at the level of the dorsomedial and ventromedial nuclei, but have failed so far to find short-latency responses to stimulation of the olfactory bulb.

Our results confirm prior anatomic indications of an olfactory pathway into the lateral hypothalamus by way of the medial forebrain bundle. The multiunit character of the records with gross electrodes suggests that we were recording from a cluster of cells or fibers responsive to odors, rather than from randomly scattered cells in the area. Since we could usually isolate one or two large spikes with the microelectrodes, we assume that we were often recording from cell bodies observed in our stained sections, lying in or near the bundle of fibers. Medial-forebrain-bundle components are known to arise in the olfactory tubercle, the piriform cortex, and the amygdala, all of which have anatomically demonstrated connections from the olfactory bulb (*1*). The immediate source of the particular bundle that is associated with responses to odors and stimulation of the olfactory bulb remains to be established. The broad range of latencies observed to electrical stimulation may be due to multiple pathways.

Our results are of interest for the study of hypothalamic mechanisms in that they show a strong olfactory input that, at least in the anesthetized rat, is localized in a ventrolateral portion of the medial forebrain bundle. How this sensory input relates to the elicitation of feeding and sexual behavior by hypothalamic stimulation, or to the phenomenon of intracranial self-stimulation, is an intriguing line for future research. The localized character of the system should make it readily amenable for study with small lesions and discrete stimulation.

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Puromycin and Retention in the Goldfish

Abstract. *A first experiment compared the behavior of goldfish injected with puromycin immediately after each of a weekly series of brief discriminative training sessions in the shuttlebox to that of appropriate controls. Discrimination was not prevented, nor was escape from shock impaired, but probability of response to the conditioned stimuli, both positive and negative, was reduced substantially. These results suggest that puromycin interferes with the consolidation of conditioned fear. The null outcome of a second experiment, in which all training was given in a single long session instead of a series of short sessions, suggests (contrary to recent indications) that consolidation begins in the training session. The conditioned-fear hypothesis is supported by the results of a third experiment in which the animals were shocked upon entering a goalbox to which they had previously learned to swim for food; animals injected with puromycin, immediately after the shock, entered the goalbox more readily 1 week later than did appropriate controls.*

Agranoff and his co-workers have found that goldfish injected with puromycin immediately after a brief session of simple avoidance training in the shuttlebox perform less well 3 days later than do control animals injected either with saline immediately after training or with puromycin after some delay (*1*). One may reasonably conclude from these results that the drug somehow interferes with retention, but whether the interference is general or restricted to certain products of shuttlebox training—to fear conditioning, to the selection of the instrumental response, or merely to sensitization—remains to be determined. Although (at higher dosages) the Agranoff effect is large in relative magnitude, it is small in absolute magnitude (because little is learned in a single brief training session) and therefore does not lend itself easily to functional analysis. In our first experiment we used repeated training and injection in an effort to produce a greater effect, and a somewhat more elaborate (discriminative) procedure designed to permit closer inquiry into its cause.

In a set of fully automated shuttleboxes (*2*) we trained 52 goldfish, 7.5 to 10 cm in length (snout to base of caudal peduncle), living in individual tanks, their water being filtered, aerated, and controlled as to temperature; 6 training days were 1 week apart. On each training day, 20 trials were given, with a mean intertrial interval (in darkness) of 1.5 minutes. The conditioned stimulus was light, either amber or green; the interval between conditioned stimulus and unconditioned stimulus was 5 seconds; and the unconditioned stimulus was a train of 0.25-second pulses of low-voltage a-c shock (1.5 seconds between pulses) with a maximum duration of 15 seconds. In each session the lights of the two colors were presented equally often in quasirandom (Gellermann) order. Light of the "positive" color (amber for half the animals and green for the others) was followed by shock after 5 seconds if the animal failed to respond to it; response to the light turned off the light and forestalled the shock (avoidance), while response after the onset of shock turned off both light and shock (escape). Light of the "negative" color (the alternative color in each instance) was terminated either by response or automatically after 5 seconds (if the animal failed to respond within that time). The position of each animal in its shuttlebox was monitored continuously by photocells, and the conditioned stimulus was presented only in the compartment that the animal happened to occupy at the start of any trial. The response required to avoid or to escape the shock was swimming to the opposite compartment.

One group of 16 animals (the O-Puro group) were injected intracranially by Agranoff's technique with 170 μg of puromycin dihydrochloride in 10 μl of saline immediately after training on each day. A control group of 18 animals (the O-Saline group) were injected with saline alone immediately after training, and a second control group of 18 animals (the 24-Puro group) were injected with puromycin 24 hours later.

In Fig. 1 the performance of the three groups is plotted in terms of

mean probability of response to the positive and negative stimuli on each day. The two control groups performed in much the same way, responding with increasing probability to both stimuli, and more to the positive than to the negative. The performance of the O-Puro group also improved, but its level of response to the two stimuli was significantly lower than that of the combined controls in all stages of training (F, 22.96; df, 1/50; P <.01). The difference was small initially and increased as training continued; over the six sessions the rate of acquisition in the O-Puro group was only half that in the controls.

The level of intertrial responding also was somewhat lower in the O-Puro animals than in the controls; on blank trials (the same in all respects as other trials except that there were no stimuli) in the last three sessions, the probability of response was about .10 for the O-Puro animals and about .20 for the controls. The probability of escape was not, however, affected by treatment; the O-Puro animals responded to the shock as promptly as did the control animals whenever they failed to avoid.

These results confirm Agranoff's finding that puromycin impairs retention in the shuttlebox, although they show that the 170-μg dose does not entirely prevent consolidation. They also tell us something about the locus of the effect. With the discriminative procedure as a control for sensitization, we know that the pairing of stimuli has produced some conditioning; that is, differential response to positive and negative stimuli shows that the training has not merely lowered the threshold for shuttling. From the independent measures of escape and avoidance we may assume, in fact, that the principal effect of the drug is on the classical-conditioning component of the training.

Other experiments with the goldfish make it clear that the avoidance response in the shuttlebox is activated by a mediating process (called "fear") which is evoked originally by shock and connected to the conditioned stimulus, the probability of avoidance increasing with the intensity of the fear (3). If puromycin simply interfered with the consolidation of conditioned fear, all the experimental results would follow: the positive stimulus would evoke less fear, less fear would generalize to the negative stimulus and to intertrial stimuli, and the probability of response to all these stimuli

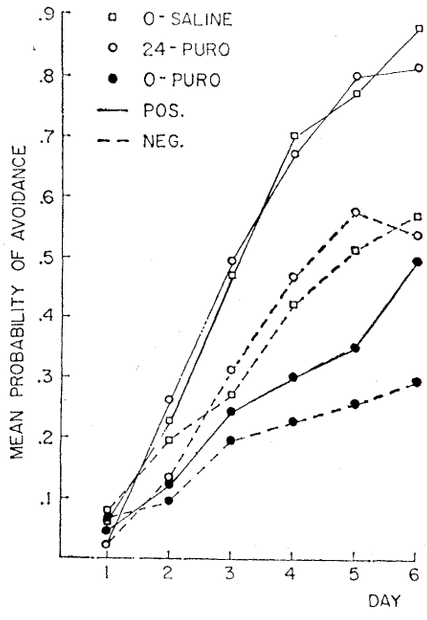

Fig. 1. Response of three groups to the positive and negative stimuli on each of the 6 training days.

therefore would decline proportionally.

Before proceeding to a test of this hypothesis we attempted in a second experiment to magnify the Agranoff effect by use of a single long training session instead of a series of short sessions (the practical advantages of a one-session procedure being many). In Agranoff's earlier experiments the training sessions were kept short in order to limit the opportunity for consolidation during training; in consequence the control animals learned little, and the difference between experimentals and controls was necessarily small. Recent work by Davis and Agranoff has suggested, however, that the process of consolidation with which the puromycin is presumed to interfere may not begin until the animal is removed from the training situation (*4*). If that is so, it should be possible to produce a substantial puromycin effect in a single long training session (in which control animals learn as much as they do in repeated short sessions).

In our second experiment, fresh animals were trained in the shuttlebox as before, except that 90 trials were given (half with the positive stimulus and half with the negative) in a single 2-hour session, the interval between conditioned stimulus and unconditioned stimulus being 20 seconds, as in Agranoff's experiments. There were a O-Puro group of 22 animals and a combined control group of 26 animals (half 24-Puro and half O-Saline). By the end of the training session, both groups were responding with a probability of about .90 to the positive stimulus and about .60 to the negative. In tests made 1 week later, both groups responded as they had at the end of training; their performances were indistinguishable. The results suggest that consolidation does after all begin before the animal is removed from the training situation.

To test the hypothesis that puromycin interferes with the consolidation of conditioned fear, we trained a fresh sample of 18 goldfish in a third experiment. The apparatus was a gray "runway," 1 m by 8.75 cm, with a white goalbox, 20 by 25 cm, filled with water to a depth of 12.5 cm.

On each trial an animal was carried in a container of clear plastic from its living tank to the starting end of the runway; then a guillotine door, 12.5 cm from the starting end, was lifted, and the animal was permitted to swim to the goalbox. As it entered, a clump of Tubifex worms was squirted into the water of the goalbox by an automatic feeder (*2*), and after eating the worms the animal was carried in the plastic container back to its living tank. The measure of performance was the time between the lifting of the guillotine door and the animal's entry into the goalbox.

There were 28 such training trials at the rate of one or two daily, in the course of which the swimming times decreased to a mean level of about 25.2 seconds. On trial 28 the animals were not fed in the goalbox, but shocked intermittently for 30 seconds before ten of the animals were injected immediately with 170 μg of puromycin; the rest were injected immediately with saline. All animals were rested for 1 week before being given three more rewarded trials in the runway, one daily for 3 days. The mean swimming time on those 3 days was 25.6 seconds for the O-Puro animals and 52.7 seconds for the O-Saline animals. The increase in swimming time (logarithmically transformed to normalize the distributions) was significantly greater in the O-Saline than in the O-Puro condition (t, 2.12; P <.05).

In this third experiment, injection of puromycin produced better performance than did injection of saline, which fact demonstrates that the effect of the drug is not simply to depress performance. The situation was one in which performance would be depressed by conditioned fear, and the outcome was

predicted on the assumption that the animals injected with puromycin would be less fearful. Here then is further evidence that puromycin interferes with the consolidation of conditioned fear. Whether its effects on retention in goldfish are still more general remains to be demonstrated in experiments with different dosages, different loci of injection, and different tasks.

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Hormone-Mediated Nutritional Control of Sexual Behavior in Male Dung Flies

Abstract. *Male* Scatophaga stercoraria *must prey on other Diptera before they display sexual behavior, develop the accessory cells of their ejaculatory ducts, and achieve full elongation of the testes. The corpus allatum also becomes enlarged after predation and is necessary for the onset of sexual behavior and development of reproductive organs. The complex diet consumed during predation apparently activates the corpus allatum, and the hormone released acts independently on organ growth and sexual behavior.*

It is widely stated that male insects require no special nutrition for reproduction, but vitamin E recently has been shown to be necessary for normal spermiogenesis in the cricket *Acheta domestica* (*1*), and spermatophore production in the bug *Rhodnius prolixus* ceases after long periods of starvation (*2*). Deprivation of food has also been shown to reduce development of male internal reproductive organs in several insects (*3*), yet the influence of nutrition on male sexual behavior apparently has not been examined.

In the predatory yellow dung fly, *Scatophaga stercoraria* (L.) (Diptera, Anthomyiidae), I have found that both sexes generally must be sexually mature to be attracted to cattle dung, on which both insemination and oviposition occur, and a period of predation on small Diptera in pastureland vegetation is prerequisite to sexual maturity. Laboratory studies revealed that males, maintained from emergence on sucrose and water, never attempted to copulate with females; at first they ignored or avoided them, and after several days they ate them. Males having access to *Musca domestica* L. from emergence continued to avoid or ignore females for about 4 days before abruptly starting to copulate with them; ability to copulate sometimes preceded ability to inseminate by as much as 1 day. The onset of sexual behavior did not require the presence of females; sexual responses could be elicited by other males or by inanimate objects.

Comparison of dissections of ten prey-supplied and ten prey-deprived males 8 days after emergence with dissections of ten newly emerged males revealed that deprivation of prey did not prevent spermiogenesis. But the testes of prey-deprived males were not so elongated as those of prey-supplied males (t, 2.89; d.f., 18; $P < .01$; two-tailed test), though testes of the former were much longer than those of newly emerged males. The accessory cells that surround the ejaculatory duct (measured as the greatest diameter of the ejaculatory duct) were slightly smaller in prey-deprived males than in newly emerged males (t, 3.10; df., 18; $P < .01$; two-tailed test), whereas in prey-supplied males the accessory cells were far larger than those in newly emerged males. Similarly, the corpora allata of prey-supplied males were usually much larger than those of prey-deprived males of similar age, or those of newly emerged males.

These observations suggested that in male *Scatophaga* the corpora allata might control both development of accessory cells and sexual behavior as in the locusts *Schistocerca gregaria* (*4*) and *Locusta migratoria* (*5*); the corpora allata might also control the rate of spermiogenesis. Furthermore the apparent nutritional activation of the corpora allata in male *Scatophaga* closely resembles the case in female insects, in which corpora allata hormone, as well as available yolk precursors, is necessary for vitellogenesis; it is released in response either to gut expansion (*6*) or to the presence of nutrients in the gut or hemolymph (*7, 8*).

To demonstrate the role of the corpora allata in male sexual maturation in *Scatophaga*, seven males were allatectomized within 4 hours of emergence, with fine forceps through a neck incision; then each was allowed access to ten *Musca*, the dead being replaced daily, in a 340-cm^3 predation carton, with water and sucrose available. Sham-operated and unoperated controls were each divided into prey-supplied and prey-deprived groups. Eight days after emergence all males were tested for response to females in the presence of dung (but pairs were separated before insemination could occur); they were then dissected for measurement of the ejaculatory ducts, testes, and corpora allata.

Predation rates were roughly the same in all three prey-supplied groups, but none of the seven allatectomized or 13 prey-deprived males attempted to copulate with females, whereas all six sham-operated, prey-supplied males attempted copulation, as did five of the ten unoperated, prey-supplied males. The diameters of ejaculatory ducts of allatectomized males were all a little larger than those of 17 prey-deprived controls (including four not used in mating tests) (t, 9.25; d.f., 22; $P < .01$; two-tailed test), but were much smaller than those of all but two of the prey-supplied controls.

The mean length of testis of allatectomized males was not significantly greater than that of prey-deprived controls (t, .90; d.f., 22; $P > .03$; two-tailed test), but was significantly shorter than that of prey-supplied controls (t, 3.61; d.f., 21; $P < .01$; two-tailed test). Examination of the retrocerebral complexes of allatectomized males showed that in all instances the corpora allata had been mainly or completely removed, while the corpora cardiaca and hypocerebral ganglia remained intact. The corpora allata, measured as the product of the long and short diameters in sagittal view, were all larger in 14 prey-supplied controls than in seven prey-deprived controls (I used only preparations in which accurate measurements could be made). Two prey-supplied controls had corpora allata