the 95 percent confidence limits were narrower for the second group, this dose schedule was employed for testing the lyase reactors.

The change in circulating phenylalanine levels produced by reactors with low (HP-315) and high (HP-312) activity were compared (Fig. 2). Because of the sudden initial decrease of phenylalanine and the large variation among controls, it was difficult to evaluate further changes produced by the reactors. Under the conditions of these experiments, the phenylalanine levels obtained with the reactors overlapped those of the control group.

We therefore employed a different method to produce sustained hyperphenylalaninemia in dogs. We adapted to dogs and monkeys a method previously used in rats (*9*) to induce experimental PKU. We administered orally 200 mg of phenylalanine per kilogram of body weight daily, and 100 mg of *p*-chlorophenylalanine (a phenylalanine hydroxylase inhibitor) per kilogram of body weight every third day, resulting in hyperphenylalaninemia within 3 to 4 weeks. With this method it was possible to raise circulating phenylalanine to 11 to 29 mg per 100 ml of blood. The variation among animals was of no major concern, since, in the assay employed, each animal served as its own control.

Multitubular lyase reactors were inserted into arteriovenous shunts placed in heparinized dogs with experimental PKU (Fig. 3). Temporary shunts were prepared on both sides of four animals. Because of the variation among animals, phenylalanine is expressed as the percentage of initial values obtained before application of the reactor. After 15 minutes of exposure to the reactor, circulating phenylalanine decreased to 18 percent of the initial concentration. After the animals were treated for 30 minutes, phenylalanine started slowly to rise, but stayed below 40 percent of the initial value. After the reactor was removed, the rise continued, but was still approximately 50 percent 5 days after reactor application.

The rise within hours after sudden depletion is probably due to the release of phenylalanine from erythrocytes and tissues, in order to reestablish the equilibrium between circulating and stored phenylalanine. The later, slow rise of phenylalanine is the result of continuous dietary administration of phenylalanine and *p*-chlorophenylalanine.

On the basis of these experiments, it seems feasible to remove blood phenylalanine by multitubular enzyme reactors. Suitable enzyme reactors could become clinical tools for the management of PKU patients, particularly at times of infection and pregnancy-induced exacerbation.

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15 May 1978

## Tail Pinch Versus Brain Stimulation: Problems of Comparison

Koob *et al.* (*1*) claim that, since tail pinch and brain stimulation produce behaviors with common properties, "both manipulations may act through the same mechanism." This conclusion is not warranted by the authors' experiments. The results were interpreted in terms of qualitative similarities between the behavioral effects of electrical stimulation of the hypothalamus and tail pinching with a paper clip. However, the fact that the two types of stimulation can induce behaviors with common properties in no way constitutes evidence that they exert their behavioral effects through a common mechanism. An additional difficulty is that it is impossible to determine the magnitude of the effect of tail pinch, since unpinched control animals were not included. Certainly, wood-gnawing and eating have measurable latencies and durations in unpinched rats, and such measurements should have been reported.

Nevertheless, Koob *et al.* demonstrated that while the tail of a rat is being pinched, behaviors with the following properties occur. (i) The nature of the behavioral response is somewhat arbitrarily determined by goal objects. (ii) The response changes gradually with latencies decreasing and durations increasing over time. (iii) New habits will be learned in order to execute responses during tail pinching. The authors argue that these characteristics apply to the behavioral effects of both tail pinch and electrical stimulation of the brain. We agree that these characteristics may apply to behaviors produced by generalized behavioral activation. We are equally certain that arousal or generalized activation can be produced by electrical stimulation of certain brain regions. However, we are complelled to point out that electrically elicited behaviors cannot be universally accounted for in terms of arousal-related processes.

With regard to the first two points, critical distinctions between the behavioral effects of brain stimulation and responses to generalized arousal are evident from research over the past several years in which we have electrically stimulated the hypothalamus in rhesus monkeys free to move and interact socially during stimulation.


0036-8075/78/0901-0839$00.50/0  

The first contention of Koob *et al.* is that a relatively arbitrary, environment-dependent behavioral plasticity governs the nature of the behavior exhibited. However, in a number of species, electrical stimulation of specific sites in the hypothalamux can induce behavioral responses that are not solely dependent on characteristics of the test environment (*2*). Results from our own experiments demonstrate that it is possible to elicit qualitatively dissimilar responses within the same animal by simply stimulating different hypothalamic areas. For example, electrical stimulation of the dorsomedial nucleus of the hypothalamus of an adult male rhesus monkey will induce sexual behavior directed toward a receptive female. When, in the same setting, at any time before or after the evoked sexual response, one delivers the electrical stimulus in another area of the hypothalamus (for example, the ventromedial nucleus or the lateral preoptic area) it is possible to induce an aggressive response directed toward the same female who was the object of the sexual response. These results suggest to us a degree of specificity for some behaviors elicited by electrical stimulation of the brain that stands in contrast to the nonspecific, situationally dependent responses of eating, gnawing, biting, and licking that can be induced by painful stimulation, by tail pinch, or by electrical stimulation of a number of areas of the central nervous system, including regions of the hypothalamus.

A second contrast between our findings and those observed with tail pinch is that electrically elicited sexual and aggressive responses virtually always occur the first time an appropriate stimulus is presented. These responses are characterized by latencies that remain stable over many months. Latency to physical contact, from the onset of stimulation of aggression-producing sites, is usually less than 0.1 minute, even on the occasion when the behavior is first elicited. This short latency includes times spent chasing a fleeing target animal. Furthermore, the duration of the stimulation-bound attack is determined by the duration of the stimulation, because the attacks cease promptly when the stimulating current is turned off. Flynn and his colleagues have likewise reported that latencies to stimulus-bound attack remain stable and depend on stimulus characteristics. The exception is that latency to actual physical contact (rather than to directed movement) tends to increase slightly over days of stimulation (*3*).

Valenstein *et al.* (*4*) have demonstrated plasticity of behavioral responses to electrical stimulation of the hypothalamus under certain conditions. Such plasticity is not without limits. Valenstein has pointed out, for example, that aggression, but not eating or gnawing, can be induced in the rat by stimulating the ventromedial hypothalamus (*5*). It is likely that plasticity of the type described either by Valenstein or by Koob *et al.* is limited to oral responses involving chewing or gnawing. Our studies of electrically elicited social behavior in primates contrast starkly and qualitatively with such studies of oral behaviors. We believe that this contrast emphasizes the usefulness of electrical stimulation of the brain as a tool for examining neural systems underlying specific behavioral patterns.

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9 December 1976; revised 22 July 1977

Koob *et al.* (*1*) have suggested that the arousing consequences of mechanically induced tail pinch are sufficient to motivate both the learning of a T-maze discrimination and its reversal. Although the ascription of motivating properties to tail pinch is clearly established by their results, the subsequent interpretation of these findings in terms of an arousal mechanism may in fact be premature.

Tail pinch is a psychophysically complex stimulus, and it embodies both arousing and aversive properties. For example, all eight rats in our laboratory subjected to a 20-second tail pinch from a hemostat showed vocalizing, attempted flight, and aggressiveness (biting, directed at the hemostat or, when possible, the experimenter). Such signs of pain were also present in the experiment of Koob *et al.*: "Rats that ran to the incorrect goal box characteristically vocalized, defecated, and chased their tails."

It is therefore of interest that (i) biting and gnawing, the major consummatory responses to tail pinch reported by Koob *et al.*, may be elicited by aversive stimulation such as shock (*2*), (ii repetitive aversive stimulation increases a variety of unconditioned behaviors similar to those reported (for example, unconditioned response sensitization, pseudoconditioning) (*3*), and (iii) a variety of aversive stimuli may motivate learning (*4*). One possible alternative explanation of tail-pinch learning might therefore rest upon the reduction of an aversive state, if, for example, chewing or biting a goal object reduced the pain of tail pinch. Other forms of peripheral stimulation are known to reduce pain (*5*).

It is possible that tail pinch and other aversive events energize a fairly general adaptive coping response, the purpose of which is aversion reduction. A prepotent and species-specific defense response (either fight or flight) may thus eventually give way to other responses (including gnawing) if the initial responses are unsuccessful. In fact, Koob *et al.* show such behavior to occur.

Since tail pinch is both arousing and aversive, and since aversion may account for the reported phenomena with at least the same parsimony as arousal, no compelling reason exists for the choice of either possible interpretation. It might be noted that the studies of brain stimulation, which Koob *et al.* suggest support their hypothesis, also demonstrate both arousal and some degree of motivation, be it incentive or aversive in nature (*6*). Given the aversive properties of an arousing stimulus (such as tail pinch) and the arousing properties of an aversive stimulus (such as shock), both factors might be argued to be inextricably related and jointly necessary for the learning described by Koob *et al.* Furthermore, neither factor by itself has yet been adequately dissociated and shown to be sufficient for learning.

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27 December 1976; revised 9 August 1977

Perachio and Herndon have made one important technical point, which concerns the amount of eating and gnawing by unpinched rats, and two theoretical points: (i) the specificity of electrically induced sexual and aggressive behavior in contrast to the apparent nonspecificity of tail-pinch-induced behavior, and (ii) the short latencies of electrically induced sexual and aggressive behavior compared to the time taken for tail-pinch-induced oral behavior to emerge.

With regard to eating by unpinched rats, a 2-minute habituation period was given in the open field before each tail-pinch trial. During these habituation periods, only an occasional bite at the food was observed; the same result has been obtained with rats tested with pieces of wood. Unpinched rats were not run in the original maze study, but control trials have since been run with both food and wood. In 100 trials, a group of six rats did not show a side preference, and eating or gnawing was confined to an occasional bite. There were no significant eating or gnawing by unpinched rats, and tail pinch was necessary to produce learning of the T-maze.

Perachio and Herndon state that our conclusion (*1*) that both tail pinch and electrical brain stimulation may act through a common mechanism is not warranted on the evidence provided. We are not suggesting that tail pinch and brain stimulation produce absolutely identical effects, but rather that they share the property of inducing activation in a nonspecific way and that this nonspecific activation may be all that is necessary to produce new learning.

The ability of electrical brain stimulation to produce sexual and aggressive behavior may indicate that it is more specific in its effects than tail pinch or electric shock, which may produce only "nonspecific, situationally dependent responses of eating, gnawing, biting, and licking." However, sexual behavior and aggressive behavior can be produced by electric shock (*2*), and tail pinch may also elicit sexual behavior and maternal behavior (*3*). The relative strength of these responses compared to tail-pinch-induced oral behavior should be investigated.

Perachio and Herndon also suggest that the short, stable latencies they see with electrically induced sexual and aggressive behavior indicate that this behavior is qualitatively different from tail-pinch-induced behavior; we propose that the difference is merely quantitative. Most rats eat the first time they are pinched (*4*), and the decrease in latency occurs rapidly during the first four or five sessions (*1*) until the rats start eating as soon as the pinch is applied; the eating generally stops as soon as the pinch is removed. The rats' reactions are often defensive in nature; for example, they sometimes run around the open field, bite at the clip, or lick their tails until they come across a food pellet whereupon they bite at the food and then settle down in a normal eating posture. The decrease in latency thus reflects the replacement of competing defensive responses by eating; the rats learn to overcome the aversive effects of the pinch, and once they have learned, the eating latency remains low on subsequent trials. The same is true of behavior elicited by electrical stimulation of the lateral hypothalamus; the decrease in latency with experience probably reflects the rats' overcoming the aversive effects of the stimulation (*5*). At some sites, however, electrical brain stimulation may produce only very small aversive effects, especially since the level of stimulation can be carefully controlled so that very short latencies may be seen from the outset. Therefore, differences in latency may reflect the aversiveness of the stimulation; this is a quantitative rather than a qualitative difference.

Nevertheless, the representation of different motor routines in the brain (for example, eating versus sexual behavior) means that it might be possible to stimulate selectively the neuronal systems that specifically code these routines and separate them anatomically. Different behavior elements can be elicited by stimulating different sites, but different responses are associated to different degrees. For example, in the opossum, hypothalamic stimulation that elicits eating will also elicit mating, but not threat (*6*). The most obvious anatomical separation is between eliciting approach responses from the lateral hypothalamus and eliciting defensive responses from the medial hypothalamus (*7*). Similar results have been obtained with stimulation in the mesencephalon and pons (*8*), which suggests that this lateral grouping of approach responses and medial grouping of defensive responses extends to lower brain structures.

Therefore, it is not possible to decide whether the particular response elicited from a particular site on any one occasion is determined by preferential activation of a neuronally discrete system interdigitated with other systems or by the environmental influences on the animal during the activation of a single, nonspecific system. Moreover, since a nonspecific stimulus, tail pinch, is sufficient to produce a variety of different responses, it is not necessary to hypothesize the existence of separate motivational systems. Thus, when the lateral hypothalamus is stimulated a general approach system may be activated, and when the medial hypothalamus is stimulated a general defensive system may be activated. At the same time, connections to specific motor routines may be activated, which may account for the different elements of specific behavior patterns that can be produced by stimulation at different sites.

Animals will self-stimulate from electrodes in the lateral hypothalamus and will escape from stimulation of the medial hypothalamus; these effects may be the result of activation of these same approach and defensive systems, which appear to retain their lateral and medial organization throughout a large part of the brain (*9*). Further, a rewarding lateral hypothalmic site can be made aversive merely by increasing the current. In general, low-intensity stimulation is pleasurable and high-intensity stimulation is aversive; the relative intensity of a stimulus may to a large extent determine whether it is rewarding or aversive (*10*). Thus, a low-intensity stimulation or arousal may activate the approach system and be interpreted as rewarding, and high-intensity stimulation may activate the defensive system and be interpreted as aversive.

Katz makes the important point that the behavioral effects of tail pinch may be due equally to aversive consequences or to appetitive consequences. The question is whether tail-pinch-induced behavior can be regarded merely as a coping response to an aversive stimulus or as genuine appetitive behavior. If the behavior is merely a coping response to a stressful stimulus, then reducing the stress with a minor tranquilizer should reduce the tail-pinch-induced behavior.

Robbins, Phillips, and Sahakian (*11*) have demonstrated that chlordiazepoxide (Librium) actually increases tail-pinch-induced eating at doses that have no effect on eating during control trials with no pinch. This result suggests that the eating is produced by an appetitive component of the tail pinch, which is unmasked from the inhibitory, aversive component by the effect of the drug. The same interpretation has been applied to the effects of tranquilizers on eating induced by electrical stimulation of the lateral hypothalamus (*5*).

We agree in the most part with Katz's comments, but we cannot agree with the proposal that arousal (or incentive) and aversion are "inextricably related and jointly necessary for the learning," since, if their effects cannot be separated, parsimony requires that they be reduced to a single activating or motivating process. Reward and aversion may be the sensations associated with an approach or withdrawal response, but an association must be formed between these emotional sensations and the activating process. The learning of inappropriate associations may explain studies showing that squirrel monkeys and cats will work for electric shock (*12*), and hungry rats and squirrel monkeys will work to postpone food presentation (*13*).

All that is necessary then for learning is activation, which can be defined as stimulus change; whether an animal learns to approach or avoid will depend on the situation. Although they are not identical, tail pinch and brain stimulation both activate the animal, as does deprivation. Perhaps the nonspecific component of deprivation is all that is necessary to motivate learning; the particular sensations of hunger or thirst are merely cues in the presence of which learning occurs.

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8 May 1978

## H-Y Antigen Gene Loci

Detection of the H-Y antigen in the heterogametic sex of vertebrates is probably one of the most important discoveries to have been made in recent years concerning differentiation of gonadal tissues; however some exceptions have been noted which raise many questions about the strict localization of the genes responsible for expression of this antigen to only the short arm of the Y chromosome (p). The exceptions include Sxr mice, some *Myopus schisticolor* XY embryos, and human XX males and true hermaphrodites (*1*, *2*). Additionally, Koo *et al.* reported a case of a human female who was H-Y$^+$ and whose karyotype was 46,X,der(X),t(X;Y) (*3*). This derivative chromosome was reportedly composed of the short arm (p), centromere, and the proximal long arm (q) of an X that was translocated to a Yq.

Arguments to explain these exceptions have been made for pericentric inversions of the Y, two loci on the Y, or an unseen translocation of some Y material elsewhere; the basis for these ideas seems to be the belief that the H-Y antigen must be confined to the "male chromosome," at least in mammals. Wachtel (*2*) admits that the dosage effect of supernumerary Y's ". . . is not easily reconciled with the existence of a Y-situated regulator . . . ." The ideas of multiple gene copies and loci that are distributed throughout a genome are not new ones, but are not adequately treated as a viable explanation for the unexpected cases of H-Y antigen expression or lack of it.

Studying mammalian chromosome evolution reveals that the X has remained more stable, while the Y shows great variability between species and the human races (*4*–*8*). This fact, together with the similarity of X and Y prometaphase banding patterns, random inactivation of X material (Lyon's hypothesis), and the homology that must exist between the X and the Y, suggests that the Y may be an evolutionary breakdown product of an originally sexually bipotential X. This would also be consistent with evolutionary divergence of the amphibian and the H-Y$^-$ homogametic *Xenopus laevis* male.

Consequently, the X would host an H-Y operon that is normally repressed by the X-situated regulator. The Y may have lost its regulator, with probable mutation of the operator so that it is insensitive to repression. The X would normally not express H-Y antigen except in the case of a mutated operator or regulator. This would account for the citations above and the reduced amount of H-Y antiserum binding in human XX males and true hermaphrodites (*1*, *2*). The gene dosage effect of Y's is now also explained.

In conclusion, while I must agree that the H-Y antigen genes are located on the Yp, other evidence requires that additional loci be considered. H-Y antigen studies of persons with partially deleted or ring X chromosomes may prove just as informative if not more so than confinement to presumed unusual Y's.

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7 February 1978


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