Axon-Sparing Brain Lesioning Technique: The Use of Monosodium-L-Glutamate and Other Amino Acids

Abstract. *Infusions of monosodium-L-glutamate into the rostral hypothalamus, believed to contain neurons mediating satiety, produced persistent hyperphagia and obesity, thus suggesting that a brain lesion had been produced. Similar infusions into the caudal hypothalamus, believed to contain unmyelinated axons of passage that mediate satiety, failed to alter food intake or body weight. Histological examination of the affected tissue confirmed the behavioral evidence that suggests that this technique spares axons but destroys cell bodies. Infusions of several other amino acids also damaged neurons while sparing axons of passage.*

A major difficulty in the interpretation of behavioral and physiological changes caused by brain lesions is that the lesions produced by traditional methods [electric currents, radio frequencies, and transmitter-specific neurotoxins (*1*)] damage axons of passage as well as the cells and synapses within the area of the brain being studied (*2*).

Experimental differentiation between fibers of passage and cell bodies is provided in part by knife-cut techniques. Knife cuts damage axons that traverse the plane of the cut, while cell bodies that are not attached to the severed axons are spared. A complementary technique that would permanently inactivate cell bodies or synapses in a particular area while sparing fibers of passage should lead to a clearer understanding of regional brain function.

We have tested a variety of potential lesioning substances, the first and most notorious being the dietary additive monosodium-L-glutamate (MSG). In order to evaluate selectivity we used a neural system whose route has been mapped and whose destruction leads to "hypothalamic" hyperphagia and obesity. The literature on this brain lesion syndrome has been reviewed (*3*). The neurocircuitry of the system that must be damaged in order to produce hyperphagia has been mapped by means of a variety of approaches including asymmetrical knife cuts (*4*). These "satiety" fibers ascend (or descend) in the lateral hypothalamus in or alongside the medial forebrain bundle (MFB). At its rostral end, at the coronal level of the paraventricular nucleus (PVN), the satiety system turns toward the midline, where it presumably terminates (or originates). Injections of norepinephrine (NE) in the region of the PVN, presumably acting at NE-sensitive synapses, elicit vigorous eating (*5*).

These studies provided tentative verification of the system we worked with: (i) fibers that pass longitudinally in or adjacent to the MFB and (ii) synapses in the rostro-medial hypothalamus, near the PVN. A lesion that spares fibers of passage should produce overeating only when placed at the coronal level of the PVN, while nonselective lesions or knife cuts should be effective when placed more caudally.

Using adult female Charles River CD rats, we slowly infused 5 or 10 μl of 1 percent MSG (*6*) into the brain at the rate of 1 μl per minute. Infusions were into one of two locations. One location (Fig. 1, location *B*) was the cellular area ventrolateral to the PVN where electrolytic lesions most effectively produce hyperphagia and obesity (*7*). The second, a site chosen to damage axons of passage, was 2 to 3 mm more caudal than the first and was adjacent to the ventromedial nucleus (Fig. 1, location *D*). On the other side of the brain we repeated one or the other infusion, or placed a knife cut at either of two locations at which bilateral knife cuts are known to produce hyperphagia and obesity (*8*). We placed one knife cut parasagittal and adjacent to the PVN in order to sever axons leaving or entering the cellular area before they turned longitudinally (Fig. 1, location *A*). The other knife cut was coronal, transecting much of the hypothalamus (and longitudinal fibers of passage) at the same coronal level as the more caudal MSG infusion site (Fig. 1, location *C*).

As reported earlier (*9*), unilateral damage to satiety neurocircuitry produced only minimal excess weight gains (Table 1). As predicted, infusions into the cellular area produced large weight gains when combined with contralateral in-

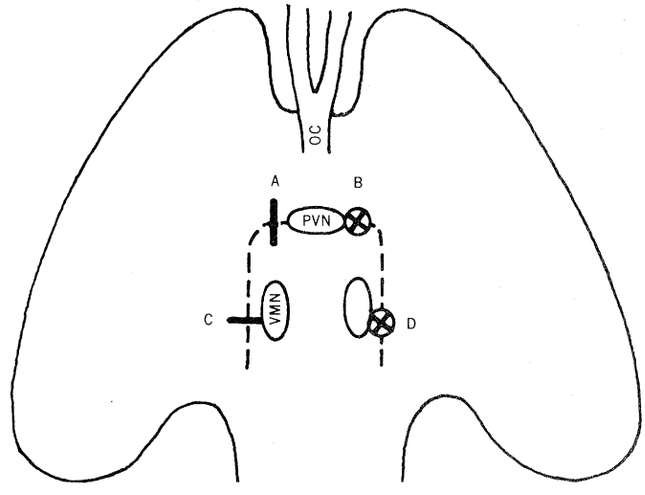

Fig. 1. Horizontal plane of a rat brain showing sites of injections and knife cuts. Symbols: *A*, anterior hypothalamus parasagittal knife cut; *B*, anterior hypothalamus injection site; *C*, mid-hypothalamus coronal knife cut; *D*, mid-hypothalamus injection site; PVN, paraventricular nucleus; and VMN, ventromedial nucleus.

Table 1. Excess weight gains in rats infused with 1 percent (0.059*M*) MSG. Changes in weight gain were calculated from the postoperative weight gain (grams per day for 2 weeks) minus the preoperative weight gain (grams per day for 1 week). The rats were fed a high-fat diet (*4*).

Procedure	*N*	Location in Fig. 1	Change in weight gain
MSG in anterior hypothalamus plus parasagittal knife cut	8	B and A	4.67 ± .53*
MSG in anterior hypothalamus plus frontal knife cut	8	B and C	4.92 ± .62*
Bilateral MSG in anterior hypothalamus	8	B and B	3.33 ± .28*
MSG in anterior hypothalamus plus MSG in mid-hypothalamus	4	B and D	0.24 ± .09
MSG in mid-hypothalamus plus parasagittal knife cut	4	D and A	0.68 ± .37
Unilateral MSG in anterior hypothalamus	2	B	0.41 ± .34
Unilateral parasagittal knife cut	2	A	0.54 ± .32
Saline (0.85 percent) in anterior hypothalamus plus parasagittal knife cut	2	B and A	0.84 ± .34
Controls with sham operations	10		0.87 ± .07

*Differs significantly from controls $P < .001$, Mann-Whitney U test.

fusions into the cellular area or with either type of contralateral knife cut. In contrast, infusions into the more caudal area, containing axons of passage, were ineffective no matter what type of contralateral lesion they were combined with. Thus, the 1 percent MSG infusion produced lesion-typical behavioral effects if it was placed in the cellular area but not if it was placed among the axons of passage. The knife cuts, in contrast, were effective at both locations. We have previously shown electrolytic lesions to be behaviorally effective at both locations (*9*).

Since our slow (1 μl per minute) infusions caused permanent changes in food intake in the same direction as those that occur after electrolytic lesions at the same site, but only if the infusions were made in locations containing cell bodies, we conclude that there was permanent synaptic or cellular dysfunction, or both, in the vicinity of the injection, and that fibers of passage were spared.

The rats were killed 2 to 6 weeks after surgery. When examined by light microscopy, sections of the brain stained with cresyl violet had the appearance of normal brain tissue near the cannula. The hole, scarring, and tissue distortion generally seen with lesions produced by electric currents or radio frequencies were absent. Only a slender needle track identified the infusion site. At high magnifications, a greater-than-normal complement of glial cells and a paucity of neurons was seen near the MSG infusion sites. The blood vessels near these sites appeared intact. Similar gliosis, with numerous astrocytes and microglia filled with neuronal debris but with normal vascularity, occurs in the ventromedial and arcuate nuclei after systemic injections of MSG (*10*). The brains of saline-infused controls only had needle tracks and the narrow band of gliosis attributable to the needle insertion.

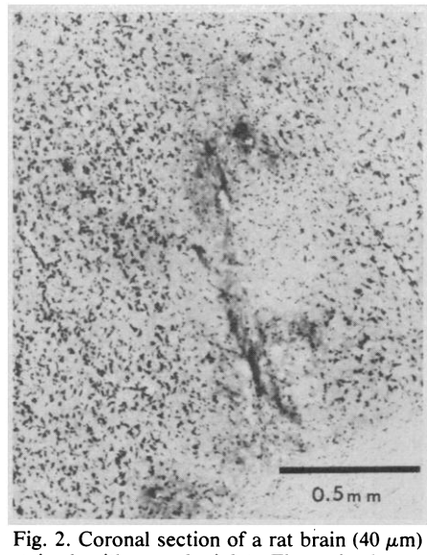

Fig. 2. Coronal section of a rat brain (40 μm) stained with cresyl violet. The animal was killed 6 hours after surgery. See text for discussion.

The clearest picture of the neuronal destruction is observed on brain sections taken from animals killed 6 hours after the infusion of 5 μl of 1 percent MSG. As shown in Fig. 2, a small cell-free cylindrically shaped area (0.5 mm in diameter, 0.9 mm long) surrounds the needle track. The margins of the cell-free area show no sign of gliosis. Just 6 hours later (12 hours after surgery) microglia have migrated throughout the infusion site, and by 24 hours after surgery dense gliosis occurs in the area of the infusions (*11*). In contrast, sections from animals killed 6 hours after the infusion of 5 μl of physiological saline showed a cell-free area only 0.15 mm in diameter. This damage was of uniform width and appearance for the full extent of the needle track and thus appears to be due to the needle itself.

Weigert-Weil staining for myelin proved to be of little use in revealing damage because the fibers in question are poorly myelinated. Fink-Heimer staining for degeneration was performed on the brains of rats killed 14 days after surgery. Except for a very small ring of damage that could have been caused by the cannula penetration itself, no axonal damage and numerous normal axons were seen at the infusion sites. In contrast, after parasagittal knife cuts, Fink-Heimer staining revealed a profusion of degenerating fibers.

The cellular neurotoxicity of our localized infusions of MSG is further supported by reports of cellular brain damage occurring after systemic administration of MSG (*12*). Histologically, the cells of the arcuate nucleus are the most sensitive to systemic MSG (*10, 12, 13*). Also, localized injections of MSG into the motor cortex produce convulsions only when they are placed in cellular areas, not in fiber areas (*14*). Electrophoretic application of glutamate to the cerebral cortex induces localized neuronal degeneration (*15*). It has also been shown that glutamate-induced cortical spreading depression affects only neuronal cell bodies

Table 2. Excess weight gains in rats infused with MSG and other solutions.* Weight gains were calculated by subtracting the preoperative weight gain (grams per day for 1 week) from the postoperative weight gains (grams per day for 2 weeks). The procedures were: 1, MSG in the anterior hypothalamus plus parasagittal knife cut (B + A in Fig. 1); 2, MSG in the mid-hypothalamus plus parasagittal knife cut (D + A in Fig. 1); 3, MSG in the anterior hypothalamus plus frontal knife cut (B + C in Fig. 1); and 4, MSG into the anterior hypothalamus (B in Fig. 1) bilaterally.

Amino acid	Concentration (*M*)	Procedure							
		1 (B + A)		2 (D + A)		3 (B + C)		4 (B + B)	
		N	Gain	*N*	Gain	*N*	Gain	*N*	Gain
MSG (from Table 1)	0.059 (1 percent)	8	4.67	4	0.68	8	4.92	8	3.33
L-Aspartate	0.059	2	−0.29	1	−3.72	1	−0.92		
L-Glycine	0.059	4	0.16	2	−1.36				
L-Glycine	0.100	2	5.00	2	0.89				
L-Glycine	0.150	1	5.78	1	2.29				
L-Leucine	0.015	2	2.85	2	4.85				
L-Leucine	0.030	1	6.19	1	5.46				
L-Leucine	0.059	3	0.23	2	0.80	2	0.22	1	−1.57
D-Tryptophan	0.059	7	5.25	4	1.55			1	0.20
L-Tryptophan	0.030	2	5.50	2	0.29				
L-Tryptophan	0.059	4	1.79	1	1.03	1	−0.40	3	1.04
L-Tryosine	0.059	3	2.11						
D-Glucose	0.22 (5 percent)	2	6.04						
D-Glucose	0.059	5	4.78	2	2.89				
Urea	0.059	2	7.52						
Corn oil	Undiluted	2	9.30						

while sparing fibers of passage and glial cells (*16*).

While the literature concerning glutamate-induced cortical spreading depression might be viewed as conflicting with our claim of a permanent and localized behavioral change, we think that even if temporary subcortical spreading depression was induced, it would not contradict our finding of localized permanent damage (*17*).

To determine whether the ability to damage cells while sparing axons was unique to MSG, unilateral injections of other monosodium amino acids—as well as a fat, a sugar, and urea—at either the cellular or the fiber of passage site, were combined with contralateral parasagittal knife cuts. A differential effect, damaging cells but sparing fibers, was inferred from observing obesity after infusions in the site containing cells but not the site containing fibers. Molar equivalents of 1 percent MSG (0.059*M*) were used in 5- or 10-μl doses. Many of the doses were then increased or decreased as we searched for a differential effect on fibers and cells. Table 2 shows that L-aspartate, urea, and undiluted corn oil caused large holes at the infusion site and were therefore nondifferentiating; L-tyrosine was extremely difficult to dissolve and thus not practical; and L-leucine did not differentiate at the concentrations used. A good behavioral differentiation, confirmed by light-microscopical examination of brain tissue stained with cresyl violet, was obtained with 0.03*M* L-tryptophan, 0.059*M* D-tryptophan, and 0.10*M* glycine. D-Glucose (0.059*M*) did not show very good differentiation at the concentration used but caused persistant hyperphagia, and the cells appeared normal when examined at low magnifications. The greater toxicity of the natural (L) isomer of tryptophan suggests that the selectivity for somatic damage may be a result of active uptake and concentration. Since several amino acids produced differential effects on fibers and cells, we conclude that no one transmitter system was uniquely affected (*18*).

This new lesioning technique should help us determine what areas of the brain contain the cell bodies that control specific behaviors, and may eventually prove advantageous when therapeutic brain lesions are indicated. The brain damage that follows central infusion of any one of several metabolites suggests that such damage might also be caused by diets overloaded with any one of many naturally occurring, or even essential, substances. We have demonstrated with our localized infusions that when brain damage does occur, the histological picture can remain deceptively benign upon casual inspection.

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6. The 1 percent MSG solution was made by dissolving 1 g of MSG (Sigma) in 100 ml of distilled H_2O; NaCl was then added to make the solution isotonic. The needle used for infusions was a stainless steel hypodermic needle (outer diameter, 0.3 mm) with a conical tip. Fluid exited via a side hole 1 mm from the tip (KF-730, Style 5, Hamilton).
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11. E. L. Simson and R. M. Gold, in preparation. Note that a large (5 μl) volume of infusate produced only a small (~0.25 μl) cell-free area. Possible explanations of this 20-fold difference include rapid uptake of the amino acid into cells, or dilution of the amino acid to lower than toxic levels as the infusate spreads and mixes with extracellular fluid.
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17. Electrophysiological effects of glutamate in cerebral cortex [L. K. Kaczmarek and W. R. Ader, *J. Neurobiol.* **5**, 231 (1974)] showed recovery of membrane potentials at approximately 150 minutes after superfusion with 15m*M* glutamate. During this time period our animals were still anesthesized. Our animals showed appetitive behavioral changes that lasted at least 6 weeks.
18. Glutamic acid is a precursor of the neurotransmitter γ-aminobutyric acid (GABA). Both are found in high concentrations in the hypothalamus [Y. Okada, C. Nitsch-Hassler, J. D. Kim, I. J. Bak, R. Hassler, *Exp. Brain Res.* **13**, 514 (1971)]. Within the hypothalamus, the anterior hypothalamus has the highest concentration of GABA [H. Kimura and K. Kuriyama, *J. Neurochem.* **14**, 903 (1975)]. If there are specialized cells in the anterior hypothalamus that produce or store GABA, then the behavioral result that we observed with MSG might be due to an overloading of the storage or synthetic system. Such damage would presumably produce a permanent change in GABA stores. With this in mind we undertook an amino acid analysis of the hypothalamus of rats that had been rendered hyperphagic as a result of bilaterally injected MSG. The hypothalami after removal were ground in a Potter-Elvehjem glass-teflon homogenizer with 8 ml of a *p*H 2.2 lithium citrate buffer, *p*H 2.2 [J. V. Benson, M. J. Gordon, J. A. Patterson, *Anal. Biochem.* **18**, 228 (1967)] and spun at 40,000 rev/min for 90 minutes in an ultracentrifuge under vacuum at 2 to 4°C [T. Gerrtison, M. L. Rehberg, H. A. Waisman, *Anal. Chem.* **11**, 460 (1965)]. Amino acids were separated with lithium citrate buffers being used for the basic amino acids on a Beckman 1200 analyzer. The technique was such that some 40 ninhydrin-positive compounds could be separated and quantitatively estimated. The analysis showed no significant differences between injected rats and controls in any of the ten measured amino acids (alanine, aspartate, cysteine, GABA, glutamate, glutamine, glycine, serine, taurine, and threonine). The absence of any generalized amino acid depletion despite a loss of neurons suggests that neurons have been replaced by glia.
19. We thank D. Atkinson, A. Pellett, R. Rufner, and F. Spencer for technical assistance. Supported by PHS grant MH 26251 to R.M.G. Portions of this paper were presented to the Eastern Psychological Association Meeting in New York, April 1976.

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Regenerating Afferents Establish Synapses with a Target Neuron That Lacks Its Cell Body

Abstract. *When the axons of crayfish tail-fan mechanoreceptor neurons are severed, the axons regenerate into the central nervous system and after 2 to 6 weeks reestablish functional contacts with their standard interneuronal target cells. Removal of the cell body and hence the genes of the largest of these interneurons does not interfere with the successful reestablishment of synapses between it and its afferents.*

Recent studies have demonstrated that in various arthropod neurons all of the major signaling functions of neurons—reception, conduction, and transmission—can survive for months after removal of the neuron soma and hence the cell's genetic apparatus (*1-3*). Thus arises the question of what functions are disrupted in such "somaless" neurons. A plausible possibility is that routine maintenance activities can persist in absence of the soma, while activities outside the normal "line of duty" might not.

This reasoning led us to investigate whether the soma of a target neuron is required in order for regenerating af-