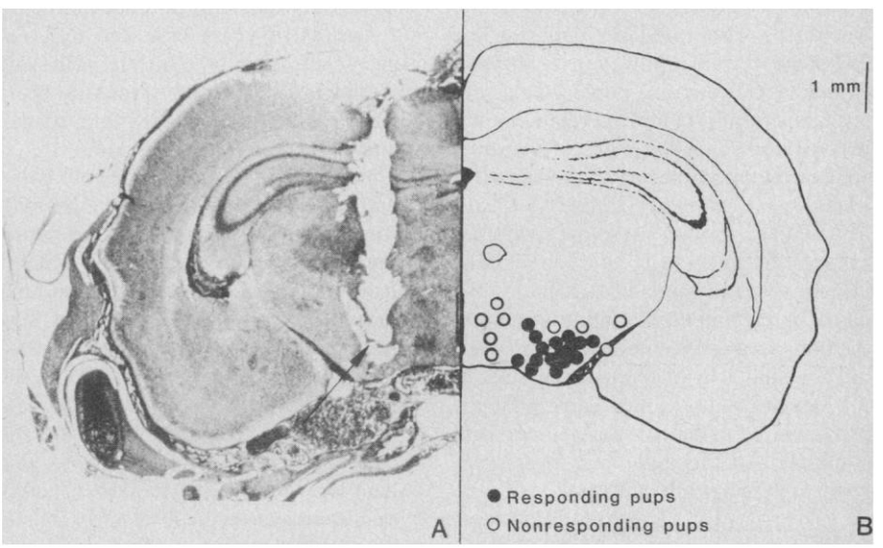

Fig. 2. (A) Representative coronal section showing electrode track. Tissue damage around the track caused by the removal of the electrode is characteristic. Arrow denotes our estimation of the location of the electrode tip. Brain was cut within decalcified skull. (B) Diagrammatic representation of electrode location in responding and nonresponding 3-day-old pups. Locations in the area of the medial forebrain bundle support the performance of the operant task.

versus 163.3; matched pair $t(5) = 4.194$, $P < .01$). For the first 8 hours of the test, response rates were similar; then responses to the paddle that produced stimulation increased (Fig. 1B). Individual animals required varying amounts of time to form the discrimination. Those whose initial rate of responding was high acquired the discrimination more rapidly. Some animals learned in as little as 3 hours; others took as long as 15 hours. In general, pups did not increase their rate of responding to the paddle with the reward until they had received approximately 75 stimulation trains. This was also the case in the experiments with a single paddle.

Electrode placement was verified histologically, as shown in Fig. 2. Only electrodes in the medial forebrain bundle in the area of the lateral hypothalamus support the performance of the operant task. This is a site which, in the adult, has been shown to support very high rates of intracranial self-stimulation (*12*). Placements that were either medial or dorsal to this location were ineffective (*13*) during the pretest even at a current range of 30 to 80 μA, and the response rates of these animals did not increase. Thus there is a strong correlation between electrode placement, behavior during the pretest, and learning the operant response.

This work indicates that stimulation of the medial forebrain bundle can reinforce behavior in 3-day-old rat pups. Brain sites that support self-stimulation in adults correspond to projections of catecholamine pathways, and reinforcement is thought to be mediated by the activation of dopamine neurons (*14*). Development of central norepinephrine and dopamine systems, however, is far from complete in 3-day-old pups. Density of terminals is only 15 to 40 percent of adult levels (*1*) and, on the basis of axotomy studies, neuronal activity is not present in dopamine pathways as late as 6 days of age, even though these pathways are capable of generating and conducting impulses (*15*). Either self-stimulation behavior is mediated by another system in the pup, an unlikely possibility considering the similarity of supporting site, or the development of the catecholamine pathways is sufficient to mediate self-stimulation at this age when these pathways are electrically activated.

TIMOTHY H. MORAN
MARK F. LEW
ELLIOTT M. BLASS
Department of Psychology, Johns Hopkins University, Baltimore, Maryland 21218

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7. Only pups weighing between 10.0 and 11.0 g were used in all experiments.
8. The stereotaxic coordinates, taken from the confluence of the midsagittal suture and lambdoidal fissure, were 2.0 mm anterior to the bregma, 1.2 mm lateral to the midline, and 6.5 mm below the skull surface.
9. Results of the threshold test were used as the basis for including pups in the study. Pups responded to brain stimulation by displaying a progressive behavioral sequence. In response to single stimulation trains pups showed mouthing and chewing. Vigorous activation, licking, and stretch responses were emitted to multiple stimulation trains. These responses were a reliable predictor of successful performance in the operant task.
10. Response rates of yoked pups decreased slightly over the test, a result which is not surprising since yoked controls were essentially being reinforced for remaining on the floor of the cup.
11. In some instances pups emitted fewer than 20 responses in the first 10 hours of the test period. Results from these pups were discarded from the study.
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16. We wish to thank M. Williams for her expert histology. This work was supported by grant AM 18560 from the National Institute of Arthritis, Metabolism, and Digestive Diseases (to E.M.B.).

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A Brain for All Seasons: Cyclical Anatomical Changes in Song Control Nuclei of the Canary Brain

Abstract. *Male canaries that have reached sexual maturity can, in subsequent years, learn new song repertoires. Two telencephalic song control nuclei, the hyperstriatum ventrale, pars caudale, and nucleus robustus archistriatalis are, respectively, 99 and 76 percent larger in the spring, when male canaries are producing stable adult song, than in the fall, at the end of the molt and after several months of not singing. It is hypothesized that such fluctuations reflect an increase and then reduction in numbers of synapses and are related to the yearly ability to acquire new motor coordinations.*

The song of adult male canaries is a motor skill learned by improvisation (*1*) and by imitation of other males (*2*), in either case requiring intact hearing and access to auditory information (*3*). A male canary has the potential to learn on successive years new and different song repertoires (*4*). In the following experiment I have tried to identify brain changes in adulthood that relate to this yearly learning of a motor skill.

First-year male canaries (*5*) hatched in April develop stable adult song by mid-January, when 9 months old. The song patterns developed at that time last for the duration of the breeding season, until approximately mid-June. Canaries sing little if at all during the summer months. A total absence of song characterizes the period of the molt, lasting roughly from mid-August to mid-September. As the molt ends, male canaries start to sing once more, first in the tentative, highly variable manner typical of early plastic song. By early January, birds well into their second year of life have developed a new, stable song repertoire (*4*).

In the experiment described here, 21 male canaries hatched in mid-April were used. At 10.5 months of age they were caged singly. Nine of these birds were killed the following April, when 12 months old. These birds were then in full reproductive condition and were producing stable adult song. The remaining 12 canaries were paired with females and allowed to breed (*6*), then killed 5 months later, in mid-September, toward the end of the molt, when 17 months old (*7*). Blood (1/2 ml) was obtained by intracardiac puncture before birds died (*8*). The testes and brain were removed after perfusion (*9*).

Spring and fall volumes were obtained for each of the following brain structures (*10–12*): two telencephalic nuclei involved in song control, the hyperstriatum ventrale, pars caudale (HVc), and the nucleus robustus archistriatalis (RA) (*13*); two discreet midbrain nuclei not known to be involved in song control, nucleus rotundus (Rt) and spiriformis medialis (SpM) (*14*); and the caudal forebrain at the level of HVc, referred to subsequently as caudal forebrain volume (*15*). This last measurement was taken in order to get an impression of the size of the telencephalon over the rostro-

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