but do not cause an increase in the release of catecholamines do not produce behavioral stimulation or an increase in ICS in rats [see (8)]; also S. M. Schanberg, J. J. Schildkraut, I. J. Kopin, *Biochem. Pharmacol.* **16**, 393 (1967); S. H. Snyder, A. I. Green, E. D. Hendley, *J. Pharmacol. Exp. Ther.* **164**, 90 (1968); E. B. Sigg, *Can. Psychiat. Ass. J.* **4** (suppl.), 75 (1959); L. Stein and J. Seifter, *Science* **134**, 286 (1961).

22. S. H. Snyder, K. M. Taylor, J. T. Coyle, J. L. Meyerhoff, *Amer. J. Psychiat.* **127**, 199 (1970).

23. L. A. Loizou, *Brain Res.* **15**, 563 (1969); N.-E. Anden, A. Dahlström, K. Fuxe, K. Larsson, L. Olson, U. Ungerstedt, *Acta Physiol. Scand. Suppl.* **67**, 313 (1966); K. Fuxe, T. Hokfelt, U. Ungerstedt, *Int. Rev. Neurobiol.* **13**, 93 (1970).

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A Neuronal Inhibition Mediated Electrically

Abstract. *When the goldfish Mauthner cell fires an impulse there is a simultaneous hyperpolarization of adjacent medullary neurons as far as 200 micrometers from its soma. This hyperpolarization is due to an inward transmembrane flow of some of the current generated by the Mauthner cell spike and is sufficiently large to block spikes evoked both directly or transsynaptically.*

"Electrical inhibition" can be mediated in a number of vertebrate and invertebrate systems where neuronal elements are electrotonically coupled (*1*), but since the coupling is often weak, its excitatory effects generally predominate. In addition, the extracellular currents generated by nerve cell impulses can also decrease the excitability of adjacent neurons (*2*); again, however, the effects of such ephaptic currents have generally been found to be facilitatory (*3*). The clearest case of a significant electrical inhibition described so far, that of the Mauthner cell, has been attributed to a failure of impulse propagation in the neurons mediating this effect (*4*). We report here a presumably related case where the extracellular currents set up by the action potential of a single neuron can impose an inhibitory hyperpolarization on the membrane of neighboring cells.

Experiments were performed on goldfish (14 to 22 cm in length) paralyzed with Flaxedil (1 μg per gram of body weight) and perfused through the mouth with dechlorinated tap water. The methods used for exposure of the medulla and for electrical stimulation of the eighth nerve and spinal cord were similar to those described elsewhere (*4*, *5*). Intracellular recordings from the Mauthner cell (M-cell) and from adjacent neurons located as far as 200 μm from its soma were obtained with single- or double-barreled micropipettes filled with 0.6*M* K_2SO_4 or 3*M* KCl. A Howland pump (*6*) was used to inject current intracellularly.

Antidromic invasion of the M-cell produced by a spinal cord stimulation results in a characteristic all-or-none extracellular negativity which is followed by a later positivity, the well-known (*4*) "extrinsic hyperpolarizing potential" (Fig. 1A). The negativity is as large as 20 to 40 mv in the axon cap and falls off steeply in amplitude as the recording electrode is moved away from the maximum focus. Surprisingly, we have found when recording from cells located in regions where the extracellular field is no more than a few millivolts that there is a corresponding but significantly larger intracellular negativity, as illustrated in Fig. 1B. Subtraction of the extracellular field (Fig. $1B_2$) from the intracellular potential (Fig. $1B_1$) indicates a net membrane hyperpolarization of 6.4 mv. Usually lower values were found (Fig. 1C); in 93 cells the average was 2.01 mv, with a range of 0.7 to 6.4 mv. In all the investigated neurons it had the same threshold, all-or-none character (Fig. 1, B and C), latency, and peak time (Fig. 1, A and B) as the M-cell field. Since the M-cell has the fastest conducting axon in the goldfish spinal cord (*5*) this hyperpolarization cannot be mediated through chemical synapses (*7*); it is therefore necessarily brought about by an inward transmembrane flow of the current generated by the M-cell spike, and it may be termed a "passive hyperpolarizing potential" (PHP).

If this hypothesis is correct, the PHP should decrease membrane excitability and should be relatively independent of membrane potential. Both characteristics were demonstrated. As shown in Fig. 1, D and E, when adequately timed the PHP did block the generation of spikes either directly evoked by depolarizing current pulses (Fig. 1D) or synaptically induced by stimulation of the ipsilateral eighth nerve (Fig. 1E). The latter effect could already be observed extracellularly prior to penetration of the neurons. Finally, when the PHP was made to interact with a subthreshold excitatory postsynaptic potential (EPSP) the two summated algebraically. The insensitivity of the PHP to changes in membrane potential produced by applied transmembrane currents is illustrated in Fig. 2: the size and time course of the PHP evoked during large hyperpolarizing

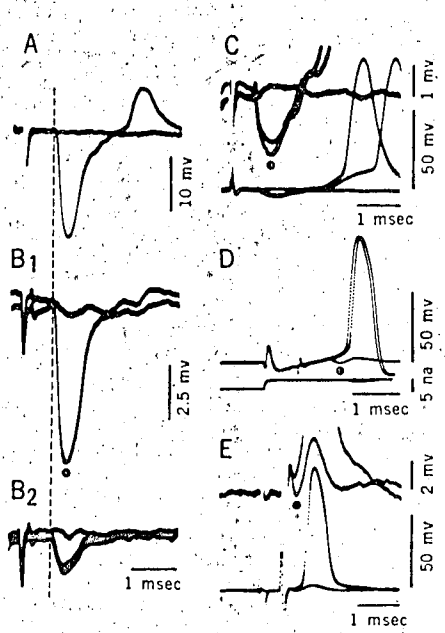

Fig. 1. Evidence that impulses evoked in the M-cell can generate inhibitory PHP's in adjacent neurons. (A to C) The spinal cord was stimulated at strengths straddling the threshold for M-cell antidromic activation; several sweeps are superimposed on each record. (A) Antidromic field potential extracellularly recorded in the axon cap. (B_1) Intracellular recording obtained during the same experiment as in (A) from a neuron located 75 μm caudal to the axon cap; the spinal stimulus evoked a PHP. (B_2) Field potential recorded outside that cell. The vertical dashed line indicates that the potentials in (A) and (B) had the same latency. (C) Another example of an intracellular PHP, followed in this case by an EPSP which fired the cell. The records in (D) and (E) are from two different neurons; in each record, sweeps were superimposed without and with spinal stimulation. (D) A spike evoked by a depolarizing current pulse (lower trace) was blocked by an adequately paired PHP. (E) A spike synaptically evoked by stimulation of the ipsilateral eighth nerve was similarly blocked; its failure unmasked a now subthreshold EPSP [the upper and lower traces in (C) and (E) are recordings of high a-c and low d-c gain, respectively]. In all records positivity is upward, and the PHP is indicated by a filled circle.

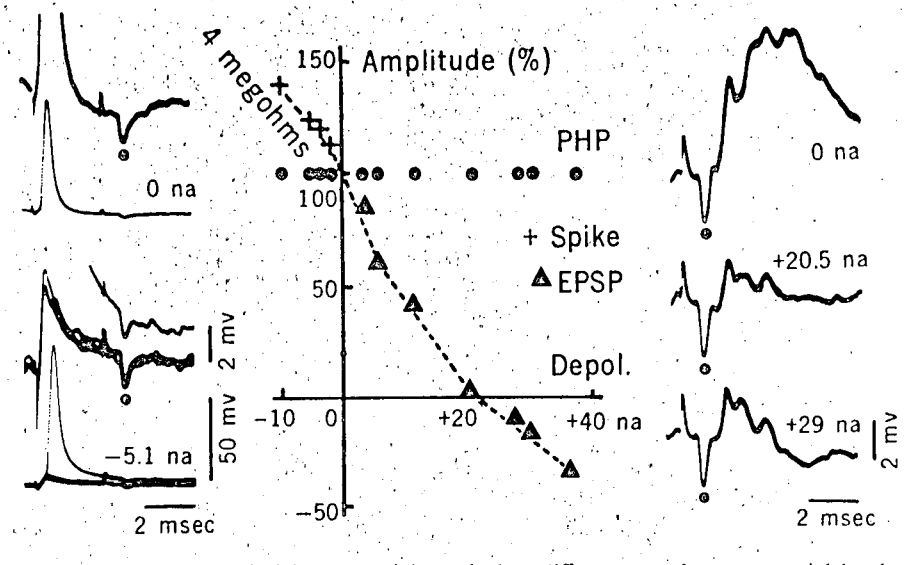

Fig. 2. Passive hyperpolarizing potentials evoked at different membrane potential levels. Series of responses recorded intracellularly through one barrel of a double microelectrode from two different neurons are shown on each side of the graph. The membrane potential was changed by passing a d-c current through the second barrel (the current amplitude is indicated on each series). (Left) The unit is activated synaptically by a stimulation of the ipsilateral eighth nerve; a subsequent stimulation of the spinal cord evokes a PHP (records at both high and low gain are shown). A hyperpolarization of about 20 mv as indicated by increased spike height does not affect the size of the PHP. (Right) A spinal stimulus produces a PHP followed by an EPSP. A depolarization of the neuron sufficient to cause a polarity reversal of the EPSP leaves the PHP unaffected. The values of the recorded potentials on the graph, expressed in percentages of the control values (determined at the resting potential) are plotted against the corresponding applied currents. The resistance of the neuron on the left is 4 megohms, as determined from changes in spike height produced by hyperpolarizing currents. Positive currents are depolarizing (*Depol.*) and negative hyperpolarizing.

and depolarizing shifts remained unchanged. It should be stressed that the input resistance of these neurons as measured both by changes in spike height (*8*) and by applied pulses was not low; it ranged from 1.9 to 7.0 megohms (mean, 4.15 megohms; number of cells, 38).

The evidence so far obtained suggests that cells exhibiting PHP's belong to two neuronal populations: (i) neurons of the vestibular nuclei, as indicated by their short-latency action potential following stimulation of the ipsilateral eighth nerve (Figs. 1E and 2), and (ii) interneurons in the recurrent collateral network (*4*) which feeds back inhibition onto the M-cell, since in some of the investigated neurons a spinal cord stimulation just sufficient to activate the M-cell axon produced a delayed all-or-none EPSP (Figs. 1C and 2). Whether an immediate inhibition of these two groups of neurons by the M-cell spike—that is, during mediation of the startle response (*9*)—could be of functional significance remains to be clarified.

It can be postulated that the PHP is generated by an intracellular channeling of some of the current flowing back to the axon cap during the M-cell spike (Fig. 3). This presumably is possible only if processes of the neurons exhibiting PHP's lie along the lines of return current flow, for example, if these neurons send processes into the axon cap. Whether this intracellular channeling of the current is simply a consequence of the high extracellular current density (*10*) associated with the M-cell spike or involves in addition morphological specializations requires further investigation. Finally, Fig. 3 suggests that at least some of the inhibited neurons mediate the previously described electrical inhibitions of the M-cell itself (*4*). If this is indeed the case, we have a situation with two neurons effectively coupled electrically, but in such a manner that the polarity of the signal in one cell is inverted as it is transmitted to the other (*11*).

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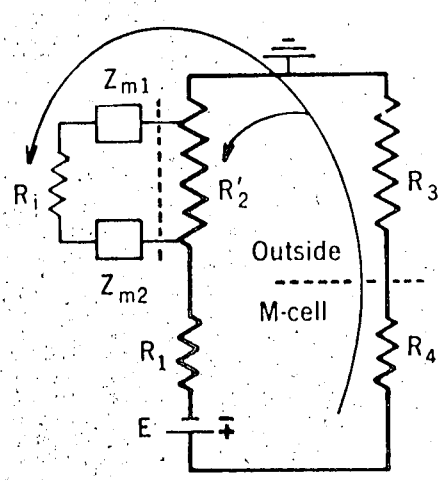
Fig. 3. A model for PHP generation. The schematic is an expansion of that proposed (*5*) to explain the extracellular fields associated with the M-cell spike. The lower half represents the M-cell, R_1 and E being, respectively, the membrane resistance and the driving electromotive force of the activated portion of the cell, and R_4 the passive resistance of the inactivated membrane. R'_2 and R_3 are the extracellular resistances associated with these two parts of the M-cell. In parallel with R'_2 is a simplified model of a cell exhibiting a PHP. Some of the current generated by the M-cell spike flows inward across one membrane of the cell, producing a hyperpolarizing potential, or PHP, across this membrane, and exits from the cell at a point presumably within the axon cap. This latter region of the cell should be depolarized during the M-cell spike. Z_{m1} and Z_{m2} are the effective impedances of these two portions of the cell, the PHP being generated across Z_{m1}, and R_i is the internal resistance of the cell. This model must be regarded as tentative, since it ignores the distributed, three-dimensional characteristics of the neuronal elements and tissue involved.



References and Notes

1. M. V. L. Bennett, *Ann. N.Y. Acad. Sci.* **137**, 509 (1966); ———, G. D. Pappas, E. Aljure, Y. Nakajima, *J. Neurophysiol.* **30**, 180 (1967); L. Tauc, in *Progress in Brain Research*, K. Akert and P. G. Waser, Eds. (Elsevier, Amsterdam, 1969), vol. 31, p. 247.
2. A. Arvanitaki, *J. Neurophysiol.* **5**, 89 (1942); B. Katz and O. H. Schmitt, *J. Physiol. London* **100**, 369 (1942); H. Grundfest and J. Magnes, *Amer. J. Physiol.* **164**, 502 (1951).
3. H. Grundfest, in *The Structure and Function of Nervous Tissue*, G. H. Bourne, Ed. (Academic Press, New York, 1969), vol. 2, p. 463; P. G. Nelson, *J. Neurophysiol.* **29**, 275 (1966).
4. T. Furukawa and E. J. Furshpan, *J. Neurophysiol.* **26**, 140 (1963).
5. E. J. Furshpan and T. Furukawa, *ibid.* **25**, 732 (1962).
6. P. W. Gage and R. S. Eisenberg, *J. Gen. Physiol.* **53**, 265 (1969).
7. B. Katz and R. Miledi, *Proc. Roy. Soc. Ser. B* **161**, 483 (1965); M. V. L. Bennett, Y. Nakajima, G. D. Pappas, *J. Neurophysiol.* **30**, 209 (1967).
8. K. Frank and M. G. F. Fuortes, *J. Physiol. London* **134**, 451 (1956).
9. D. M. Wilson, *Science* **129**, 841 (1959).
10. C. A. Terzuolo and T. H. Bullock, *Proc. Nat. Acad. Sci. U.S.A.* **42**, 687 (1956).
11. A preliminary communication of these results was presented at the 26th annual meeting of the Society of General Physiologists, Woods Hole, Mass., September 1972; H. Korn and D. S. Faber, *J. Gen. Physiol.*, in press.

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