

ency, independent of the response to the mother's surface, which contributes to the normal massing of young on the female (8).

Examination of the body surface of an adult female *Lycosa punctulata* with a scanning electron microscope (9) revealed the presence of at least two different types of hairs on the abdomen and posterior carapace. Relatively few long, smooth hairs project rather steeply above a dense layer of shorter hairs with knobbed tips (Fig. 2a), the latter corresponding to Graefe's "knobbed hairs" (3). Prominent longitudinal rows of curved spines run the length of the knobbed hairs (Fig. 2b). Examination of an adult male *L. punctulata* revealed no knobbed hairs, although some spiny but sharp-tipped hairs occur. Males possess primarily short, smooth hairs, which are rare on the abdomen of adult females.

Histological preparations of the female abdomen (10) indicated that the knobbed hairs were not innervated, nor did their structure suggest any secretory function. The few long, smooth hairs are triply innervated and exhibit the same ultrastructure as other spider mechanoreceptors (11). They probably play a role in maternal care, since the climbing of young onto the female's abdomen provides a mechanical stimulus which triggers the dropping of the egg sac and inhibits prey capture behavior in the mother during the period of brood care (2).

Since the knobbed hairs are found on female lycosids only after the final molt and are absent in females of the closely related family Pisauridae (3), it is likely that they are associated with the brood care behavior peculiar to the Lycosidae. Our experiments indicated that newly emerged spiderlings would not settle on a denuded abdominal surface for a period of days. As the most abundant of the two protruding structures on the surfaces chosen for settlement, the knobbed hairs probably trigger the attachment response of the young which form the inner layer of the brood. Indeed, the structure of the knobbed hair suggests an important role in attachment itself, the interstices between the spines providing good grasping sites for the tarsal claws of the spiderlings. Scanning electron microscope examination of young lycosids mounted on their mother (12) indicated such a relationship (Fig. 2c). Since the welfare of the outer layers of the brood ultimately depends on the ability of the innermost layer of spiderlings to hold

tightly to the mother, the need for a grasping surface is obvious. Conceivably, knobbed hairs also facilitate the subsequent attachment of silk draglines by the spiderlings.

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4. We used the large wolf spider *Lycosa rabida* for the experimental portion of our study. Spiders were collected as immature individuals and, when adult, mated in the laboratory. Emergence of young occurred about 1 month after egg sac construction.
5. When tested by the Mann-Whitney U test, the difference in data between young with and without 1 day of prior experience on the female was not significant ($P = .075$). However, the group of young without 1 day of prior experience on the female did not settle on the cloth until after 9.4 ± 7.7 days, whereas the group of young with 1 day of prior experience on the female showed initial settlement after 2.2 ± 1.5 days.
6. It may be that remounting spiderlings lay draglines as they wander over the abdomen, which would then serve for attachment in the shaved area. Newly emerged young, having settled in hairy regions, may not have wandered extensively over the shaved areas during the first few days, since they had never been forced to vacate their original sites.
7. G. A. Higashi and J. S. Rovner, in preparation.
8. We also noted alternating periods of settlement and absence of young on the cloth substratum; that is, either a mass of young were present on the cloth or no young at all were present. This finding constitutes further indication that the aggregation tendency plays a role in determining site selection.
9. The carapace and abdomen were exposed to glutaraldehyde and osmium vapors for several days. They were then coated with gold (without prior dehydration) and immediately examined with a JEOL JSM 2 or a JSM 50-A scanning electron microscope.
10. Small pieces of the dorsal abdomen of female *Lycosa punctulata* were fixed in cold, cacodylate-buffered glutaraldehyde, then fixed in OsO_4 and embedded in Epon. Thick sections stained with methylene blue were used for light microscopy; thin sections were contrasted with uranyl acetate and lead citrate for electron microscopy.
11. R. F. Foelix and I-W. Chu-Wang, *Tissue Cell* **5**, 351 (1973).
12. Female lycosids with young were immersed in liquid nitrogen or kept in a freezer (-20°C) for several days. They were then processed as described in (9).
13. We thank I-W. Chu-Wang for collaboration on the scanning electron microscope, C. H. Page for valuable discussions, and R. Braun for assistance in obtaining G. Graefe's unpublished dissertation. Supported in part by NSF grant GB-35369 to J.S.R.

30 August 1973

Trigeminal Deafferentation and Feeding in the Pigeon: Sensorimotor and Motivational Effects

Abstract. *The role of oral sensations in the control of hunger and thirst in the pigeon was studied in birds in which the trigeminal sensory nerves had been sectioned bilaterally. Trigeminal deafferentation, although it does not impair drinking, disrupts both the neurosensory and the motivational control of feeding behavior. These two types of deficit may be experimentally dissociated.*

It has been suggested that, in mammals, oropharyngeal sensations are not essential either for the arousal of hunger and thirst or for the regulation of body weight (1). This generalization is derived primarily from experiments with a combination of intragastric feeding techniques and operant conditioning procedures (2) rather than from studies of feeding behavior in surgically deafferented animals. The location and peripheral distribution of the pigeon trigeminal nerves permits deafferentation of the oral region without affecting motor function. I found that trigeminal deafferentation in the pigeon not only disrupts the consummatory response of eating but also affects motivational processes underlying the arousal of hunger and the regulation of body weight, and has these effects without impairing drinking.

Anatomical and electrophysiological

studies indicate that the trigeminal nerve in the pigeon innervates the orbit, beak, and buccal cavity, exclusive of the tongue (3, 4). Section of one or more trigeminal sensory branches was done under a dissecting microscope. In ophthalmic and mandibular denervations, sections approximately 10 to 15 mm long were removed, whereas maxillary sections were somewhat less extensive. Control surgical procedures were identical in all respects except for actual nerve section.

In the first experiment, deafferentation effects on the consummatory response of eating were studied in a group of eight food-deprived birds maintained at 80 percent of their free-feeding weight to ensure a constant level of responsiveness to food during testing. Birds were tested three times weekly in an observation chamber con-

Table 1. Effects of trigeminal deafferentation on food intake, body weight, and feedometer responses; *N*, number of animals.

Group	<i>N</i>	Aphagia (days)		Weight loss (%)		Number of days below free-feeding weight	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
Control	8	0.38	0.4	3.1	1.1	3.9	2.3
Two-branch	8	2.9	0.8	8.9	1.1	24.0	9.7
Three-branch*	7	7.0	2.4	18.2	6.0	64.1	32.2

* Because of the intubation, data for case 61 are not included (Fig. 2).

taining a dish with 20 kernels of grain (milo) and a water tube. The efficiency of the consummatory response was quantified by counting the number of pecks made and the number of grains consumed within a 10-minute period. The ratio of pecks to grains consumed was calculated for each bird and provides a baseline for evaluation of denervation effects on feeding efficiency. Before surgery, such ratios were low and stable (group mean, 1.49; standard deviation, 0.14). There was no significant increase after control surgery, but the ratio increased strikingly with progressive increases in the extent of bilateral trigeminal denervation (Fig. 1). While feeding efficiency improved with time, significant impairment was still evident after several weeks of testing. A motion picture analysis of feeding behavior at 64 frames per second indicates that pecking and swallowing were performed normally after deafferentation. The reduction in feeding efficiency was caused by a disruption of mandibulation—the process by which grain is grasped at the beak tip and moved to the back of the mouth before swallowing (5). No effects on drinking were seen.

In a second experiment, the effects of trigeminal deafferentation on intake and weight regulation were studied in an additional group of 24 pigeons, maintained in their home cages with free access to food and water and then subjected to surgical control procedures or to bilateral section of two or three trigeminal sensory branches (two- and three-branch groups, respectively). In addition to data on intake and body weight, obtained from all three groups, a feedometer was used to monitor feeding behavior in the three-branch group. Pecks into the food magazine interrupted a light beam triggering a photodetector circuit whose output was connected to a digital counter (6). The feedometer thus provided a direct measure of the pigeon's responsiveness to food, which

could be assessed independently of its food intake. Moreover, because consummatory response deficits increased the number of pecking responses required to obtain a unit quantity of grain, the efficiency of feeding in the home cage could be measured by calculating the ratio of feedometer responses to grams of food consumed.

Surgical control procedures produced only minor and transient effects, whereas bilateral section of trigeminal sensory nerves was followed by a disruption of food intake whose extent and persistence was approximately proportional to the number of branches sectioned (Table 1). Denervated birds exhibited periods of aphagia (absence of food intake) ranging from 5 to 40 days, followed by extended periods of hypophagia. During the first week after resumption of eating, median

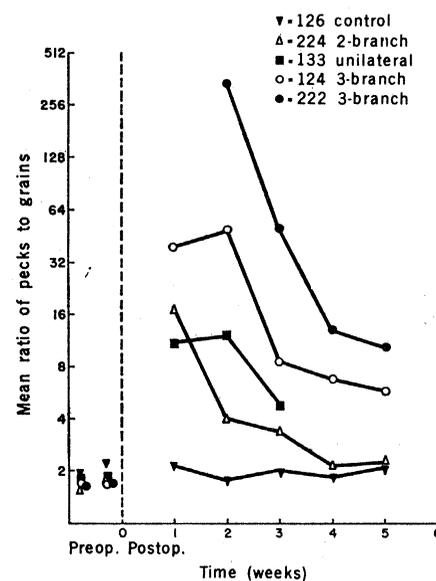


Fig. 1. Effects of varying degrees of trigeminal deafferentation on efficiency of the consummatory response of feeding in the pigeon. Representative data are shown for one surgical control bird and four birds with unilateral or bilateral section of two or three sensory branches. Case 222 made no feeding responses during the first postoperative (Postop.) week; Preop. preoperative.

food intake (calculated as percentage of the preoperative value) was 94 percent for the control group, 74 percent for the two-branch group, and 58 percent for the three-branch group. Statistical analyses by a Wilcoxon matched pairs, signed ranks test (7) indicate that only the reductions in the experimental groups are significant ($P < .01$).

Birds in all three groups resumed drinking within 24 hours. Although the water intake of denervated birds was reduced during the first postoperative week (two-branch group mean, 93 percent; three-branch group mean, 67 percent), it was normal or higher by the second postoperative week (means of 104 and 111 percent for two- and three-branch groups, respectively). With respect to water intake, the denervated birds did not differ from normal pigeons subjected to total or partial food deprivation for equivalent periods (8).

The rate of weight loss in aphagic birds was comparable to that of normal pigeons during food deprivation. However, even after food intake had returned to the preoperative value, many birds did not show the degree of compensatory overeating typical of deprived pigeons allowed free access to food (8), and their body weights remained below preoperative values for extended periods.

By examining the relation between food intake and feedometer responses, it is possible to determine whether the decreased food intake after deafferentation is due to disruption of consummatory response patterns or to reduction in responsiveness to food. In the former case, we would expect many feedometer responses but little or no ingestion of food during the immediate postoperative period. In the latter case, absence of food intake would be paralleled by a significant reduction in the number of feedometer responses during the period of aphagia.

The data for the eight deafferented birds in the three-branch group indicate dramatic reduction in feedometer responses during the period of postoperative aphagia. Before surgery, the mean number of daily feeding responses per bird was 2775 (range, 1906 to 3869). During the postoperative period of aphagia, means for individual birds ranged from 88 to 180 responses per day. In the week immediately after resumption of food intake, the ratios of feedometer responses to food consumed were ele-

vated in some birds, which indicates a reduction in feeding efficiency as reported in experiment 1. However, although efficiency improved over the next few weeks, food intake remained insufficient to permit the recovery of body weight to preoperative values. Figure 2 illustrates these results for the case with the greatest deficit.

These two experiments demonstrate that trigeminal deafferentation not only disrupts the consummatory response of eating, but also affects motivational processes underlying arousal of hunger and regulation of body weight. These results suggest that trigeminal inputs have both a phasic and a tonic function (9) in the control of feeding in the pigeon.

The consummatory response deficits are consistent with our electrophysiological studies of the pigeon's trigeminal system. Single-unit analyses of the central projections of the trigeminal nerve indicate that it provides inputs from the beak and buccal cavity that signal the presence of a kernel of grain at the beak tip, provide complementary information about the static position of the kernel and its movement within the mouth, and monitor the extent of mouth opening (4, 10). Deafferentation effects on the consummatory response may thus reflect a disruption of phasic sensory inputs involved in the somatosensory control of mandibulation.

The reduction in responsiveness to food—that is, the decline in the initiation of feeding responses—is not readily explicable in these terms, because pecking is probably under the exteroceptive control of visual rather than somatosensory inputs. At least three hypotheses may be advanced to account for the data. (i) Deafferentation produces abnormal or painful sensations in the oral region that diminish during recovery. This explanation is unlikely in view of the persistence of drinking and the finding that deafferentation, while it reduces the pigeon's responsiveness to the reinforcement (food), does not impair performance of an operant key-pecking response (6). (ii) By reducing the efficiency of the feeding response, deafferentation abolishes the primary reinforcement normally provided by food intake and leads to extinction of the pecking response to food. This hypothesis is incompatible with the fact that responsiveness is lowest immediately after nerve section and that intake and body weight remain low despite gradual im-

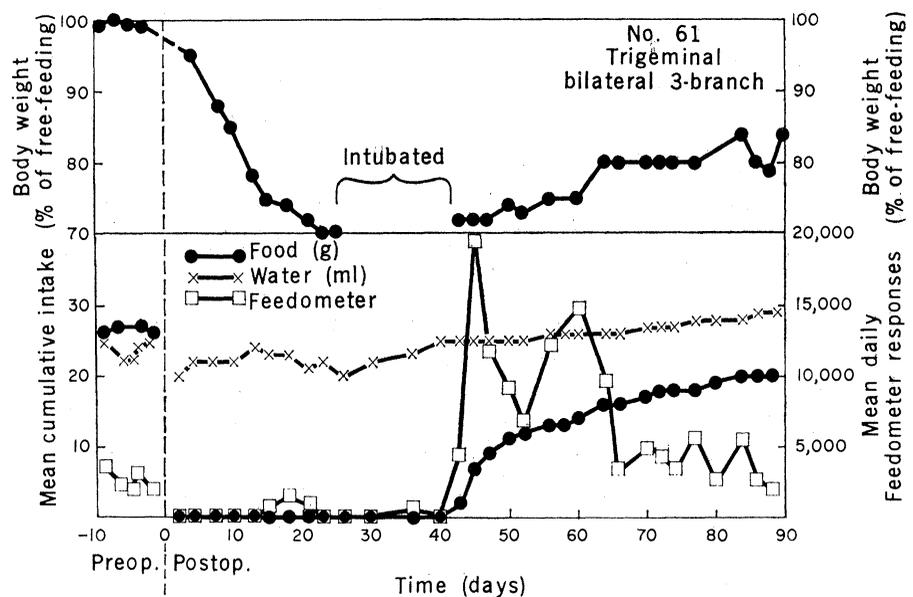


Fig. 2. Effects of trigeminal deafferentation on intake and responsiveness to food in the case showing the greatest deficit. In the week before surgery, case 61 made a total of 27,089 feeding responses for 179 g of food (ratio of responses to food obtained, 151) and drank 170 ml of water. During the first postoperative week, it made only 445 feeding responses, obtained no food, but drank 150 ml of water. In the next 5 weeks, 4,797 feeding responses were recorded, with no measurable food intake. For a portion of this period, the bird was maintained on a liquid diet delivered by intubation. During the seventh postoperative week the bird made 76,310 feeding responses and obtained 66 g of food (ratio, 1156). Although feeding efficiency improved gradually over the next few weeks, there was no compensatory overeating, and body weight stabilized at about 80 percent of its preoperative value.

provement in feeding efficiency. (iii) Trigeminal deafferentation reduces or abolishes the flow of sensory input that is a normal accompaniment of feeding behavior. Such input may have a tonic function in helping to maintain the responsiveness of central sensorimotor mechanisms involved in the initiation and persistence of feeding in response to exteroceptive and interoceptive stimulation. This hypothesis is consistent with the fact that lesions of central trigeminal structures in the pigeon produce a syndrome of feeding behavior deficits similar to those seen after peripheral deafferentation (11).

Our results may be related to certain species-typical characteristics of pigeon eating and drinking behavior. The profound disruptions of food intake produced by deafferentation of a single sensory system may be related to the fact that, in a granivorous bird, trigeminal stimuli would be relatively more important than olfaction or taste for the control of feeding. Similarly, the absence of deficits in drinking after trigeminal deafferentation may reflect the fact that the consummatory response patterns related to hunger and thirst are morphologically distinct in the pigeon (12).

These neurobehavioral studies of feeding in the pigeon provide addi-

tional support for the contention that oropharyngeal sensations have both sensory and motivational properties (13). They further suggest that the pigeon may be an ideal preparation for the dissociation of neural mechanisms subserving hunger and thirst and for analyses of the interaction of sensory and neural factors in the control of feeding behavior.

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19 April 1973

Acquisition and Retention of Long-Term Habituation in *Aplysia*: Correlation of Behavioral and Cellular Processes

Abstract. To examine the cellular mechanisms responsible for transition from a short-term to a long-term behavioral modification, a rapid training procedure was developed for producing long-term habituation of the defensive withdrawal of gill and siphon in *Aplysia*. Four ten-trial training sessions, with 1½-hour inter-session intervals, produced habituation that was retained for more than 1 week. This 5-hour procedure could be applied to a test system in the isolated abdominal ganglion where the cellular changes accompanying the acquisition of long-term habituation can be examined. During acquisition, intracellular recordings were obtained from L7, a major gill and siphon motor neuron, and the pattern of stimulation used in the behavioral experiments was applied to an afferent nerve. Acquisition was associated with a progressive decrease in the complex excitatory synaptic potential produced in L7 by afferent nerve stimulation. When retention was tested 24 hours later, the synaptic decrement was still evident. Thus, a behaviorally meaningful stimulus sequence, consisting of only 40 patterned stimuli, leads to changes in synaptic effectiveness lasting one or more days in a neural pathway involved in short-term habituation of this reflex.

Cellular studies in several invertebrates have shown that short-term habituation and dishabituation involve changes in the synaptic effectiveness of excitatory synaptic connections [(1-3); for vertebrates, see (4)]. For ex-

ample, in the marine mollusc *Aplysia*, habituation of the defensive gill-withdrawal reflex involves a change in effectiveness of the excitatory synapses made by mechanoreceptor sensory neurons onto motor neurons and inter-

neurons (1). A similar locus and mechanism have been found in studies of habituation of escape responses in crayfish (2) and may also be involved in the cockroach (3).

The short-term behavioral modifications so far analyzed last several minutes to several hours (1-4). Whether similar synaptic changes characterize long-term behavioral modifications that endure days and weeks is not known. Recently, Carew *et al.* (5) described long-term habituation in *Aplysia*. Four consecutive days of siphon stimulation led to the acquisition of long-term habituation of both gill and siphon withdrawal that persisted for more than 3 weeks. We now report that, in the isolated abdominal ganglion, acquisition of long-term habituation is correlated with a pronounced decrease in excitatory synaptic input to a major motor neuron, L7, which persists for at least 24 hours. This neurophysiological investigation was possible because of a new behavioral procedure for habituation training that produces, within a few hours, long-term habituation that lasts more than a week.

Carew *et al.* (5) found that the time course of retention of long-term habituation depended on the pattern of stimulation: Massing 40 trials in one session was not as effective as spacing 40 trials (10 per day) over four daily sessions. However, it was not determined whether training sessions separated by less than 24 hours might also be effective. Since this would be technically advantageous for cellular neurophysiological studies, we first examined whether long-term habituation could be produced in a single day.

We used the training procedures of Carew *et al.* (5) with one modification: Four habituation training sessions were presented as before, but successive sessions were separated by 1½ hours rather than by 24 hours, so that all the training sessions necessary for acquisition could be given in 1 day. The withdrawal reflex was produced by an 800-msec jet of seawater to the siphon of an unrestrained animal (6). The duration of the siphon component of the withdrawal reflex (the time during which the siphon was completely hidden between the parapodia) was measured by an observer using an electric timer. A single training session consisted of ten trials, with an intertrial interval of 30 seconds. All animals were maintained in individual aquariums for at least a week before the

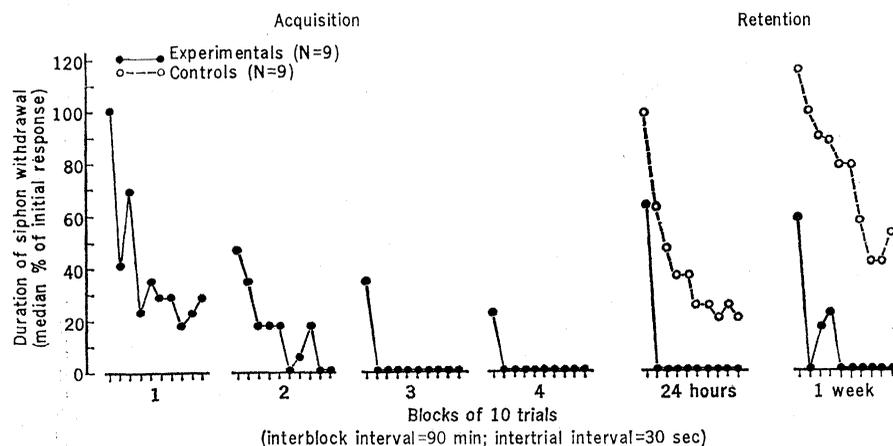


Fig. 1. Acquisition and retention of long-term habituation of siphon withdrawal. Data are expressed as percentage of the median of each group initial response (block 1, trial 1), which was 17 seconds for the experimentals and 19 seconds for the controls. For statistical analysis, the duration of siphon withdrawal for each animal was summed for trials 1 to 10; this measures the total time an animal spent responding in the habituation session. Intergroup statistical comparisons were made by means of Mann-Whitney U tests, and intragroup comparisons by means of Wilcoxon matched-pairs, signed-ranks tests. After four blocks of siphon-habituation training (acquisition), experimentals exhibited significantly greater habituation than controls in retention testing after both 24 hours and 1 week ($P < .001$ for both tests).