comes: at what level of concentration does a minor member of a pair of duplicate gene products become sufficiently functionless so as to be invisible to selection? We are inclined to believe that level has been reached in the case of A_2 where the contribution of this component to overall hemoglobin concentration is quantitatively less than day-to-day variation in total concentration. Nonetheless, even if the variation of δ sequence and the contemporary polymorphism of A_2 are largely or entirely attributable to non-Darwinian evolution, it is still necessary to posit selective constraints upon β in order to explain the lesser variation in β than δ (Fig. 2). These constraints upon β have apparently differed in several evolutionary epochs in that detectable variation of β among the New World primates studies has only occurred since the taxonomic divergence of these species. The fact that δ variation is less in the latter interval than in an earlier time suggests, on the presumption of δ neutrality, that the postspeciation interval among the New World primates examined has been shorter in duration than the prespeciation period.

Despite the uncertainties attached to our presumptions, and thus to our interpretations, we regard the situation of duplicate genes with products of major and minor concentration as one that provides a potentially useful model for assaying the relative influences of Darwinian and non-Darwinian factors in evolution.

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16. Our finding of an unusual number of sites (7 of 21) with two or more changes in closely related chains and species (Fig. 2) is at variance with the random distribution of about 484 changes in globin obtained from a less closely related group of animals (*9*). If hypermutability is a property of particular codons rather than particular positions, then this phenomenon would tend to be self-eliminating and would not be observed when data from very diverse species are compared.
17. Alternative explanations for the findings at position 5 include (i) a complex succession of nonhomologous recombinations between β and δ, and (ii) homozygosity for a mutation affecting the amino acid acceptor site of transfer RNA used as this position. In either case, the mutation would need to have taken place after divergence of man and New World primates from the stem line.
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20. Threonine occurs at β-12 and histidine at β-117 in both brown lemur (*18*) and rhesus (*19*). It is believed that these residues have been preserved in both animals since their divergence from the primate stem line. These residues are assigned to the archetype (Fig. 2), since it is supposed that the ancestors of lemurs diverged before and those of rhesus after the branching between the progenitors of man and New World primates.
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24. The following abbreviations are used: Ala, alanine; Arg, arginine; Asn, asparagine; Asp, aspartic acid; Gln, glutamine; Glu, glutamic acid; Gly, glycine; His, histidine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Tyr, tyrosine; Val, valine.
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Lateral Hypothalamic Stimulation: Inhibition of Aversive Effects by Feeding, Drinking, and Gnawing

Abstract. *The opportunity to engage in feeding, drinking, and gnawing behavior facilitated by localized hypothalamic stimulation can delay the onset of the aversive effects of the stimulation and may completely suppress them. This suggests that the aversive effects of the stimulation are due to the excessive arousal of a drive.*

Electrical stimulation of some brain sites gives rise to both rewarding and punishing effects (*1–3*). Short durations of stimulation are rewarding, while longer durations become punishing. Cats and rats with electrodes in these parts of the brain typically make one response to turn on the stimulation, wait for a few seconds, and then very quickly make another response to shut it off. The on-off sequence may be repeated over 80 times during a 10-minute period (*4*). This pattern of intracranial self-stimulation is usually interpreted as indicating that the stimulation is initially rewarding (as indicated by the fact that the animals turn it on), but soon becomes aversive (thus motivating the animals to turn it off) (*5*).

While there have been a number of studies concerned with the effects of stimulation parameters, such as intensity (*3, 6*), on the preferred duration of stimulation, there is a lack of experiments attempting to analyze the nature of the aversive process itself. One clue as to the aversive quality of the stimulation may be gleaned from an observation of the responses elicited by the stimulation. Electrodes whose stimulation gives rise to both rewarding and punishing effects frequently lie in and around the lateral hypothalamic area. This is the same area whose stimulation facilitates a variety of motivational activities (*1, 7*), such as feeding, drinking, gnawing, biting, sexual behavior, grooming, wheel-running, exploration, and various components of maternal behavior (nest building and pup retrieval).

Many of these behaviors induced by brain stimulation have been shown to be

Table 1. Durations (medians) of brain stimulation selected by each rat in the empty shuttle box and in the presence of appropriate goal objects (food, water, or wood). The intensity in microamperes is a root-mean-square value. Feeding, F; drinking, D; and gnawing, G.

Rat No.	Intensity (μa)	Behavior	Median ON durations (sec)	
			Empty	Goal object
47	50	F	14	22
52	60	F	7	12
67	55	F	20	107
89	21	F	45	101
47	50	D	14	31
67	55	D	17	233
91	37	G	4	17
119	30	G	9	31
120	50	G	20	34
125	25	G	9	32
Medians			14	33.5

associated with motivational states or drives (hunger and thirst), as indicated by the fact that the stimulated animals are motivated to engage in instrumental responses to procure appropriate goal objects (food and water) (*8, 9*). The stimulation that arouses these drives is almost invariably found to be strongly rewarding when administered at moderate intensities (*7*). It has been suggested that the stimulation is rewarding because of its drive-induction properties (*7*). It could also be that stimulation which gradually shifts from reward to punishment does so because of an excessive buildup of drive. That is, a mild intensity of drive induction might be rewarding and a strong intensity, aversive.

If brain stimulation becomes aversive because of an excessive arousal of a drive to engage in a particular kind of behavior, the performance of such behavior might counteract the aversive nature of the drive. Thus, given an opportunity to engage in the behavior whose drive is aroused by brain stimulation, a rat might never develop the motivation to terminate the stimulation. This report concerns an experiment designed to test this hypothesis.

A large number of naive albino and hooded rats were implanted with two monopolar, stainless steel electrodes aimed at the perifornical area of the lateral hypothalamus on each side of the brain (*10*). A screw mounted in the skull served as indifferent electrode. The animals were maintained individually in cages in which Purina rat food and tap water were always freely available. After allowing at least 3 days for recovery after the operation, each rat was tested in a cage similar to its home cage, with food, water, and wood freely available. Each hypothalamic electrode was tested separately to see if stimulation at the electrode site induced feeding, drinking, or gnawing. A sine-wave current (60 hz) was passed between the deep electrode and the indifferent electrode at intensities varying from 5 to 60 μa. The current intensity was gradually raised until a rat began to feed, drink, or gnaw or until its behavior became so disorganized that it could not do so.

The main experiment was conducted in a shuttle box measuring 78 by 30 by 36 cm high. The floor of the box was mounted in its center on an axle running widthwise. Microswitches under each end of the floor controlled the onset and offset of the brain stimulus. The switches were operated by the weight of the rat as it ran from one side of the box to the other, thus depressing one side of the floor. One side was designated as the ON side and the other the OFF side, corresponding to whether the operation of the microswitch under the floor put the stimulation on or off. To depress a microswitch the rat had to progress to within about 16 cm of one of the ends of the box; this varied slightly from rat to rat, depending on the animal's weight (*11*).

To find a stimulation intensity which would be likely to produce shuttling in the empty shuttle box, the animals exhibiting stimulation-induced feeding, drinking, or gnawing behavior were tested again after the food, water, and wood had been removed from the test cage. Each rat was given up to 15 seconds of continuous stimulation at the threshold intensity for eliciting the behavior. If the animal attempted to leap out of the test cage after 10 to 15 seconds of stimulation, then the stimulation was terminated and this value was used in the shuttle box. If this escape behavior did not occur at the threshold intensity, then the current was raised in 2- to 3-μa steps until an intensity was found which reliably produced escape behavior within 10 to 15 seconds of stimulation. This intensity was then selected for testing in the shuttle box. If escape behavior failed to occur at 60 μa or less, the animal was discarded. Thus, two criteria were used to determine whether a rat would be used in the shuttle box experiment. (i) The stimulation had to induce feeding, drinking, or gnawing behavior at an intensity between 5 and 60 μa; and (ii) the stimulation had to induce escape within 15 seconds at an intensity between the threshold for the behavior and 60 μa. Eight animals met both criteria; two of them both ate and drank upon stimulation, two only ate, and four gnawed.

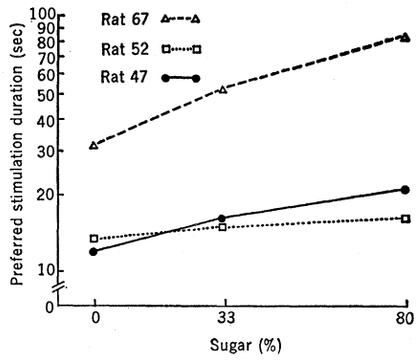

Fig. 1. Median duration of feeding-inducing brain stimulation selected by each rat as a function of the percentage of sugar in the available food. Stimulation intensity was the same as in Table 1.

Each of these rats was placed individually into the OFF side of the box and given an opportunity to learn how to control the onset and offset of the current during four 20-minute periods. If a rat failed to cross over to the ON side of the box within any 5-minute period, it was placed there by the experimenter. All the rats learned how to operate the shuttle box and their preferred durations of current on and current off stabilized within four training periods.

Then the rats were given one pair of tests on each of 4 days (*12*). On each day one test was conducted in the empty box and the other in the presence of the appropriate goal object. The tests were separated by at least 1 hour, and their order of administration was varied from day to day. The tests for the feeders and drinkers were of 5-minute duration; for the gnawers, 10 minutes. Of the two feeders that were also drinkers, one was given all the food tests before the water tests, and the other was tested with food and water available on alternate days. On the goal-object tests, the food, water, or wood was equally distributed on both sides of the shuttle box, to control for novelty effects. Thus, the entire floor of the shuttle box would be covered with a thin layer of Purina food powder or water, or wooden sticks would be placed on each side of the box. At the beginning of each test, an animal would be placed in the OFF side of the box facing the ON side and given one warm-up trial. At the end

of the test, if the animal was on the OFF side it was removed immediately; if on the ON side, it was not removed until it crossed over to the OFF side. The animals received no signal indicating the end of the 5- or 10-minute session.

In the empty shuttle box the median preferred ON durations were 4 to 20 seconds for seven of the rats and 45 seconds for one rat (*13*). The introduction of appropriate goal objects into the box produced a dramatic change in all the animals' behavior. Instead of the restless searching, rearing, and sniffing which was characteristic of their behavior on the ON side of the empty box, each rat calmly engaged in feeding, drinking, or gnawing behavior while the current was on in the presence of appropriate goal objects. Their median ON times increased by at least 50 percent and in most cases by more than 100 percent. Their range was now 12 to 233 seconds (Table 1). While eating the food or drinking the water, the rats would typically stroll slowly around the box, occasionally appearing to accidentally enter deep into the OFF side, thereby terminating the current. In fact one of the feeders (rat No. 89) and one of the drinkers (rat No. 67) seemed never to terminate the stimulation intentionally, but only accidentally by wandering into the OFF side while feeding or drinking (*14*). The combined median ON duration for all the rats was 14 seconds under the empty condition and 33.5 seconds under the goal-object condition (*15*).

Thus for all the rats the onset of the aversive effects of the brain stimulation was delayed, and for two of them it appeared to be almost completely suppressed by performance of the behavior facilitated by the stimulation. To evaluate the role of sensory input in suppressing these aversive effects, three of the feeders were given additional tests with powdered foods containing 0, 33, and 80 percent sugar (*16*). Each rat was given three 10-minute tests per day, one with each concentration of sugar. The tests were separated by 3 hours. One hour before each test, the food mixture to be used in the test was placed into the rat's cage to insure that the rat would be satiated on the test food. Each rat was tested for six consecutive days; each day the tests were administered in a different order. The results were clear for all animals: the greater the concentration of sugar in the food, the longer the preferred ON duration (Fig. 1). This trend was statistically significant for each rat (*17*). Thus the greater the palatability of the food, the greater the inhibition of the aversive effects of hunger-inducing brain stimulation.

These data are consistent with the hypothesis that the aversive effects of hypothalamic stimulation are due to an excessive arousal of a drive, and that these effects can be inhibited by the engagement of the consummatory behavior facilitated by the drive. Most previous studies have emphasized the role of stimulation parameters and brain locus as the primary determinants of the reinforcing effects of brain stimulation (*1*). However, four experiments have shown that the affective tone of hypothalamic stimulation is also a function of the environment in which the subjects are tested (*18*): if the environment lends support to activities facilitated by the stimulation, then its rewarding effects are augmented. The present experiment complements the previous ones by demonstrating that in such environments the aversive effects of stimulation are diminished.

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11. This testing apparatus was designed for the experiment because it provided for simplicity of the responses for initiating and terminating the stimulation and for freedom of movement for the rat while the stimulation was on or off.
12. There were three exceptions to this. Rat No. 91 dislodged its electrode after two tests in the empty box and one test with wood, and rats Nos. 89 and 120 stopped engaging in stimulation-induced feeding and gnawing, respectively, after their second pair of tests.
13. Rat No. 89 initially had much shorter ON times (median, 29 seconds), but then developed the habit of chasing and chewing its hind legs. This greatly increased its ON times (median, 60 seconds), presumably because chewing decreased the aversive effects of the stimulation.
14. Rat No. 67 appeared to intentionally terminate the stimulation on only one of its food tests, but only accidentally on the other three tests. All its terminations on the water tests appeared to be accidental.
15. It should be noted that this result is obtained with food and wood only if they are not easily transportable. However, if food pellets or small pieces of wood are used, then the preferred ON times decrease by more than 50 percent and the animals transport the food or wood from the ON side to the OFF side. This is probably related to the fact that in the rat's natural environment "hoarding" successfully competes with feeding when hungry rats find food in an insecure place. In this experiment the ON side is insecure or potentially dangerous (the stimulation becomes aversive if allowed to continue too long); so the rats hoard portable objects to the OFF side (where there is never any aversive stimulation), thus obscuring the present phenomenon.
16. Purina rat food powder was thoroughly mixed with various amounts of brown sugar. For rats Nos. 47 and 67 these tests were conducted about 6 months after the first experiment, by which time their preferred ON durations had slightly changed.
17. $P < .001$ by a nonparametric trend analysis [G. A. Ferguson, *Nonparametric Trend Analysis* (McGill Univ. Press, Montreal, 1965)].
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19. This research was begun at the Massachusetts Institute of Technology, continued at the University of Michigan and McGill University, and completed at Rutgers University. Supported in part by NIH grants to S. L. Chorover (MH-07923), S. E. Glickman (MH-13253), D. Bindra (MH-03238), and J. Olds and J.M. (MH-31258 and MH-14410), and by NSF grant GB-7370 to J.M.

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Compulsive Sexual Activity Induced by p-Chlorophenylalanine in Normal and Pinealectomized Male Rats

Abstract. p-*Chlorophenylalanine depletes brain serotonin and induces long-lasting sexual excitation in male rats. The effect of* p-*chlorophenylalanine is potentiated by pargyline. Administration of 5-hydroxytryptophan to rats treated with* p-*chlorophenylalanine plus pargyline blocks the sexual excitation.* p-*Chlorophenylalanine also elicits sexual excitation in pinealectomized rats; this effect is not mediated by the lack of indole hormones in the pineal but may be the consequence of depletion of 5-hydroxytryptophan in the brain and the resulting imbalance between 5-hydroxytryptophan and catecholamine activity in the central nervous system.*

Several studies suggest that brain serotonin (5-hydroxytryptamine, 5-HT) and the pineal hormones (melatonin and 5-methoxytryptophol) inhibit the estrous cycle and sexual behavior in female rats. Thus, Meyerson concluded that serotoninergic mechanisms inhibit the copulatory behavior (lordosis re-