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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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-

ferents to be attracted by and form functional synapses on that neuron (*4–6*). This specific question was addressed because (i) gene products are generally considered essential for developmental processes, and (ii) recent work on arthropod neurons (*3, 7*) has made it feasible to do experiments that will answer this particular question.

Interneuron A (hereafter **A**) of the crayfish abdominal nerve cord (*8*) receives chemically transmitting terminals of tail fan mechanoreceptor afferents that arrive through ipsilateral nerve roots 1 to 5 of the last abdominal ganglion. The soma of **A** lies contralateral to its axon and dendritic field at the caudal margin of the ganglion. It can be reliably disconnected from the neuron by a caudally placed midline incision or by removing a wedge of tissue that contains the soma, and after such removal the somaless neuron usually survives and continues to fire normally in response to water currents for more than 8 weeks (*3*).

In the present experiment we compared the ability of cut afferents to regenerate and reestablish functional connections with normal and somaless **A**'s. Most of the animals were prepared as shown in Fig. 1A: Roots 1 to 5 were cut; the peripheral ends of roots 2 and 4 were tied to the central end of 3 which acted as a guide for the regenerating afferents; and the peripheral ends of roots 1, 3, and 5 were allowed to retract in a peripheral direction. Usually, the central ends of 2 and 4 were tied to muscle or skin laterally to minimize the possibility of regenerating afferent fibers of roots 2 and 4 encountering and fusing with their central stumps (*1, 9*). In a few animals the central stumps of 2 and 4 were instead cut short and each ligated tightly at its entrance to the ganglion to facilitate later identification.

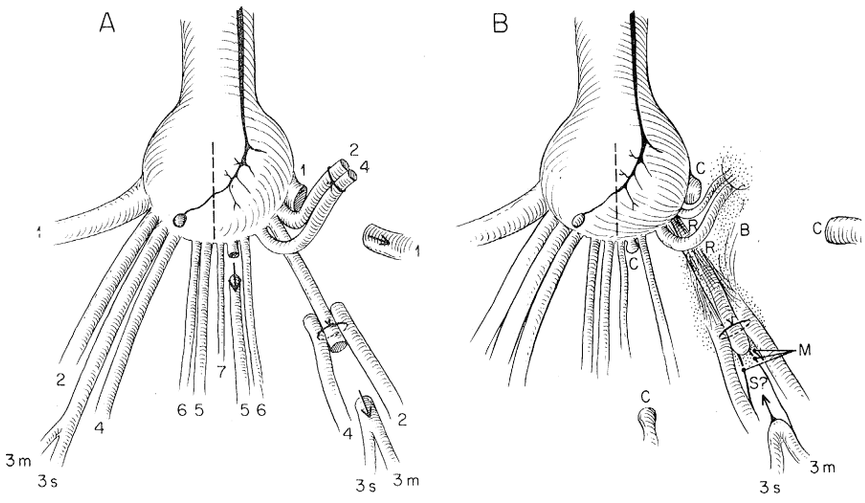

Fig. 1. (A) The operative procedures described in the text. The drawing of **A** is based on observations of cobalt-stained A's. Ganglionic roots are numbered, and sensory (*s*) and motor (*m*) branches of root 3 are indicated. The arrows on peripheral stumps indicate that the piece tended to retract. The dashed line indicates the cut made in experimental animals. Sometimes root 6, which is motor, was also cut. (B) Sketch of a terminal dissection as described in the text.

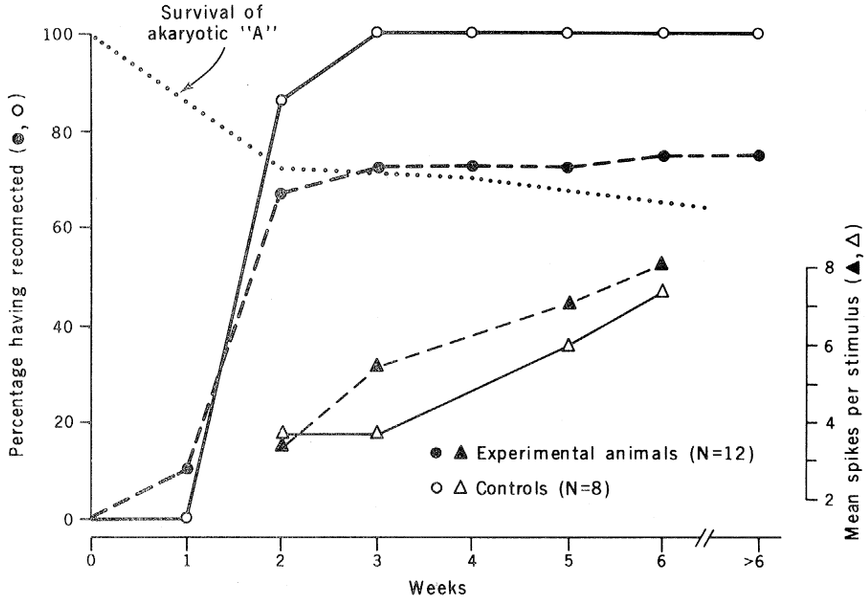

Fig. 2. Regeneration to interneuron A. Circles show the cummulative percentage of animals in which water drops could evoke at least single spikes in **A**; once an **A** had reacted to water drops it was counted as having reconnected even if it later became inexcitable. Some late loss is expected, because somaless A's eventually become inexcitable even when afferents are intact; the dotted curve indicates the percentage of normally innervated somaless **A**'s still surviving at various times after soma removal [from (*3*)].

In 12 experimental animals **A** was severed from its soma by a midline incision as shown in Fig. 1A [for details see (*3*)]; regeneration in these animals was compared with that in eight control animals in which the ganglion was left intact.

Each animal was tested at weekly or biweekly intervals for the ability of water drops, falling about 3 centimeters onto the surface of the bath containing the animal, to fire **A**. Recording from **A** was achieved by inserting insulated stainless steel electrodes through ventral skin laterally and insinuating the tips to the ventrolateral margin of the nerve cord, where **A**, which is the largest fiber in the cord except for the dorsal giants, runs close to the surface. It was identified by the large size of its spike and its strictly caudal, ipsilateral receptive field (*3*).

Figure 2 shows that in the control animals functional contact between regenerating afferents and **A** was usually made within 2 weeks and always within 3 weeks (*10*). The number of spikes produced during a 50-msec period following the first **A** spike to be evoked by a water drop increased gradually over the 6 weeks during which measurements were made, to a level comparable to that seen in normal animals. The experimental animals showed a similar pattern of recovery except that the final percentage of animals in which **A** could be fired by water drops was lower than in controls. However, since about 30 percent of somaless **A**'s typically become inexcitable within 6 weeks even when sensory roots are intact (dotted line in Fig. 2), it must be concluded that reformation of sensory connections to **A** seems to occur normally (*11*).

Although our operative procedures were designed to minimize the possibility of fusion of cut afferents (which

could preclude a necessity for regeneration of synaptic contacts per se), it was conceivable that (i) some regenerating fibers might have found their way to their central stumps and fused, or that (ii) fibers from roots 2 and 4 might have fused with the central stumps of root 3 to which they were tied.

Possibility (i) was evaluated at about 6 weeks by exposing the regenerating roots, attempting to cut all tissue connecting the peripheral with the central ends of the previously severed roots, and then retesting the ability of water drops to fire **A**. The situation encountered at the time of this "clean-up" surgery is summarized in Fig. 1B. Stumps of cut nerves other than 2 and 4 usually had smooth, clublike ends (see *C* in Fig. 1B), occasionally with small bundles of axons growing out of them. Growth out of the peripheral ends of roots 2 and 4 along the remnant of the central end of root 3 was sometimes obvious, but often the region was embedded in a mass of scar tissue. The stumps of the peripheral and the laterally tied central pieces (*R*) of roots 2 and 4 were frequently joined by a mass of vascularized scar tissue (*B*) in which regenerating axons could sometimes be seen and also sometimes by small bundles of stray regenerating fibers. The stray fibers and *B* or *R* of Fig. 1B were cut as necessary. This surgery commonly reduced the sensitivity of **A** to water drops but in no case abolished it. The average number of spikes produced after "cleanup" was 5.8 per 50 msec for experimental animals and 6.2 for controls. Upon fuller dissection at completion of testing we often found fibers joining the peripheral stump of root 3 to the region where 2, 3, and 4 were tied together. Some of these (*M* of Fig. 1B) could be seen by their course to be motor fibers growing out from the central end of root 3. But others (*S* of Fig. 1B) might have been sensory fibers from peripheral root 3 that could have fused with their counterparts in the central segment of 3. However, of the 12 animals with reactive **A**'s that were examined carefully, there were six with no indication of growth of sensory fibers that could have fused with their central stumps; moreover, the mean sensitivity of **A** in these animals (7.3 spikes per 50 msec after clean-up surgery) was actually slightly greater than that in the animals where there could have been fusion (5.2 spikes per 50 msec).

Possibility (ii) cannot account for recovery of excitability of **A**, because **A** was excited by water drops at 3.5 weeks after surgery in two animals in which roots 1, 3, and 5 were left intact until just before testing so that they did not provide cut central pieces with which regenerating roots 2 and 4 could have fused.

Finally, to rule out the possibility that our results might be accounted for by fusion of the severed neurite between **A**'s axon and soma, we removed a chunk of tissue containing **A**'s soma in six animals; in each animal regeneration of afferents to the somaless interneuron again occurred within 4 weeks. It remains conceivable that these somaless **A**'s joined with foreign somas, but in previous work in which we used the present operative techniques cobalt was not taken up by any soma when **A**'s axons and dendrites were filled with cobalt after 8 weeks of survival (*3*).

We therefore conclude that **A**'s return of sensitivity to water drop stimulation does indicate true regeneration of synapses between cut afferents and **A**, and that this regeneration is normal in time course and extent even though **A** lacks its cell body (*12*).

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4. While it seems likely that the target cell plays an important role in the reestablishment of afferent synapses on itself, the precise nature of this role is uncertain. Cajal's evidence that target structures produce factors that promote growth or sprouting leads one to suspect that regeneration might require gene-dependent synthesis of substances that attract regenerating afferents or cause them to establish synapses, or both [see S. Ramon y Cajal, *Trab. Lab. Invest. Biol. Univ. Madrid* **17**, 181 (1919), L. Guth, Transl., in *Studies on Vertebrate Neurogenesis* (Thomas, Springfield, Ill., 1960), pp. 149–200; J. Diamond, E. Cooper, C. Turner, L. Macintyre, *Science* **193**, 371 (1976); M. H. Moscona and A. A. Moscona, *Science* **142**, 1070 (1963)]. On the other hand, there is some evidence that in crustacea synapses might be formed primarily by dendrites growing to meet and form synapses on the axis cylinders of presynaptic axons [see (*5*); and W. J. Davis, *Science* **168**, 1358 (1970)]; this would place an even greater demand on the target cell. Additionally, when afferents to a target are lost, there are often signs of morphological, physiological, or biochemical dedifferentiation of the target which must be reversed upon reinnervation [see (*6*); and J. Rosenthal, in *Handbook of Neurophysiology* (American Physiological Society, in press); S. Gelfan, T. H. Field, G. D. Pappas, *Exp. Neurol.* **43**, 162 (1974); T. Gentschev and G. Sotelo, *Brain Res.* **62**, 37 (1973); D. A. Matthews, C. Cottman, G. Lynch, *ibid.* **115**, 1 (1976)]. However, in rat superior cervical ganglia and septal nuclei, postsynaptic membrane thickenings persist without obvious change despite denervation, and therefore they might not have to be reconstituted upon reinnervation [see G. Raisman, in *Neural Mechanisms of Learning and Memory*, M. R. Rosenzweig and E. L. Bennett, Eds. (MIT Press, Cambridge, Mass., 1976), p. 384]; moreover, at vertebrate neuromuscular junctions some muscle changes that normally follow degeneration of motor neuron terminals appear not to occur in the absence of protein synthesis (*6*).
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12. See (*3*) for a discussion of the possible meaning of survival of function in akaryotic neurons.
13. Supported by NIH grant R01-8108. We thank M. Letinsky for helpful discussions and P. Farel for commenting on the manuscript.

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Selective Vocal Learning in a Sparrow

Abstract. *Male swamp sparrows learn their songs; they fail to learn songs of the sympatric song sparrow. Syllables from tape recordings of both species of sparrow were spliced into an array of swamp sparrow–like and song sparrow–like temporal patterns. Swamp sparrows learned only those songs made of swamp sparrow syllables. They did so irrespective of whether the temporal pattern was swamp sparrow–like or song sparrow–like. Selectivity was retained by birds reared in total isolation from adult conspecific sounds.*

It is a long-standing premise of classical learning theory that any sensory stimulus can be attached through learning to any arbitrarily chosen response. Biological approaches to animal learning have called several assumptions of learning theory into question, including the principle of equipotentiality (*1*). Vocal learning is widespread in birds, and all oscine songbirds studied to date show some degree of song abnormality when reared in social isolation (*2*). A feature of