over the outer margins of the taste bud. With increasing cornification of the tongue, a narrow channel forms between the taste pit and the oral environment. The number of taste buds per fungiform papilla also increases from one at approximately 84 days, to two at approximately 100 days of gestation, to three or more in lambs and adults. It is difficult to correlate these light microscopic anatomical changes with the electrophysiological data without the added insight that could be provided from ultrastructural studies, including observations on taste bud cell membranes and synapses.

From our studies of responses from ST chemosensitive units in fetal, newborn, and adult sheep we conclude that before structural development of taste buds is complete, taste responses can be recorded in the central nervous system; functional changes in range of responsiveness to salts and acids accompany morphological changes in taste buds. Cells in younger fetuses usually respond to lingual stimulation with NH_4Cl, KCl, and citric acid only; more units respond to HCl as development progresses, and sensitivity to NaCl and LiCl first appears in older fetuses. Therefore, taste responses seem to develop in a particular sequence, not randomly. These changes in the range of responsiveness may relate to maturation of taste receptor sites.

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4. Recordings were characterized as single-unit when neural impulses of one consistent amplitude were obtained and as few-unit when impulses of different amplitudes were obtained.
5. The mean spontaneous discharge frequency for each unit was calculated by averaging the impulse frequency during the 5-second periods preceding each lingual stimulation. The frequency of spontaneous activity for all units ranged from 0.06 to 9.10 impulses per second. Mean impulse frequency during chemical stimulation was calculated from neural activity during seconds 3 through 7 after application of the stimulus to the tongue. The first 2 seconds of response were not included, to eliminate counting errors resulting from stimulus artifacts.
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Aphagia and Adipsia After Preferential Destruction of Nerve Cell Bodies in Hypothalamus

Abstract. *Microinjections of the excitatory neurotoxin kainic acid into the lateral hypothalamus of rats produced a period of aphagia and adipsia. Kainate-treated rats displayed transient motor effects during the first hours after the injection but did not show the persisting sensory-motor and arousal disturbances typically observed in animals with electrolytic lesions in this part of the hypothalamus. Histological examination revealed a significant reduction in the number of nerve cell bodies in the lateral hypothalamus. Silver-stained material indicated no evidence of damage to fiber systems passing through the affected region. Assays of dopamine in hypothalamus, striatum, and telencephalon did not indicate significant differences between experimental and control animals. These results are in agreement with recent reports of the anatomical and biochemical effects of intracerebral kainic acid injections and suggest that the observed effect on feeding behavior is related to the destruction of neurons in the lateral hypothalamus.*

Food and water intake (hunger and thirst) are thought to be regulated by excitatory and inhibitory mechanisms intrinsic to the hypothalamus. This idea is based on an extensive body of research demonstrating that ingestive behavior is abolished or exaggerated by certain hypothalamic lesions and that food or water intake can be elicited or inhibited by electrical stimulation or microinjections of putative neurotransmitters and related compounds into the diencephalon (*1*). The "hypothalamocentric" interpretation has been seriously questioned in recent years because (i) it is not clear that the effects of hypothalamic lesions or stimulation can be ascribed to a direct effect on soma and dendrites rather than fibers of passage, and (ii) the behavioral effects of hypothalamic lesions or stimulation are not specific to ingestive behavior (*2*).

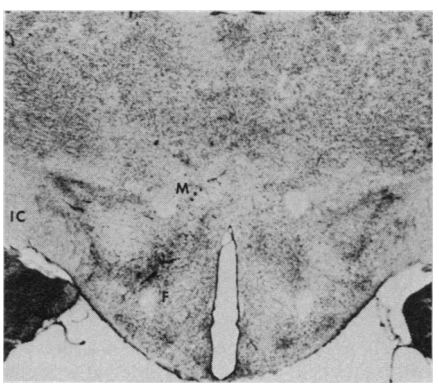

Fig. 1. Sections (50 μm) through the brain of a kainic acid-treated rat of group 7. The area damaged by the injections shows a loss of neurons and increased glia. This animal was aphagic and adipsic when killed 10 days after the injection. Cresyl violet stain; *IC*, internal capsule; *F*, column of the fornix; *M*, mammillothalamic tract.

The focus of the recent criticism has been on the lateral hypothalamus because this region is relatively cell-poor and is traversed by numerous diffuse fiber systems, several of which originate or terminate in areas of the brain (such as the globus pallidus, substantia nigra, and ventral tegmentum) where electrolytic or more selective chemical lesions produce effects on behavior that are similar to those seen after damage to the lateral hypothalamus (*3*).

In all cases, severe sensory-motor disturbances (lack of endogenous arousal, absent or impaired responsiveness to external stimuli, and akinesia) are prevalent throughout the period when the animals are aphagic and adipsic. Although it was suggested (*4*) that the sensory-motor impairments failed to correlate with the severity and persistence of the aphagia and adipsia syndrome, more recent investigators (*5*) have demonstrated parallel recovery functions and concluded that a lack of endogenous arousal or impaired arousal response to external or internal stimuli might be responsible for or contribute to the aphagia and adipsia syndrome.

We have investigated the role of some of the major fiber systems which course through the lateral hypothalamus by means of surgical knife cuts (*6, 7*). The results of these experiments indicate that

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Table 1. Effect of kainic acid injections into the lateral hypothalamus on food and water intake. In groups 1 to 6 and 8 the infusions were delivered over 1 minute. In group 7 kainic acid was infused over 12 to 15 minutes in a smaller volume to avoid spread to adjacent brain structures. Brains from animals injected unilaterally (groups 2, 4, and 6) were stained by the Fink-Heimer method to determine more clearly the damage associated with the behavioral effect.

Group	N	Kainic acid (μg)	Volume injected (μl)	Bilateral (B) or unilateral (U) injection	Days of aphagia		Days of adipsia	
					Mean	Range	Mean	Range
1	11	1.0	1.0	B	5.0	3-10	3.5	2-10
2	4	1.0	1.0	U	1.0	1	1.3	1-2
3	9	1.0	0.5	B	3.3	2-7	2.7	0-7
4	3	1.0	0.5	U	0.7	0-2	1.0	1-2
5	3	1.0	0.25	B	1.7	1-3	1.7	0-4
6	3	1.0	0.25	U	1.7	1-2	1.7	1-2
7	8	0.5	0.10	B	7.5	2-10	7.5	2-10*
8	2	†		B	0.0‡		0.0	

*Three animals were killed on day 10 before recovery of voluntary food or water intake. †Vehicle injection control. ‡Food intake of control animals did not differ significantly from preoperative baseline.

any significant interference with afferent or efferent connections of the striatum results in aphagia and adipsia as well as various sensory-motor disabilities that may or may not contribute to the observed impairments in ingestive behavior. We also find that individual knife cuts often selectively interfere with the behavioral response to specific glucoprivic or hydrational challenges, which suggests that neural mechanisms that may or may not originate in the lateral hypothalamus but course through it are much more specifically related to the regulation of food and water intake than recent hypotheses have suggested.

To investigate what role neurons intrinsic to the lateral hypothalamus play in the regulation of ingestive behavior, intracranial injections of kainic acid were administered to male rats. Kainic acid, a structural analog of glutamate, preferentially destroys cell somata without damaging axons of passage (*8*). This report summarizes the effects of slow infusions of small quantities of this substance into the lateral hypothalamic region (*9*).

All kainic acid injections produced transient motor effects beginning approximately 20 minutes after the injection and lasting approximately 5 hours (*10*). Bilateral injections resulted in vigorous treading of both forelimbs, tilting up of the head, and occasional rolling motions along the long axis of the body that were initiated by head rotation. Forelimb treading was reduced when the animal was held with the head higher than the body. Unilateral injections produced treading of a single forepaw and a head tilt contralateral to the injection site. Intermittent propeller-like motions of the tail and body rolling in the direction contralateral to the injection were also observed. All signs of overt motor disturbances had completely disappeared 12 hours after the injection. After this transient phase of apparent motor excitation, there were no indications of motor dysfunctions, somnolence, or impaired arousal in response to environmental stimuli. In fact, when placed into an open field together with other experimental and control rats (including rats that were akinetic as a result of electrolytic lesions in the dorsolateral hypothalamus), the kainate-treated animals explored avidly, interacted apparently normally with conspecifics, and displayed exaggerated startle responses.

The results of kainic acid injections into the lateral hypothalamus on food and water intake are summarized in Table 1. Animals in groups 1, 3, and 5 received bilateral injections of 1 μg of kainic acid in varying volumes of artificial cerebrospinal fluid (CSF). Animals in group 1, which received a relatively large volume (1 μl) over approximately 1 minute, were subsequently aphagic and adipsic for 2 to 10 days. Smaller volumes delivered over the same infusion period (groups 3 and 5) produced significant but less persistent effects. The most persistent aphagia and adipsia (mean, 7.5 days) were obtained when a smaller dose of kainic acid (0.5 μg) dissolved in only 0.1 μl of CSF was very slowly infused over 12 to 15 minutes (group 7).

Examination of the histological material by light microscopy (*11*) revealed that when large volumes were infused over 1 minute (group 1) the damaged area (as indicated by glial proliferation and nerve cell loss) was not confined to the lateral hypothalamus, but included portions of the dorsomedial hypothalamus and subthalamic and thalamic nuclei. The smaller volumes injected in groups 3 and 5 produced more limited damage but tended to spread dorsally along the cannula track and thus destroy only part of the lateral hypothalamic area. In group 7, unpredictable spread was avoided by use of small volume and slow infusion rate. This procedure provided excellent localization of the visible consequences of the injection within the lateral hypothalamic area (Fig. 1). Kainic acid injection markedly reduced the

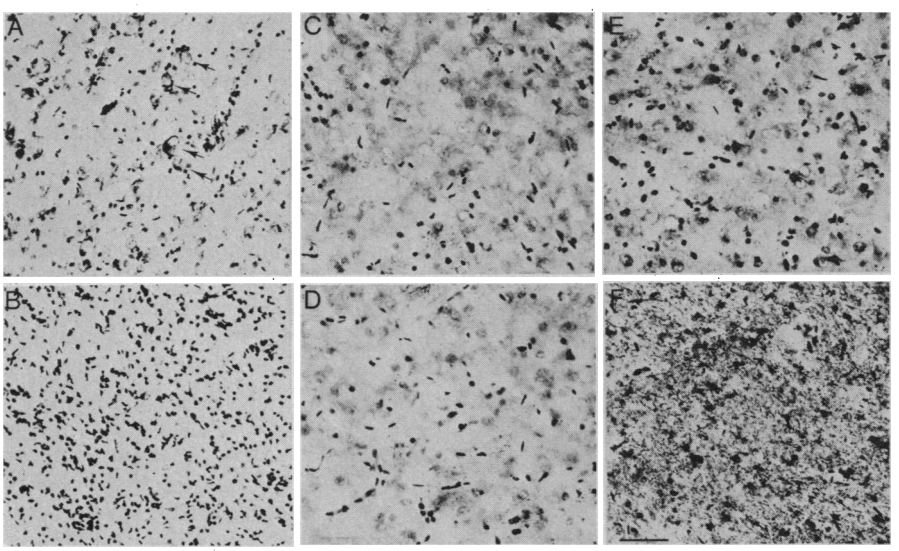

Fig. 2. (A and B) Lateral hypothalamus contralateral (A) and ipsilateral (B) to a kainic acid injection (2 μg in 1 μl of CSF, 6-day survival time). Intact cells (arrows) are in clear evidence contralateral to the injection (A), while an absence of neurons and intense glial proliferation characterizes the injection site (B). (C and D) The head of the caudate nucleus contralateral (C) and ipsilateral (D) to the kainic acid injection (1 μg in 0.5 μl of CSF, 4-day survival time). Only minimal degeneration debris associated with fibers traversing this nucleus is present; this indicates minimal damage to the dopamine fibers, which innervate the caudate and pass through the lateral hypothalamic area. (E and F) Lateral septal nucleus contralateral (E) and ipsilateral (F) to the kainic acid injection. Only background levels of degeneration are seen contralateral to the injection (E), with heavy fiber and terminal degeneration occupying this nucleus on the side of the injection (F). Calibration, 50 μm.

number of nerve cell bodies in the lateral hypothalamic region (Fig. 2).

Fink-Heimer silver staining of tissue obtained from animals that received unilateral injections was undertaken to collect data on the hypothalamic projections that may have been affected by the kainic acid lesion and to estimate the amount of damage done to fiber tracts traversing the lesion site. No significant fiber degeneration was detected in the fornix, internal capsule, and mamillothalamic tract. Fibers positioned on the lateral fringe of the medial forebrain bundle at the level of the lateral hypothalamus comprise portions of the ascending dopamine systems, which innervate the neostriatum, olfactory tubercle, and other forebrain structures. Scattered degeneration was observed, in some cases, in these structures, but we could find no differences between kainic acid-treated, vehicle-injected, and uninjected implanted controls (Fig. 2). Thus, we have no evidence that dopaminergic axons of passage were significantly affected by the kainic acid injection. One forebrain structure that did exhibit heavy terminal degeneration was the lateral septal nucleus (Fig. 2, E and F). This pattern is consistent with autoradiographic data tracing the primary ascending projections of the lateral hypothalamus (*12*).

In view of recent suggestions (*5*) that some or all of the effects of lateral hypothalamic lesions on food and water intake might be due specifically to an interruption of the dopaminergic nigrostriatal projection system, the effects of kainic acid injections on brain dopamine were assessed. We selected ten animals that had been aphagic and adipsic for 3 to 10 days after intrahypothalamic injections of 0.5 μg of kainic acid in 0.1 μl of vehicle and ten unoperated controls and dissected their brains into hypothalamus, striatum, and telencephalon (these animals are not included in Table 1 because no histological data were obtained). The three regions were then assayed (*13*) for dopamine. The results of the assay indicated no significant damage to dopaminergic projections to any of the three regions examined. The mean dopamine concentrations (nanograms per gram of tissue) ± standard error were as follows: in hypothalamus, 186.3 ± 19.1 in treated animals and 126.4 ± 20.8 in controls; in striatum, 8582.8 ± 501.7 in treated animals and 8648.1 ± 432.8 in controls; and in telencephalon, 584.7 ± 40.4 in treated animals and 617.3 ± 27.1 in controls.

Our results are in good agreement with earlier reports (*8*) of cytological and biochemical effects of intracranial injections of kainic acid that have indicated a selective destructive effect on neuronal cell bodies. Taken together, our histological, biochemical, and behavioral results are consistent with the suggestion that aphagia and adipsia produced by kainic acid injections are related to the selective destruction of lateral hypothalamic neurons, whereas the behavioral depression associated with the classic lateral hypothalamic syndrome may be related to incidental interruption of the nigrostriatal, pallidonigral, or other pathways passing through this region.

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7. Knife cuts were made along portions of the lateral border of the diencephalon, across lateral and medial aspects of the medial forebrain bundle or specific noradrenergic components of it in the tegmental area, across medial, lateral, or ventral connections of the globus pallidus and caudate nucleus, and through portions of the hypothalamus itself.
8. Several cyclic glutamate-related compounds including kainic acid are powerful excitants of neurons in the central nervous system [G. A. R. Johnston, D. R. Curtis, J. Davies, R. M. McCulloch, *Nature (London)* **248**, 804 (1974); (*14*)]. A number of investigators (*14, 15*) have presented extensive histological and biochemical evidence that microinjections of kainic acid into mammalian brain tissue (striatum and diencephalon have been the principal targets) destroy somal and dendritic components of nerve cells with little detectable effect on axonal material and terminal arborizations. The mechanisms by which kainic acid destroys neural soma and dendrites is poorly understood. It is assumed that the destructive effects of this compound may be due to the inability of neurons to terminate its activity by reuptake or metabolic mechanisms [M. J. Schmidt, J. F. Thornberry, B. B. Molloy, *Brain Res.* **121**, 182 (1977); J. R. Simon, J. F. Contrera, M. J. Kuhar, *J. Neurochem.* **23**, 1 (1975); R. M. McCulloch, G. A. R. Johnston, C. J. A. Game, D. R. Curtis, *Exp. Brain Res.* **21**, 515 (1974)]. Whether kainic acid affects all neurons equally is also uncertain. Both anatomical (*14, 15*) and biochemical data [E. G. McGeer and P. L. McGeer, *Nature (London)* **263**, 517 (1976)] suggest that not all neurons are destroyed by kainic acid injections, and there is some indication [R. M. Herndon and J. T. Coyle, *Science* **198**, 71 (1977)] that neurons with glutamic acid receptor sites may be preferentially affected.
9. Stainless steel cannulas of 30-gauge diameter were stereotaxically lowered into the brains of adult male albino rats under sodium pentothal anesthesia. The cannulas were positioned so that their tips terminated just dorsal to the region of the dorsolateral hypothalamus where electrolytic lesions produce aphagia and adipsia most effectively. Kainic acid (Sigma, St. Louis, Mo.) was dissolved in artificial cerebrospinal fluid as described by K. A. C. Elliot and H. H. Jasper [*J. Neurosurg.* **6**, 140 (1949)]. This solution is such an excellent buffer that the *p*H of our kainic acid injections was in the normal physiological range (7.0 to 7.4). A volume of 0.1, 0.25, 0.5, or 1.0 μl, containing 0.5 or 1.0 μg of kainic acid, was then gradually infused over a period of 1 or 12 minutes through a Hamilton gas syringe; the cannula was then removed. Control injections of the artificial CSF vehicle were made in two animals. Food and water intake was monitored beginning 12 hours after surgery, when all motor effects had subsided. All animals that refused food or water for 48 hours after the injection were intubated intragastrically with small quantities of a liquid diet until voluntary ingestive behavior returned. Four animals died after several days of aphagia and adipsia despite this supplementary feeding regimen.
10. In a preliminary study, animals that received kainic acid injections through permanently implanted cannulas (after a 7-day period of recovery from surgery) displayed feeding and feeding-related behaviors shortly after the injection, an observation that supports the hypothesis that the initial effect of kainic acid and related compounds is excitatory. These animals showed the transient motor impairments described above and were probably not capable of sustained feeding. They did, however, make repeated attempts to ingest dry pellets or drink liquid diet from a tube beginning a few minutes after the injection. The quantities of food ingested were insignificant but the consistent presence of ingestive behaviors in animals beset by severe involuntary motor spasms was nonetheless impressive.
11. Animals with bilateral kainic acid injections of various volumes and concentrations were killed several days after their food and water intake returned to control levels (at least 15 days after injection). The animals were perfused with a 10 percent formol saline solution, and their brains were removed and stored in formalin. Frozen sections (30 μm) through the region of the kainic acid injection were later stained with cresyl violet. Animals with unilateral injections were killed 3, 7, or 10 days after the treatment. Alternate sections were stained with thionin and by the Fink-Heimer procedure as described [R. M. Clavier and A. Routtenberg, *Brain Res.* **105**, 325 (1976); Y. H. Huang and A. Routtenberg, *Physiol. Behav.* **7**, 419 (1971)].
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