tion of the kind of perturbation employed and the species composition of the communities affected (*22*). The most responsive species will be those specialized for, and limited by, the niche parameters influenced by the environmental modification (*13*). Species with different critical niche dimensions will be affected less by the same modification.

Although MacArthur (*2*) argued that stability of a population should increase as the number of food species available to it increases, our results suggest that increased diversity at the plant and herbivore levels generates decreasing stability at the next higher trophic level. This is similar to Watt's (*23*) results from examining herbivorous insects. In our experiment, the only reasonable explanation of the lower stability in the old field consumers appears to be the possibility that fertilization preferentially enriched palatable food classes at the primary level. Documentation of such an enrichment would require careful determination of food preferences at the herbivore level, and the availability of these food classes within the plant communities.

The third aspect of stability, the long-term damping effect, is not reported here because such evidence will require several years to obtain and, at any rate, will not affect the interpretation of the first two components of the stability definition.

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Taste Pathways in Rat Brainstem

Abstract. *By means of a combination of electrophysiological and anatomical procedures, the projections of the anterior portion of the solitary nucleus were traced to the parabrachial nuclei in the pons, structures hitherto not considered to be included in the taste pathway. Responses to taste stimuli were recorded from this pontine area. Lesions in the pontine taste area resulted in degeneration of fibers reaching the lingual area in the thalamus.*

The end point in any sequence of food-seeking behavior is either ingestion or rejection. Gustatory stimuli act as a final arbiter of the consummatory behavior. In addition, gustatory stimuli have innate hedonic value—they are either positively or negatively reinforcing. Despite the importance of gustatory sensibility to behavior, the neural substrate for taste has remained poorly understood compared to that of the visual, auditory, or somatosensory systems. Neuroanatomy texts are hesitant about the central gustatory pathways, but nevertheless perpetuate the notion that the taste system joins the direct lemniscal pathways of the spinal and trigeminal somatosensory systems (*1*). We now have evidence that the taste pathways to the thalamus are neither direct nor lemniscal in the strict sense, but are mediated by cell groups in the pontine tegmentum not previously identified as components of the central taste pathway. The cells in this pontine taste area project to the thalamus, providing the basis for the thalamic and cortical representation of gustatory sensibility. In addition, this pontine taste area is potentially the first link in a polysynaptic pathway through which gustatory information could reach the limbic system areas associated with feeding and drinking behavior.

Gustatory information reaches the brain through the seventh, ninth, and tenth cranial nerves which synapse primarily in the nucleus of the solitary tract (NST) (*2*). In the thalamus, the projection area for the tongue is known to be localized in the medial third of the ventrobasal nucleus. Neurons responding to gustatory stimuli occupy the most medial portion of this lingual projection area (*3*). In 1923 Allen (*4*) described the ascending projections of the NST in the guinea pig as crossing to the contralateral medial lemniscus and distributing with this tract in the ventral and lateral thalamic nuclei. Stimulation of the chorda tympani [lingual branch of the facial nerve (VII)] or the glossopharyngeal nerve (IX), however, produces bilateral evoked potentials in the thalamus of rats, cats, and monkeys (*5*). The receptive fields of thalamic neurons responding to gustatory stimuli have been found to be ipsilateral on the tongue (*6*). We have now resolved this discrepancy between the anatomical and physiological evidence regarding the secondary pathways of the gustatory system by combining neural recording and degeneration techniques.

Rats anesthetized with pentobarbital were placed in a stereotaxic instrument fitted with blunt ear bars to avoid rupturing the tympanic membrane; the skull was trephined at a point 13 mm posterior to the bregma and 2 mm lateral to the midline. A fine wire electrode (67 μm in diameter), introduced through the overlying cerebellum, recorded neural activity from the rostral medulla while the tongue was stimulated with 0.25M NaCl, water, and a fine paint brush. Light brushing of the incisors, lips, chin, cheek, and whiskers were also routinely used as stimuli (*7*). When a response to tongue stimulation was obtained, a permanent record was made with a resistor-capacitor integrator and several other gustatory stimuli were tested (*8*).

When testing was complete, a small

electrolytic lesion (0.3 to 0.5 mm in diameter; 10 to 25 μa/20 sec) was made through the recording electrode. Four to 9 days after the lesion was made, the rats were deeply anesthetized and perfused intracardially with physiological saline and 10 percent formalin; one or two series of sections separated by 0.3-mm intervals were stained for degenerating fibers and axon terminals with the Fink-Heimer method. The lesions were localized in a separate series of sections by staining normal cells and fibers with the Fink-Schneider method (*9*).

Gustatory responses were recorded from the medullas of seven rats. In all cases of response to taste stimuli the lesion was found in the rostral tip of the NST in the region receiving the facial nerve afferents (Fig. 1). Responses to tongue cooling (two cases) were also located in the region. In contrast, when chin hairs or the lips were touched, the jaw was stretched, the vibrissae were passively moved, or air was blown into the nose, responses were recorded from sites lateral, medial, or ventral to the taste-responsive region.

In the area of the medulla from which responses were recorded, lesions unavoidably damage fibers of passage from surrounding structures afferent and efferent to the reticular formation and cranial nerve nuclei. This report will emphasize degeneration specific to cases of responses to taste stimuli and tongue cooling. A more extensive description of the degeneration from all responsive areas is in preparation (*10*).

In none of the cases of response to taste stimuli or tongue cooling were degenerating fibers seen entering either the ipsilateral or contralateral medial lemniscus. Instead, a stream of degenerating fibers ipsilateral to the lesion could be followed rostrally toward the pons into the supratrigeminal region, a reticular area dorsal to the trigeminal motor nucleus (*11*). After lesions were made through electrodes recording responses to taste stimuli or changes in tongue temperature (Fig. 2A, upper brain section), a field of terminal degeneration was found in a small-celled area surrounding the brachium conjunctivum (BC) as it enters the brainstem (Fig. 2A, lower brain section). In cases not responding to tongue stimulation there was no terminal degeneration in this area.

Most medullary lesions resulted in degeneration of fibers entering the trigeminal motor nucleus and ventral supratrigeminal area. In some instances a few degenerating fibers were seen in the ipsilateral central tegmental bundle, but these could not be traced beyond the pontomesencephalic junction. In some cases degenerating fibers were seen entering the BC and distributing to the red nucleus and the contralateral intralaminar and ventral thalamic nuclei. None of these fibers, however, passed through the lingual area of the thalamus.

Since in all cases the electrode tract (or tracts) passed through the deep cerebellar and vestibular nuclei, two cases with deep cerebellar lesions were examined. In these cases degenerating fibers entered the BC and distributed to the red nucleus and contralateral thalamus. No terminal degeneration or degenerating fibers entered the small-celled area surrounding the BC or the lingual area in the thalamus.

Dense fiber and terminal degeneration in the parabrachial grey area occurred only after lesions were placed through electrodes recording taste or temperature responses from the tongue. Since fibers of passage unrelated to gustatory sense could account for the degeneration observed, the region around the BC was explored electrophysiologically while the tongue was stimulated. In the two preparations attempted to date, reliable neural responses to gustatory stimuli were recorded from the dorsal pontine tegmentum. The lesions made through these electrodes were located on the dorsolateral and ventromedial border of the BC in the small-celled area which contains degenerating fibers and terminals after lesions are made in the NST (Fig. 2B, upper brain section).

Fiber degeneration resulting from these lesions entered the ipsilateral central tegmental bundle and could be traced into the thalamic lingual area (Fig. 2B, lower brain section). Degenerating fibers and terminals were also observed contralaterally in the

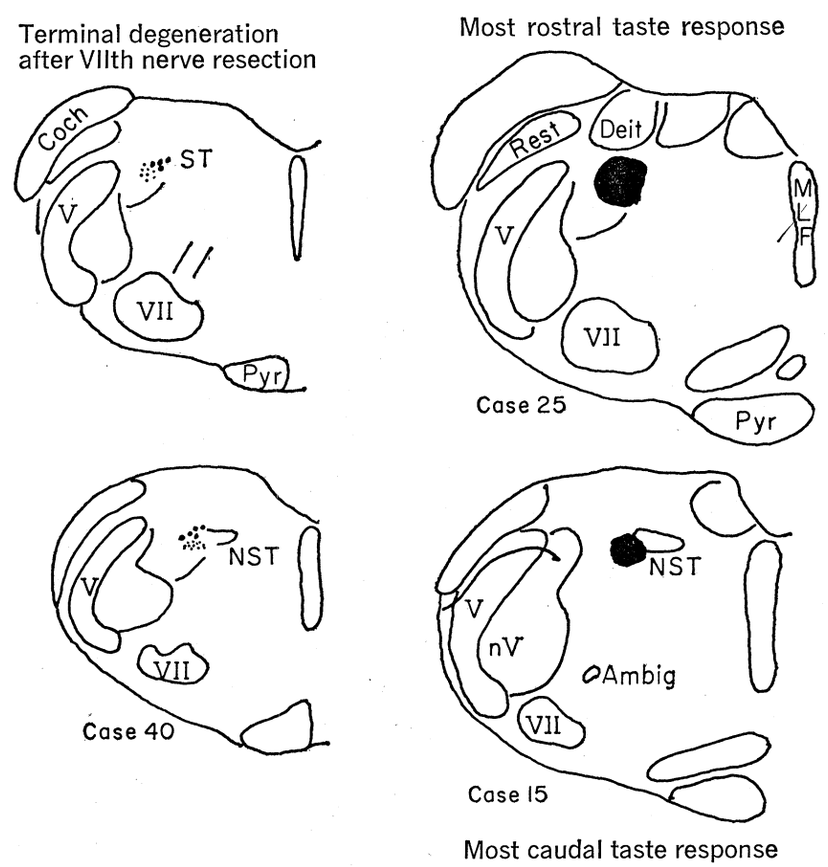

Fig. 1. A comparison of the most rostral and caudal sections containing terminal degeneration (fine dots) after resection of the facial nerve with the most rostral and caudal lesions made on electrodes recording responses to gustatory stimuli. The figures are tracings from projected sections stained by the Fink-Heimer method. *Ambig*, nucleus ambiguous; *Coch*, cochlear nucleus; *Deit*, Deiter's nucleus; *ST*, solitary tract; *NST*, nucleus of the solitary tract; *Pyr*, pyramidal tract; *V*, trigeminal nerve and its spinal tract; *VII*, motor nucleus of the facial nerve; *MLF*, medial longitudinal fasciculus; *Rest*, restiform body; *nV*, nucleus of the spinal trigeminal tract.

thalamic lingual area. The path of the fibers leading to the contralateral lingual area was obscured, however, because the lesion in both cases damaged the BC which distributes to the tegmentum and central grey matter on that side. No degenerating fibers were seen in the medial lemniscus on either side.

Preliminary electrophysiological data are consistent with the anatomical experiments indicating a polysynaptic gustatory projection to the thalamus. In three animals electrodes were implanted in the thalamus bilaterally at points which responded to gustatory stimuli. A twisted pair of electrodes was used to locate gustatory responses in the medulla. Stimulating through the bipolar medullary electrodes evoked multiunit responses on both ipsilateral and contralateral thalamic electrodes. The minimum latency of the responses was 4 msec, and they followed stimulation at 15 pulses per second poorly, if at all. Stimulation through the thalamic electrodes failed to produce any responses recorded by the medullary electrodes. A monosynaptic pathway could be expected to follow trains of up to 100 pulses per second (*12*) and to allow antidromic invasion of NST cells.

The combined electrophysiological and anatomical evidence indicates that, in the rat at least, the nucleus of the solitary tract does not project directly to the thalamus but, instead, to the cellular areas surrounding the brachium conjunctivum in the pons. These parabrachial cells, in turn, project bilaterally to the lingual area of the thalamus, apparently by way of the central tegmental bundle. This projection provides an anatomical basis for the repeatedly observed bilateral thalamic potentials evoked by taste-nerve stimulation (*5*). Fibers conducting gustatory impulses to the thalamus thus appear to ascend among the largely polysynaptic reticular pathways extending through the central pons and midbrain.

Although a pontine relay in the secondary taste pathway has not been described before, a primary pontine taste area has been inferred previously on anatomical grounds (*13*). In human material, Nageotte described the NST as extending into the pons dorsomedial to the principal trigeminal nucleus (PVN). In the cat and monkey, Rhoton found ascending components of the facial nerve terminating in the dorsomedial PVN and in a cellular area just medial to that nucleus. He could not identify such ascending primary facial afferents in the rat. This negative result was confirmed in the present series in a rat with facial nerve resection. In the cat, Bernard and Nord (*14*) recently recorded short-latency (< 3-msec) responses to chorda tympani stimulation from single units located dorsomedially to PVN. These units also responded to taste stimuli applied to the tongue. The pontine area in the cat which receives direct chorda tympani afferents may be analogous with the pontine area in rats which responds to gustatory stimuli, but a more detailed analysis in both species is necessary to resolve the issue.

Taste is intimately related to feeding behavior, but the gustatory system has yet to be anatomically related to the brain areas implicated in feeding behavior, most notably the hypothalamus. There is evidence of hypothalamic neurons which are sensitive to gustatory stimuli (*15*), but neither the thalamic nor the cortical lingual areas are known to project to the hypothalamus, or to areas related to the hypothalamus (*16*). It seems likely, therefore, that gustatory information is conveyed to the hypothalamus through pathways organized in parallel rather than in series with those leading to the thalamus and cortex.

The gustatory conduction system

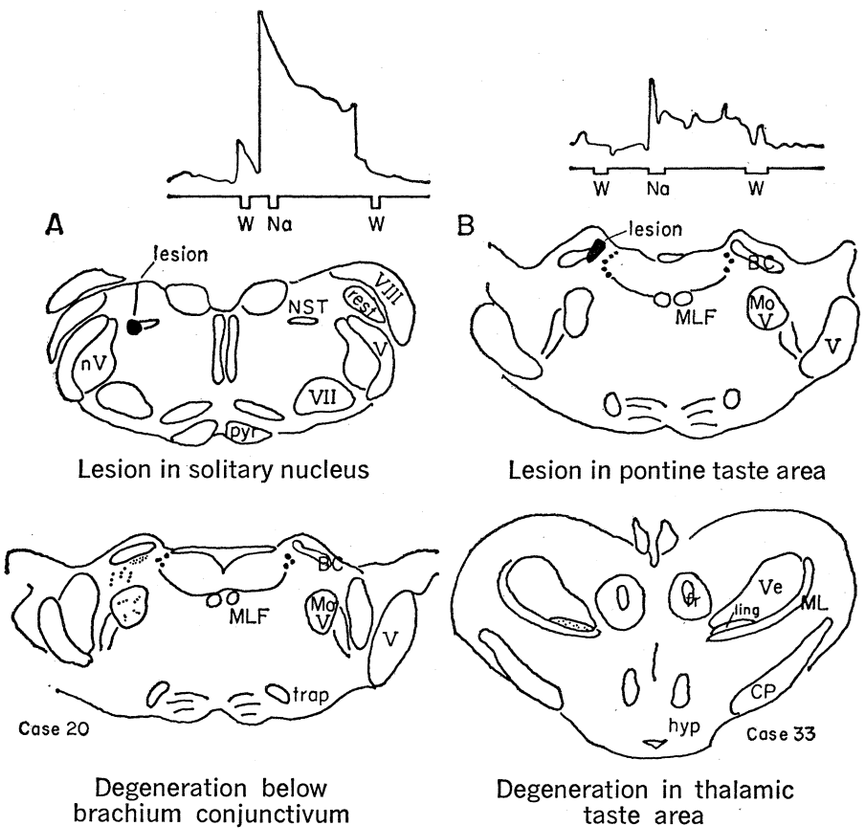

Fig. 2. The insets above the anatomical figures are examples of the integrated neural activity recorded from two electrode loci (A and B) before the lesions were made. The magnitude of the responses in A and B are only approximately comparable, because different chart recorders were used. (A) A lesion in the nucleus of the solitary tract (*NST*) at a point which responded to gustatory stimuli produced terminal degeneration (fine dots) in a small-celled area immediately below the brachium conjunctivum (*BC*). In other cases with lesions in the NST, degenerating fibers and terminals appear both dorsal and ventral to the BC. (B) A lesion in the parabrachial cellular area at a point which responds to gustatory stimuli produced terminal degeneration in the lingual projection area of the thalamus. Degenerating fibers and terminals were present bilaterally. The contralateral degeneration is not depicted, because damage to the BC has caused widespread degeneration there. *W*, distilled water washed over the tongue; *Na*, 0.25*M* NaCl solution washed over the tongue; *V*, trigeminal nerve and its spinal tract; *nV*, nucleus of the spinal trigeminal tract; *VII*, motor nucleus of the facial nerve; *MLF*, medial longitudinal fasciculus; *MoV*, motor nucleus of the trigeminal nerve; *pyr*, pyramidal tract; *rest*, restiform body; *trap*, trapezoid body; *Ve*, ventrobasal nucleus.

ascending from the pontine level through the midbrain tegmentum could be channeled in part to the hypothalamus, either by way of relays in the paramedian mesencephalic cell groups (*17*), or by way of a recently described, more lateral mesencephalohypothalamic route (*18*). The terminal degeneration in the lingual area of the thalamus after a lesion in the parabrachial nuclei is not as intense as the degeneration which results more laterally in the ventrobasal thalamic nuclei from a lesion in the principal trigeminal nucleus. This suggests that additional synaptic relays may intervene between the pontine taste area and the thalamus. Since the central tegmental pathways project both dorsally and ventrally in the diencephalon, polysynaptic gustatory fibers in these pathways provide a potential route over which taste information could reach the hypothalamus.

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8. Aside from 0.25*M* NaCl and distilled water, the taste stimuli were 0.5*M* sucrose, 0.001*M* quinine hydrochloride, and 0.003*M* hydrochloric acid. Although the responses to the various stimuli differed from one placement to the next, no differences were obvious in the anatomical results.
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Lymphocyte DNA Synthesis Inhibition

Abstract. *A specific endogenous inhibitor for lymphocyte DNA synthesis that can be isolated from the lymphoid system and which is probably cell specific is described. The inhibitor is thermolabile, is destroyed by trypsin, and has a mass of about 30,000 to 50,000 daltons.*

Bullough and Laurence proposed that epithelial cells make a specific inhibitor of the mitosis of the cell (*1*). Subsequent studies by others have indicated that these specific endogenous mitotic inhibitors might also be obtained from melanocytes (*2*), granulocytes (*3*), kidney cells (*4*), and cells of the lens (*5*) for each of these cell types, respectively. Moorhead *et al.* presented evidence that aqueous extracts of the lymph nodes from pigs can inhibit the transformation of human lymphocytes induced by phytohemagglutinin (PHA) (*6*). We now report that the lymphoid system contains noncytotoxic specific inhibitors of the incorporation of tritiated thymidine into human lymphocytes stimulated by PHA, by mixed leukocyte cultures, and by leukocytes derived from patients with lymphocytic leukemia.

Leukocytes were obtained from normal human donors and grown in medium 199 (10^6 cells per 2 ml) supplemented with 2 m*M* glutamine and 20 percent autologous human plasma; the medium also contained penicillin and streptomycin. These cultures were incubated in triplicate with PHA (PHA-p, Difco) for 66 hours at 37°C. Then 1 μc of [^{3}H]thymidine was added to the culture medium, and the cells were incubated for six more hours. The cultures were then washed once with cold saline and precipitated with 5 percent trichloroacetic acid (TCA); the precipitate was washed three times with 5 percent TCA, and then solubilized with 0.5 ml of NCS (Nuclear-Chicago) solubilizer; the radioactivity was determined in a liquid scintillation counter. The cytology and viability of the cultures was ascertained, before TCA precipitation, with vital staining (trypan blue, 0.4 percent).

Rat lymph nodes and spleen, and calf thymus and spleen, were homogenized at 4°C in 0.15*M* NaCl (10 ml/g) and homogenized by sonication for 1 minute and then centrifuged at 10,000*g* and 4°C for 1 hour. This supernatant was removed and dialyzed against 200 volumes of distilled water for 48 hours at 4°C. The precipitated euglobulins were removed by centrifugation, and the supernatant was lyophilized. This is the S_1 fraction.

Portions (1.0 mg) of S_1 derived from calf spleen and thymus and rat spleen and lymph nodes were incubated with normal human leukocyte cultures for 72 hours, and all inhibited approximately 60 to 100 percent of the [^{3}H]thymidine incorporation by lymphocytes that had been stimulated by two different batches of PHA (Table 1).

Inbred rats (Fisher) were the source of the lymph node and spleen S_1 extracts. These extracts inhibited PHA-induced uptake of [^{3}H]thymidine by cultured syngenic rat lymphocytes and cultured human lymphocytes. Thus, it would appear that the suppression of PHA-induced transformation is not due to xenogenic differences between the donors of the lymphoid extract and of the cultured lymphocytes.

Similarly prepared extracts of rat muscle and of WI-38 fibroblasts did not inhibit PHA-stimulated incorporation of [^{3}H]thymidine into lymphocytes.