folded, hooded subjects in two vans with foil-covered windows to each of four locations (A through D in Table 1) using a mutually agreeable protocol essentially identical to the one described above. None of the sites yielded statistically significant orientation by the $z$ test, although site B is significant by the $V$ test (Table 1). On the second day Baker and his colleague J. G. Mather ran the experiments, incorporating a trip to the roof of Eno Hall to allow the subjects a view of the surrounding topography. They selected a more nearly linear route to the west-northwest. Although five stops were planned (E through I in Table 1), only one van (carrying nine subjects) went to the fifth site. Orientation was statistically random at all locations (Table 1).

The experiment on the third day was again run by Baker and Mather. The 26 subjects were transported in three vans along a generally northern route from the Lawrenceville School to four sites (J through M in Table 1). Of these, 14 subjects wore magnets at the back of the head, and the polarity of the magnet was arranged so that a compass held in front of the head always indicated that north was behind the head. These individuals would be expected to indicate that they were being taken south, while the 12 controls wearing lead weights ought to indicate north as the direction of displacement. Neither group was oriented at any site, nor was there any consistent difference between the directions of their mean vectors (Table 1). The one instance of orientation at the 5 percent level out of the 34 statistical tests in Table 1 is about what ought to turn up by chance.

We believe that our consistent failures indicate that the phenomenon is neither as simple nor as robust as we had been inclined to hope. We urge others to attempt to repeat this intriguing yet technically undemanding experiment.

JAMES L. GOULD
*Department of Biology, Princeton University, Princeton, New Jersey 08544*
KENNETH P. ABLE
*Department of Biological Sciences, State University of New York, Albany 12222*

### References and Notes

1. R. R. Baker, *Science* **210**, 555 (1980).
2. ———, *Human Navigation and the Sixth Sense* (Hodder and Stoughton, London, 1981).
3. While many of Baker's methods invite criticism, the very fact that they worked so well for him and, more importantly, that magnets seemed to reduce the accuracy of orientation (without, we suppose, affecting the subjects' ability to sense such visual, auditory, topographic, olfactory, or verbal cues as we might be inclined to wonder about) serve to make his results compelling. We have been careful to incorporate them in our tests. The distances chosen are among those that gave the best results in Baker's experiments (*2*). Although we adhered to Baker's 2-year residence criterion in our first six tests, Baker subsequently reported finding no effect of residence time. Hence, we relaxed this criterion in the last two experiments. Baker's route-based navigation hypothesis (*2*) makes residence time irrelevant.
4. E. Batschelet, in *Animal Orientation and Navigation*, S. R. Galler, K. Schmidt-Koenig, G. J. Jacobs, R. E. Belleville, Eds. (Government Printing Office, Washington, D.C., 1972), pp. 61–92.
5. We thank K. Schenck for organizing the Princeton experiments, B. Presley and T. Spalding for arranging the Lawrenceville School test, and F. Dyer, C. G. Gould, H. Wildman, J. L. Kirschvink, and W. Towne for help and comments on the manuscript. We also thank W. Gergits for help in the tests at Albany. Supported by grants BNS 78-24754 (to J.L.G.) and BNS 79-23711 (to K.P.A.) from the National Science Foundation.

12 November 1980; revised 13 March 1981

## Myelinated Central Vertebrate Axon Lacks Voltage-Sensitive Potassium Conductance

Abstract. *Intracellular recordings were obtained from the goldfish Mauthner cell soma and axon before and after intracellular iontophoresis of drugs that block voltage-sensitive potassium conductance. Analysis of the action potentials generated by the axon hillock–initial segment and by the axon suggest that axonal impulses lack this potassium conductance. Thus, impulse repolarization in the Mauthner axon, and perhaps in other vertebrate central axons, may not involve an active potassium current.*

The mechanism of action potential electrogenesis at anuran nodes of Ranvier resembles that of squid giant axon. In both, depolarization results from an increase in voltage-sensitive sodium conductance and repolarization from a combination of sodium inactivation and an increase in voltage-sensitive potassium conductance ($g_K$) (*1*). Despite an early report that voltage-sensitive $g_K$ could not be detected at rat sciatic nodes (*2*), it has been generally assumed (*3*) that the mechanism of action potential electrogenesis at vertebrate nodes resembles that of squid and anurans. However, recent voltage clamp analyses of nodes from rabbit and rat sciatic nerve indicate that the predominant factors in repolarization are rapid sodium inactivation and a large leakage current, with voltage-sensitive $g_K$ playing no significant role (*4*). Pharmacological analysis of axons in the rat dorsal column also suggests that the central projections of peripheral sensory neurons repolarize without any significant contribution by voltage-sensitive $g_K$ (*5*). These findings raise serious questions about the general applicability of the classical model of action potential electrogenesis to myelinated axons. Such issues include (i) the contribution, if any, of voltage-sensitive $g_K$ to impulse repolarization in axons of neurons contained entirely within the central nervous system and (ii) the question of whether the classical model is appropriate for all nonmammalian vertebrates other than anurans. We present evidence indicating that, in the axon of a teleost central neuron, a voltage-sensitive change in $g_K$ plays no significant role in action potential electrogenesis.

Experiments were performed on goldfish Mauthner cells (M cells). The M cell is a large medullary interneuron having a myelinated axon extending posteriorly along the contralateral side of the spinal cord. Although nodes of Ranvier have not been found along the M axon, active electrogenic zones have been identified electrophysiologically, spaced at intervals of about 2 mm (*6*). Procedures for the restraint and artificial respiration of curarized fish and for exposure of the medulla have been described previously, as has electrophysiological identification of the M cell, the axon cap surrounding the axon hillock–initial segment, and the axon (*7*, *8*). We used intracellular iontophoresis to introduce drugs known to block voltage-sensitive $g_K$ in a number of excitable cells and monitored subsequent changes in the time course of the action potentials generated in the axon or in the axon hillock–initial segment. Microelectrodes were filled with a mixture of KCl and tetraethylammonium (TEA), KCl and 4-aminopyridine (4AP), or KCl and both drugs (*9*). The same electrode was used to record intracellularly, to introduce the drugs, and to block propagating action potentials with hyperpolarizing currents. For iontophoresis, a continuous depolarizing current of as much as 20 nA was most effective. Soma-dendritic penetrations were made 100 μm lateral to the axon cap. Axonal penetrations were made either 1.0 mm or 2 to 2.3 mm posterior to the axon cap—that is, on either side of the first active zone (*6*). Antidromic action potentials were evoked by stimulating the spinal cord and orthodromic action potentials by stimulating the ipsilateral posterior eighth nerve.

Iontophoresis of TEA or 4AP into the


0036-8075/81/0529-1063$00.50/0  

M cell soma (Fig. 1, site A) resulted in an immediate and pronounced effect on the duration of the antidromic action potential recorded at that site. (In Fig. 1A, compare tracings before and after iontophoresis.) Increases of 22 to 300 percent in the duration of the antidromic action potential were commonly observed after prolonged somatic injection. This effect of somatic TEA or 4AP injection was consistent with their actions on other excitable vertebrate and invertebrate cells (*10*) and was limited to the repolarization phase of the action potential. In addition, the TEA effect was dose-dependent, that is, the duration of the action potential continued to increase as more TEA was applied. Prolonged TEA iontophoresis often resulted in antidromic doublets, as the action potential from the axon hillock–initial segment had repolarization phases outlasting the axon's refractory period. Similar prolongation of the antidromic action potential after penetration of the soma with 4AP electrodes often occurred without the passage of any current. In contrast, injection of potassium ions, even when the injection current was large (50 nA) and prolonged (15 minutes), was without effect on duration of the antidromic action potential.

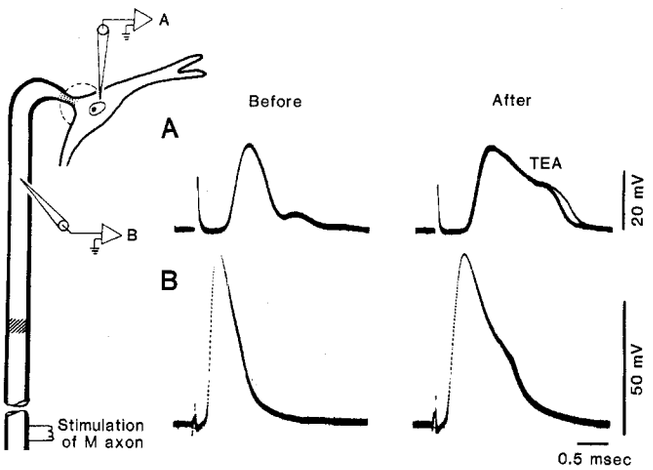


Fig. 1. Effects of TEA on the antidromic action potential of the M cell. On the left is a diagram indicating the location of the sites at which the records of (A) and (B) were made. Recording site A is in the soma and proximal dendrite, and site B is in the axon approximately 1 mm from the axon hillock–initial segment (stippled area just distal to the soma). The approximate location of the first active zone of the axon is indicated by the cross-hatching. Antidromic action potentials were evoked by stimulation of the M axon in the posterior spinal cord. The recording electrodes were also used for drug iontophoresis. (A) Somatically recorded antidromic action potentials before and after intrasomatic iontophoresis of TEA (5 nA/min). The late depolarization before iontophoresis is a synaptic response evoked by spinal stimulation. (B) Axonally recorded antidromic action potentials before and after intraaxonal iontophoresis of TEA (200 nA/min). In both cases, the rising phase was unaffected and the increase in spike duration was associated with delayed repolarization.

Since the M cell soma is not electrically excitable (*7*), the antidromic action potential recorded there is primarily an electrotonic representation of that generated at the axon hillock–initial segment. In contrast, the action potential recorded in the first few millimeters of the axon distal to the axon cap is a composite of contributions from discrete active axonal sites as well as from the axon hillock–initial segment. In order to determine the effects of TEA and 4AP on these different sites, a series of experiments were performed with the microelectrode in the rostral axon. Iontophoresis of TEA or 4AP into the M axon at distances of about 1 mm from the axon cap (Fig. 1, site B) had effects on action potential duration similar to intrasomatic iontophoresis (Fig. 1B), that is, the repolarization phase was prolonged without concomitant changes in the depolarizing phase. However, since the initial rate of repolarization was not altered by drug iontophoresis, it appeared that TEA and 4AP, while having a powerful effect on the time course of the action potential generated by the axon hillock–initial segment, might be without effect on that generated at axonal active sites.

In order to test this hypothesis, we have electrophysiologically uncoupled the axon and axon hillock–initial segment and compared the drug effects on the action potentials generated by these two regions. The results of such an experiment, in which the axon was penetrated midway between the axon hillock–initial segment and the first axonal active zone, are shown in Fig. 2, A and B. Sufficient hyperpolarization of the axon blocked the propagation of ortho- and antidromic action potentials beyond the site of current injection, revealing either the isolated axon spike in response to antidromic stimulation (Fig. 2, A1) or the isolated axon hillock–initial segment spike in response to orthodromic stimulation (Fig. 2, B1). The duration (*11*) of the isolated axon spike, 0.34 msec, was not altered by the passage of depolarizing current carrying both TEA and 4AP for a total of 360 nA/min (Fig. 2, A2), although the duration of the axon hill-

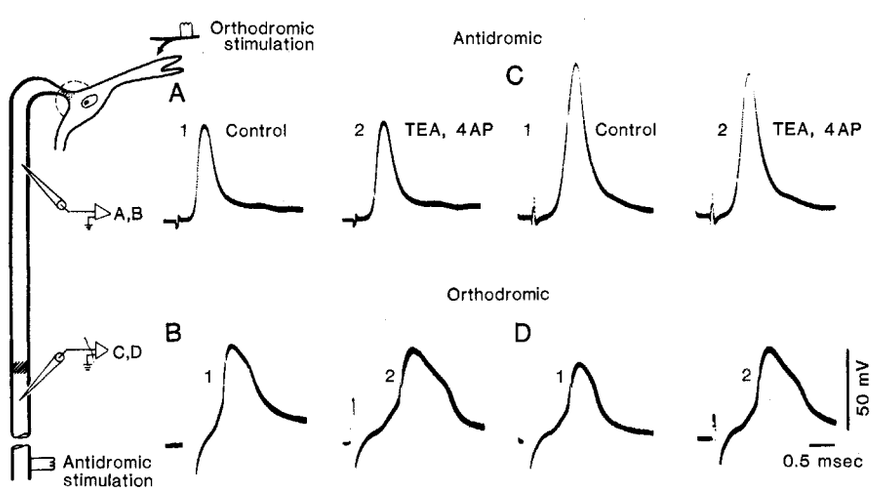


Fig. 2. Effect of TEA and 4AP of the electrophysiologically isolated action potentials generated at the axon hillock–initial segment and at axonal active zones. The diagram at the left indicates the sites at which records (A) to (D) were made. Site A,B is in the axon approximately 1 mm from the axon hillock–initial segment (stippled area just distal to the soma). Site C,D, also in the axon, is approximately 2.3 mm from the axon hillock–initial segment and distal to the first active zone of the axon (cross-hatching). Antidromic action potentials were evoked by stimulating the M axon in the caudal spinal cord, and orthodromic action potentials by stimulating the posterior seventh nerve. The recording electrodes were also used for drug iontophoresis and for passing hyperpolarizing currents to block propagating ortho- or antidromic action potentials. A1, B1, C1, and D1 were recorded before (and A2, B2, C2, and D2 after) iontophoresis of TEA and 4AP at the recording site. (A) Electrophysiologically isolated axonal action potentials recorded proximal to the first axonal active zone. (B) Axon hillock–initial segment action potential. (C and D) Electrophysiologically isolated action potentials recorded distal to the first active zone. In both experiments, only the duration of the axon hillock–initial segment action potential was increased after drug iontophoresis [360 nA/min in (A) and (B); 450 nA/min in (C) and (D)].

ock–initial segment spike had increased from 0.56 msec to 0.84 msec (Fig. 2, B2). This result might be explained if, due to preferential rostral migration of the drugs, they did not reach active zones of the axon. This possibility is unlikely since the axon narrows as it approaches the soma, and, thus, rostral diffusion would be expected to be restricted. In order to be certain that the injected drug had access to active zones of the axon, we penetrated M axons slightly distal to the first active zone (Fig. 2, C and D). After injection of both TEA and 4AP (450 nA/min) into the axon, the duration of the isolated axon spike remained unchanged (Fig. 2, C1 versus C2). The duration of the axon hillock–initial segment spike had, however, increased from 0.54 msec to 0.78 msec (Fig. 2, D1 versus D2). As the location of the first axonal active zone was probably between the injection site and the axon hillock–initial segment, it is very unlikely that the lack of effect of TEA and 4AP on the isolated axon spike resulted from failure of the drugs to reach that first axonal active site.

The distribution along the Mauthner axon of voltage-sensitive channels blocked by TEA and 4AP differs from what would be expected on the basis of most models of axonal action potential electrogenesis, which generally include voltage-sensitive potassium channels at each active site. Our data indicate that, in the axon, potassium channels sensitive to TEA and 4AP are restricted to the axon hillock–initial segment region and do not occur at active zones along the myelinated axon. It remains possible that voltage-sensitive channels, resistant to blockade by both TEA and 4AP, occur along the M axon. This possibility, if demonstrated experimentally, would be the first case of a cell's having two classes of voltage-sensitive potassium channels, with one being completely insensitive to both TEA and 4AP. Alternatively, the kinetics of voltage-sensitive potassium channels in the axon might be too slow to contribute to impulse repolarization. It seems much more likely, however, that active zones along the myelinated M axon generate action potentials much as nodes of Ranvier along rat and rabbit sciatic nerve fibers and rat dorsal column axons do (*4, 5*)—that is, without a conductance increase to any specific ion playing a role in repolarization (*12*). This interpretation of these data suggests that two mechanisms of action potential electrogenesis, having one or two active membrane conductance changes, arose early in the evolutionary history of myelinated axons; the interpretation also raises questions about the distribution of these two physiological types in vertebrate nervous systems.

CHARLES KAARS
DONALD S. FABER

*Department of Physiology, Division of Neurobiology, State University of New York at Buffalo, Buffalo 14214*

### References and Notes

1. A. L. Hodgkin and A. F. Huxley, *J. Physiol. (London)* **117**, 500 (1952); F. A. Dodge and B. Frankenhaeuser, *ibid.* **143**, 76 (1958); B. Frankenhaeuser, *ibid.* **160**, 40 (1962); ——— and A. F. Huxley, *ibid.* **171**, 302 (1964).
2. M. Horackova, W. Nonner, R. Stampfli, *Proc. Congr. Int. Union Physiol. Sci.* **7**, 198 (1968).
3. M. Cahalan, in *Physiology and Pathobiology of Axons*, S. G. Waxman, Ed. (Raven, New York, 1978), p. 155.
4. S. Y. Chiu, J. M. Ritchie, R. B. Rogart, D. Stagg, *J. Physiol. (London)* **292**, 149 (1979); T. Brismar, *ibid.* **298**, 171 (1980).
5. J. D. Kocsis and S. G. Waxman, *Nature (London)* **287**, 348 (1980).
6. P. Funch, thesis, State University of New York, Buffalo (1980).
7. E. J. Furshpan and T. Furukawa, *J. Neurophysiol.* **25**, 732 (1962).
8. D. S. Faber and H. Korn, *Science* **179**, 577 (1973).
9. Electrodes contained the following solutions: 1*M* KCl and 2*M* TEA; 2.7*M* KCl and 0.1*M* 4AP; or 1*M* KCl, 2*M* TEA, and 0.1*M* 4AP. Electrodes with both drugs were used since some voltage-sensitive potassium channels in *Aplysia* are blocked only by 4AP and TEA together [M. Klee, *Brain Res. Bull.* **4**, 162 (1979)].
10. T. Narahashi, *Physiol. Rev.* **54**, 813 (1974); J. Z. Yeh, G. S. Oxford, C. H. Wu, T. Narahashi, *J. Gen. Physiol.* **68**, 519 (1976).
11. Width at half-amplitude was the measure of duration of somatically recorded action potentials, and the time between positive and negative peaks of the differentiated record was measured as the duration of axonally recorded action potentials.
12. Two other possible indicators of voltage-sensitive potassium conductances would be a rectification in the axonal current-voltage relationship and an undershoot after the impulse. Rectification did not occur, but we have occasionally observed an undershoot associated with a conductance increase, the mechanism of which remains to be elucidated.
13. Supported in part by NIH grant NS 15335.

14 October 1980; revised 30 December 1980

## Chemical Modification of Carotid Body Chemoreception by Sulfhydryls

Abstract. *Sulfhydryl reagents cause striking augmentation of the chemoreceptor responses of the carotid body to hypoxia. This indicates that a cellular plasma membrane protein with a reactive sulfhydryl group is a constituent part of the chemoreceptor architecture and provides a means of identification, localization, and isolation of the protein.*

Chemoreception of $O_2$ in the carotid body and generation of action potentials in the chemoreceptor afferents presumably require several steps, including detection, feedback regulation, and energy transduction. Even though our understanding of the nature of $O_2$ chemoreception is incomplete, it seems clear that specific components, not shared generally by all cells, are common to cellular processes that participate in sensory mechanisms. Since cell membrane–bound systems with active sulfhydryl groups are part of the receptor architecture in several sensory systems (*1–5*), similar systems may operate in the chemoreception processes of the carotid body. Evidence for the participation of a membrane-bound system in the expression of chemoreception is indicated by the interaction of $O_2$ with dopamine (*6*) and isoproterenol (*7*), because the receptors for these catecholamines are localized on the cell membranes (*2, 3*).

If a membrane protein whose active site contains sulfhydryl groups participates in the expression of chemoreception, chemical modification of the plasma membrane protein might cause specific alterations in chemoreception. This hypothesis was tested by treating the carotid body with *p*-chloromercuriphenylsulfonic acid (PCMBS), which does not penetrate the plasma membrane, and with the alkylating agent, *N*-ethylmaleimide (NEM), which does penetrate the plasma membrane. The hypoxic response was selectively augmented by PCMBS and this effect of PCMBS was saturable, suggesting that some component of the receptor architecture is a protein with active sulfhydryl sites.

The discharge rates of the carotid body chemoreceptor afferents were studied in six cats that were anesthetized with Nembutal (30 mg/kg), paralyzed with gallamine (3 mg/kg-hour), and artificially ventilated at a maintained rectal temperature of 38°C (*8*). The external and lingual arteries were ligated and a fine arterial cannula was inserted just upstream from the carotid body for the administration of PCMBS and NEM. Chemoreceptor afferents were isolated and identified from the peripheral end of a cut sinus nerve (*8*). Airway $PO_2$ and $PCO_2$, arterial blood pressure, and carotid chemoreceptor activity were recorded continuously. The responses of a single or a few carotid body chemoreceptors to steady-state levels of $P_aO_2$ (30 to 500 torr) at a constant $P_aCO_2$ were investigated before and after several doses of PCMBS. While the cat was ventilated


0036-8075/81/0529-1065$00.50/0 