Excitability Changes of the Mauthner Cell during Collateral Inhibition

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ABSTRACT Excitability changes during collateral inhibition of the goldfish Mauthner cell (M cell) were measured directly by stimulating the cell with current pulses applied through an intracellular electrode. Excitability was suppressed during the extrinsic hyperpolarizing potential (EHP) as well as during the collateral IPSP. The inhibitory effect of the EHP was shown to be comparable in intensity to the effect of the IPSP. Excitability changes in the M cell during collateral IPSP depended on changes in the membrane conductance as well as in the membrane potential. Some simple equations are advanced which describe the excitability change during the IPSP in terms of changes in membrane potential and conductance. It was also found that invasion of antidromic impulses into the M cell was suppressed during the EHP, but not during the collateral IPSP. Conductance increase during the IPSP did not interfere with the invasion of antidromic impulses.

INTRODUCTION

It has been reported that activity of the Mauthner cell (M cell) evokes a series of inhibitory effects on the contralateral as well as ipsilateral M cell, which are believed to be associated with activity in M cell axon collaterals (13–15). The effects are most readily evoked by antidromic excitation of the cell, but orthodromic or direct excitation of the M cell is almost equally effective (15). Furthermore, the inhibition consists of different types of inhibitory processes including those acting on post- and presynaptic elements (13–15). The present communication is concerned only with the inhibition acting postsynaptically.

Two distinct types of postsynaptic inhibition are found in the M cell (15). One type of inhibition is produced in association with the "extrinsic hyperpolarizing potential (EHP)" which lowers the excitability of the M cell by hyperpolarizing the membrane in the vicinity of the axon hillock. This type of inhibition is of short duration and appears earlier than the other after the M cell has been excited. Since the EHP is a potential generated in the extracellular space, the inhibition may be called an electrical postsynaptic inhibition.

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The second type of inhibition is based on a more common mechanism of chemically transmitted inhibitory postsynaptic action. It starts with a slightly longer latency than the EHP and lasts much longer. In order to specify this latter type of inhibition, it will hereafter be called "collateral IPSP."

In the present study, the excitability of the M cell during collateral inhibition was measured by stimulating the cell directly with electric current pulses applied through the microelectrode placed inside the soma of the cell (1, 2, 10, 16). A main purpose of this experiment was to obtain a direct measure of the effects of these two postsynaptic inhibitions. Direct stimulation was found to be more suited for the present purpose than testing by ortho- or antidromic activation of the cell. Thus the intensities of the two types of inhibition were brought under direct comparison. Also from the results of direct stimulation we were able to analyze the effect of the collateral IPSP in terms of changes in membrane conductance and membrane potential.

The effects of collateral inhibition on the invasion of antidromic impulses into the M cell were also studied.

METHODS

Anatomy In most species of teleost fish the two large Mauthner cells (M cells) are the most conspicuous neurons in the brain. They are located, one on each side, deep in the medulla. As illustrated in Fig. 1 A, several distinct types of synapse cover the two large dendrites; but of particular interest is the helicoidal feltwork of fine fibers (*sp.*) that is embedded in the so called axon cap (5, 6). The axon cap is an approximately spherical structure of glial and nervous elements surrounding the axon of the M cell from its axon hillock origin to the beginning of its myelination (see broken line of Fig. 1 A). The axon of each M cell then expands to about 40 μ in diameter, decussates with its fellow, and runs down the spinal cord (Fig. 1 B).

Experimental Procedures Experiments were performed upon common Japanese goldfish (*Carassius auratus* L.), measuring about 12 cm from the nose to the tail tip. They were immobilized by intramuscular injection of flaxedil (*ca.* 1 μg/gm of body weight) and held in position in a fish chamber of an almost identical design to that described by Furshpan and Furukawa (12). The fishes were kept alive during the experiment by flowing aerated and dechlorinated tap water through the mouthpiece. The brain was exposed from above, the choroid over the open fourth ventricle teased away, and the cerebellum retracted forward to reveal the medulla.

In order to stimulate the M cells antidromically some of the body musculature near the posterior margin of the dorsal fin was removed, and two pairs of electrodes were imbedded close to the unopened vertebral column. This arrangement made it possible to deliver a pair of stimuli at different intensities. It was often possible by a careful adjustment of stimulus parameters to activate only one of the two M axons. Since collateral inhibition is evoked on both M cells even when only one of them is activated, this selective stimulation of M axons afforded us a convenient means to study the effects of collateral inhibition without its being modified by the effect of preceding spike activity.

Glass micropipettes filled with 3 M KCl or 2 M K-citrate were used for recording potentials and for passing stimulating currents. In stimulating the cell through the intracellular electrode, a bridge circuit was used in most instances so that the same electrode served for potential recording and current passing (2). But in a few instances, potential recording and current passing were done using two separate microelectrodes inserted into the same M cell.
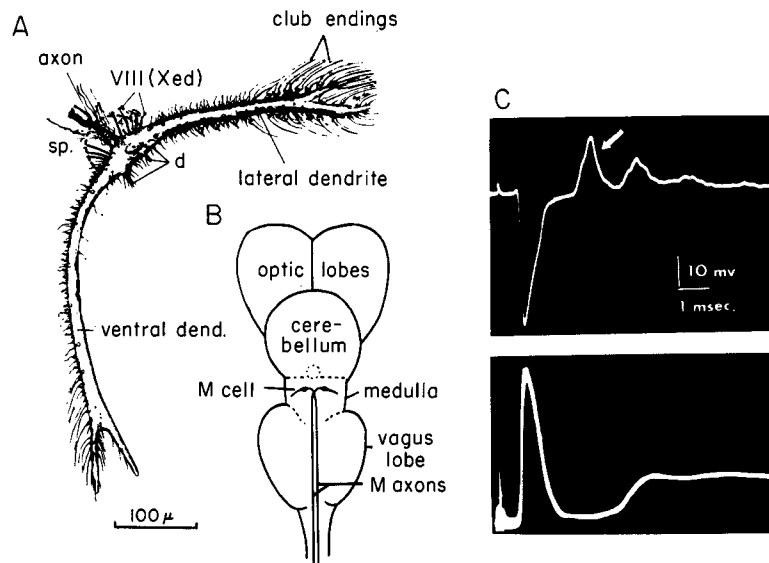

Figure 1. Structure of the goldfish Mauthner cell and potentials recorded therefrom. A, taken from Bodian (6). A semischematic drawing projected on a transverse plane. Midline is to the left, dorsal above. Note particularly the thick dendrites and the several types of presynaptic endings. *VIII* (*Xed*) = the unmyelinated club endings; *sp.* = fibers which spiral around the axon "neck"; *d* = fine dendrites. B, a schematic drawing of the location of the M-cells within the medulla as seen from above. The cerebellum is shown retracted forward to expose the subjacent medulla. The cells are more than 1 mm beneath the surface of the medulla. Note the decussation of the M axons. C, potentials recorded from the M cell following a single spinal cord shock. Upper trace, extracellular potential recorded from the vicinity of the axon hillock; lower trace, intracellular potential recorded from the soma of the same M cell. Arrow marks the EHP. Positivity upward in this and the following oscillographic records.

The microelectrode was brought near the axon hillock region of the M cell by using the negative extracellular action potential of the cell as a guide (12). The cell soma was impaled usually at a point about 50 μ lateral to the axon hillock.

Action Potentials of the M Cell Action potentials of the M cell are so unique that some preliminary account of them will be given here. Fig. 1 C shows potential changes produced by antidromic stimulation of the M cell. In the upper trace, recorded extracellularly from the vicinity of the axon hillock, the M cell spike appears as a negative

deflection of a very large size. The extracellular spike attains this large size only when recorded in the vicinity of the axon hillock. In the lower trace, recorded intracellularly from the soma, the antidromic spike appears as a positive deflection. Its size is usually about 40 mv, much smaller than the intracellular spike potential of most other neurons.

In the upper trace of Fig. 1 C the extracellular spike potential is followed by a positive wave of about 1 msec. in duration (see arrow). This extracellular positive wave is of maximum size (10 to 15 mv) in the region of the axon hillock and there is only a very small intracellular potential change that corresponds to this extracellular positivity. This means that most of the extracellular potential actually is impressed on the membrane of the axon hillock as a hyperpolarization; hence the potential is called extrinsic hyperpolarizing potential (EHP) (15). The origin of the EHP is attributable to the activity of nerve fibers that converge on the axon cap.

In the lower trace of Fig. 1 C the spike potential is followed by a slow depolarization. This is the inhibitory postsynaptic potential (IPSP) that appeared in a depolarizing direction. In the M cell the equilibrium potential of the IPSP lies very close to the resting membrane potential, hence the IPSP normally is not recorded as a potential change. In Fig. 1 C the IPSP was turned into depolarization because of a migration of Cl ions into the cell from the microelectrode (4, 15). Both the EHP and IPSP were evoked through the activity of M cell axon collaterals.

RESULTS

Excitability Changes in the M Cell as Revealed by Intracellular Stimulation

Excitability changes in the M cell during collateral inhibition were measured by stimulating the cell directly with an outward current pulse applied through a microelectrode placed inside the cell soma. The test pulse was delivered at various intervals following the conditioning spinal cord shock. The latter fired the M cell antidromically thus giving rise to collateral inhibition. At each testing interval the test pulse was varied in intensity so that it evoked a spike potential in approximately one-half of the trials.

TESTS WITH AN ELECTRODE FILLED WITH K-CITRATE   In the experiment shown in Fig. 2 an electrode filled with 2 M K-citrate was used. Citrate ions, even when they migrate into the M cell by diffusion, do not change the equilibrium potential of the IPSP. *a* and *b* show responses produced by the conditioning stimulus alone; *a* being recorded extracellularly (before the insertion of the electrode into the cell) from the vicinity of the axon hillock of the M cell and *b* from inside the cell soma. Since the strength of the conditioning spinal cord shock was so adjusted as to activate only the M cell on the opposite side, no antidromic spike appeared in records *a* and *b*. The positive deflection in *a* represents the EHP, the small positive notch recorded in *b* being its counterpart in the intracellular record (15). Although no IPSP appeared in this record as a potential change, this by no means indicates the absence of the postsynap-

tic inhibitory action. This is only because the equilibrium potential of the IPSP in the M cell lies close to the resting membrane potential. Tests with direct stimulation were carried out in *c-f*. In these records, the upper trace shows the intracellular potential change and the lower trace the stimulating current. *c* shows the control without the conditioning shock, while in *d-f* the
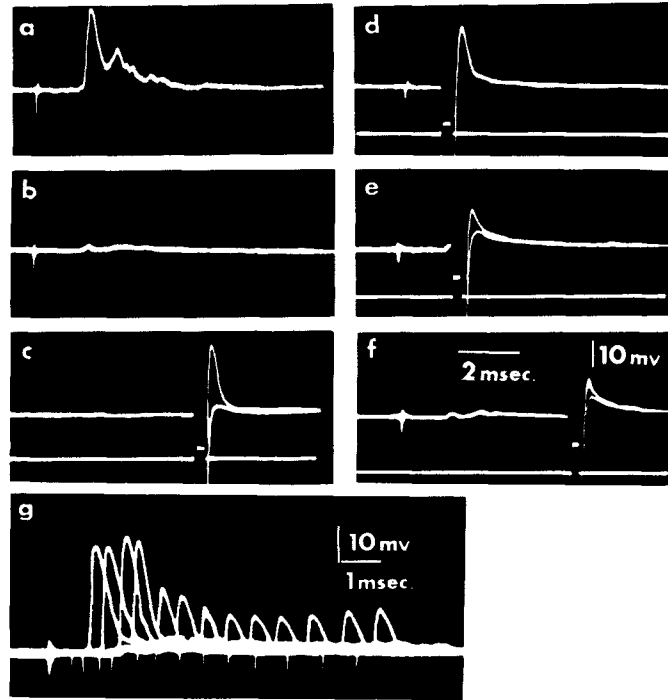

FIGURE 2. Direct stimulation of M cell through K-citrate—filled microelectrode. *a*, EHP produced by conditioning spinal cord shock that fired only the M cell on contralateral side. The same conditioning shock was applied also in *b*, and *d-g*; *b-g*, intracellular recording; *b*, conditioning shock only; *c*, direct stimulation with rectangular current pulse through the intracellular electrode; *d-f*, direct stimulation was preceded by the conditioning shock; *g*, reduction in size of antidromic spike fired at various intervals following the conditioning shock. Lower trace in *c-f*, current recording (3.3 × 10⁻⁷ A in *c*).

test pulse was applied at various intervals following the conditioning shock. It can be seen that the current strength required to fire the cell was much greater in *e* and *f* than in the control (*c*). Although direct spikes in *e* and *f* are much smaller than in the control, threshold determination could be made without much difficulty. Direct spikes were reduced in size during collateral inhibition in much the same way as were the antidromic spikes (see Fig. 2 *g*).

Fig. 2 *g* is a multiple exposure showing the effect of collateral inhibition

upon the amplitude of the intracellularly recorded antidromic spike. The same conditioning stimulus as in *b* was applied at the start of each sweep and a stronger test antidromic shock was delivered at various intervals after the conditioning stimulus. As can be seen, the amplitude of the test spike increased slightly before it was greatly diminished at longer conditioning-testing intervals. The decrease in test spike height can mostly be attributed to a shunting effect of an increased membrane conductance brought about by the inhibitory postsynaptic action (15). An early increase in test spike height was brought
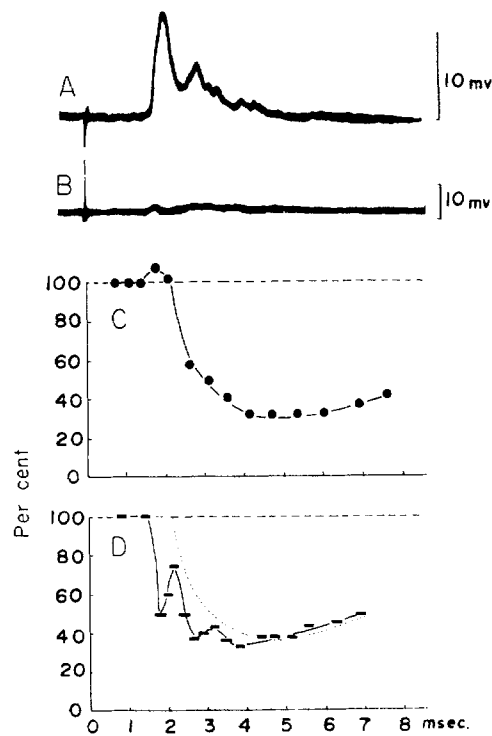

FIGURE 3. Plot from the experiment of Fig. 2. A and B, extra- and intracellular potential changes in response to a conditioning stimulus, respectively; C, amplitude of test antidromic spike (per cent of the control), plotted against intervals between conditioning shock and peak time of antidromic spikes (*cf.* Fig. 2 *g*); D, excitability; *i.e.*, reciprocals of threshold current strength. Pulses are shown as solid rectangles the length of which represents the pulse duration (0.2 msec.). Dotted line depicts the part of the lowered excitability attributable to a shunting effect of the inhibitory postsynaptic action (see text).

about by an increased transmembrane potential at the axon hillock during the EHP (15).

Results of the experiment of Fig. 2 are summarized in Fig. 3. Records A and B (same records as *a* and *b* of Fig. 2) show respectively extra- and intracellular potential changes produced by the spinal cord shock. Fig. 3 C, plotted from the record of Fig. 2 *g*, shows antidromic test spike height (expressed as per cent of the control) against intervals between the conditioning shock and the peak of each antidromic test spike. In D the excitability (reciprocal of the current strength required for threshold stimulation of the M cell) is plotted against the time interval after the conditioning antidromic shock. The test

current pulses are shown as solid rectangles the length of which represents the pulse duration. As shown in Fig. 3 D, the excitability change in the M cell took place with a rather complicated time course; first came a brief depression of excitability with its peak at an interval a little less than 2 msec., then followed a long lasting suppression with peaks at invervals of about 2.7 msec. and 3.8 msec.

The meaning of this excitability change will be considered in connection with the time course of the EHP (Fig. 3 A) and with that of the reduction in amplitude of the antidromic test spike shown in Fig. 3 C. Two factors may contribute to the change in the excitability as measured with this method of direct stimulation; *i.e.*, a change in the transmembrane potential and in the input resistance of the cell. The first peak of decreased excitability is clearly due to the EHP which hyperpolarized the membrane because it took place before any change in the input resistance of the M cell started. On the other hand, the third peak of suppression seems to be attributable to a decrease in the input resistance of the cell, because the EHP was completed by then and also because there was no change in the inside potential of the M cell as shown in B. This is also supported by the fact that the excitability was lowered approximately by the same amount as the reduction in the test spike height. The curve of Fig. 3 C was redrawn in Fig. 3 D (dotted line) after being reduced slightly in size so that its maximum coincided with the excitability change at that point. This curve thus represented the part of the lowered excitability attributable to a shunting effect of the inhibitory postsynaptic action. The difference between the continuous and dotted lines, with a time course very similar to the EHP, represents the suppression attributable to the EHP.

Results obtained on six different cells are summarized in Table I (Nos. 1–6). The excitability measured at the first peak of the EHP and during the peak of inhibitory postsynaptic action is shown as per cent of the control in columns 4 and 6 respectively. A decrease in the testing antidromic spike height during the collateral IPSP is shown in column 8. As seen from this table, the inhibitory actions of the EHP and collateral IPSP differ from cell to cell; in some cells the inhibitory action of the former is stronger while in others the relation is reversed. An apparently good agreement exists between values in columns 6 and 8. That is, the excitability was lowered during the inhibitory postsynaptic action by approximately the same amount as the reduction of the antidromic test spike height. This finding provides additional support for the interpretation that the reduction of antidromic test spike height is attributable to the shunting effect of the inhibitory postsynaptic action (15).

TESTS WITH AN ELECTRODE FILLED WITH KCL   In working with a micro-electrode filled with 3 M KCl, it was usually observed that the IPSP became

positive in sign with a lapse of time after the insertion of the electrode. This depolarizing IPSP grew for a while until it finally reached a steady potential level (15). An attempt was made to measure the excitability of the M cell during such a depolarizing IPSP.

In Fig. 4, the upper beams show intracellular potential; the lower beams, the stimulating current used. Record a shows the control without preceding
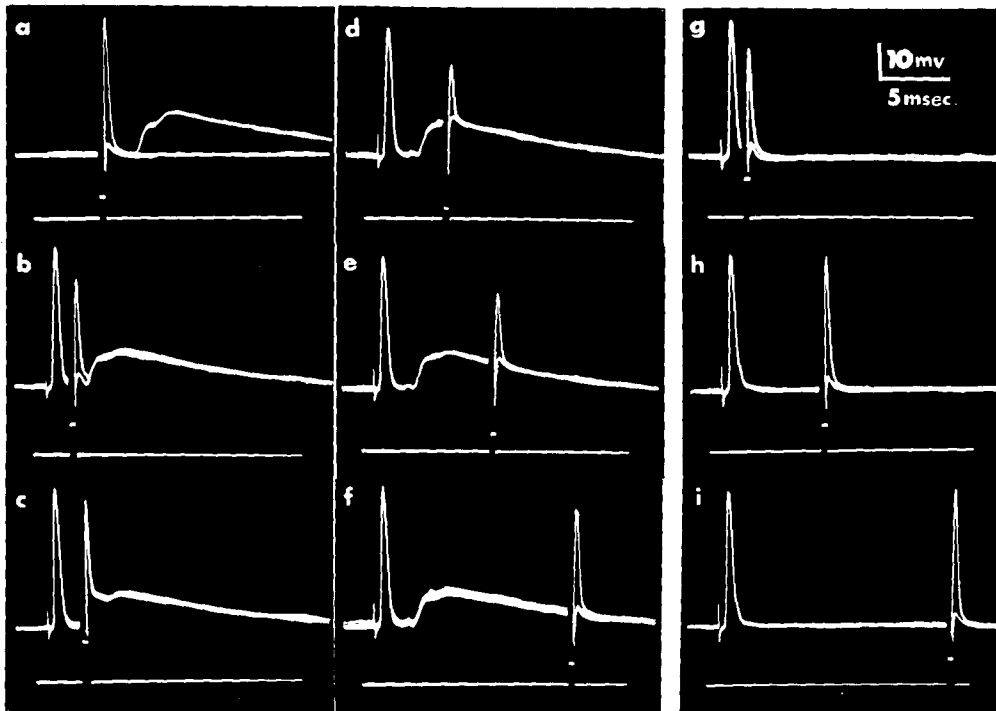

FIGURE 4. Direct stimulation of M cell through KCl-filled microelectrode. Upper trace, intracellular potential; lower trace, current recording (3×10^{-7} A in a). a, direct stimulation with rectangular current pulse through intracellular electrode; b-i, direct stimulation was preceded by conditioning antidromic shock; b-f, tested at a rate of once per 5 sec.; g-i, tested at a rate of 5 per sec.

conditioning shock. In this cell the depolarizing IPSP had an amplitude as large as 13 mv. In records b-f, the excitability was tested at various times after the conditioning antidromic shock. The conditioning shock in this instance fired the M cell under test (see first spike in each record except a). The strength of the test stimulating current was adjusted, as before, to produce a spike in about half the trials.

Fig. 5 is arranged in the same way as Fig. 3. From the top downward are shown the antidromic responses recorded extracellularly (A) and intracellu-

larly (B), antidromic test spike height (C), and the excitability of the cell measured with direct stimulation (D). The short solid rectangles plotted in Fig. 5 D are, as in Fig. 3 D, the reciprocals of the threshold current strengths expressed in per cent of the control.

The effect of refractoriness on the excitability as well as on the antidromic test spike height must be taken into account in this instance, for the condition-
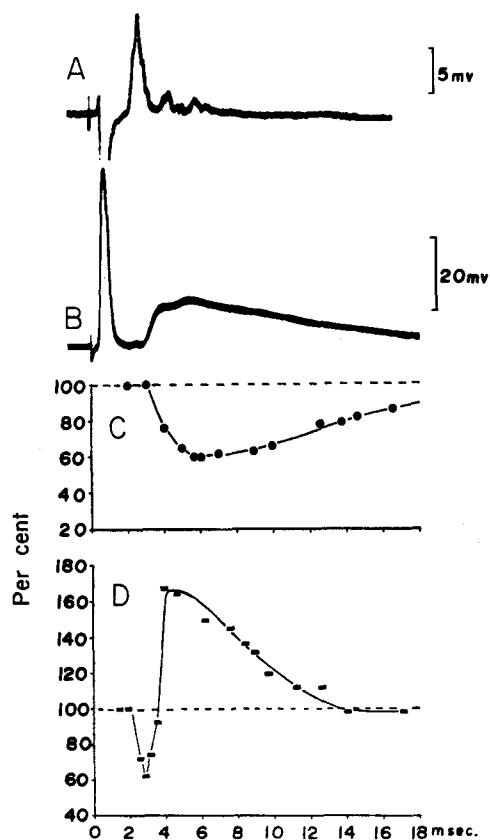

Figure 5. Plots from the experiment of Fig. 4. A and B, extra- and intracellular potential changes in response to the conditioning stimulus; C, amplitude of test antidromic spike (per cent of the control), plotted against intervals between conditioning shock and peak time of antidromic spikes; D, excitability; *i.e.*, reciprocals of threshold current strength. Pulses are shown as solid rectangles the length of which represents the pulse duration (0.4 msec.).

ing shock produced a spike potential in the M cell tested. A correction for this was made, as described below, by taking advantage of the fact that both the EHP and the IPSP were abolished at a higher test frequency of 5/sec. (15). The abolition occurs perhaps because of the fatigue of transmission at synapses located in the pathway leading to collateral inhibition.

Correction for the excitability change due to the refractoriness: The current strengths required for threshold stimulation were measured again, this time at a rate of 5/sec. As can be seen in sample records shown in Fig. 4 *g-i*, pulses stronger than

the control, shown in Fig. 4 *a*, were required in these tests. The ratios of these test currents to the control were calculated and used as correction factors. Each threshold current value obtained at a testing rate of 0.2/sec. (*cf.* Fig. 4 *b-f*) was divided by the correction factor for respective shock interval. These corrected values were used in plotting Fig. 5 D.

Correction for the spike height change due to the refractoriness: Upon testing at a rate of 5/sec., the amplitude of the test spike was measured for each conditioning-testing interval. Values thus obtained were used as controls. Test spike sizes plotted in Fig. 5 C were expressed in per cent with these controls as 100.

Values thus corrected are shown in Table I.

The initial brief dip in the excitability curve seen in Fig. 5 D corresponds in its time course to the first peak of the EHP (record A). Therefore, the initial dip must be attributed, as in Fig. 3 D, to an increase in the transmembrane potential caused by the EHP. The suppression was then followed by an increase in the excitability. The transition took place very rapidly; the excitability reached its maximum (165 per cent of the control) within a few tenths of a millisecond. Thereafter it gradually returned to the original level, the enhancement of the excitability taking place with a time course similar to that of the depolarizing IPSP. This finding, taken together with the fact that no enhancement was observed when K-citrate–filled microelectrodes were used, seems to suggest strongly that the increased excitability observed in this instance is attributable to the presence of a depolarizing IPSP.

Results obtained on five different cells are summarized in Table I (Nos. 7–11). As shown in column 4, the excitability was suppressed during the EHP by approximately the same amount as when K-citrate electrodes were used. But, as shown in column 6, an enhancement of the excitability was obtained during the collateral IPSP in all five cases listed. When compared with the size of the depolarizing IPSP listed in column 7, a tendency can be seen for stronger enhancement to accompany larger depolarizing IPSP's.

AN ANALYSIS OF THE EXCITABILITY CHANGE DURING THE IPSP   Results in the foregoing section indicate that the IPSP produces changes in the excitability of M cells by changing the membrane conductance as well as the membrane potential. An attempt will be made here to analyze the relative contributions of these two factors under different conditions.

It is assumed here that the cell is fired when the membrane depolarization exceeds a certain critical level as expressed in the following equation:

$$V_{th} = R_m \times I_{th} \tag{1},$$

where V_{th}, R_m, and I_{th} represent critical depolarization (measured from the resting potential level), input resistance of the M cell, and threshold current

required to fire the cell, respectively. During the inhibitory postsynaptic action the equation must be changed to:

$$V_{th} - V_{ipsp} = R'_m \times I'_{th} \tag{2},$$

where V_{ipsp} represents displacement of membrane potential from its resting value during the inhibitory postsynaptic action, and R'_m and I'_{th} represent input resistance and threshold current during the inhibitory postsynaptic action

TABLE I

1	2	3	4	5	6	7	8	9	10
					Values at the peak of collateral IPSP				
No.	Electrode used	R.P.	Excitability during EHP	V_{ehp}*	Excita-bility	V_{ipsp}	Anti-dromic spike size	V_{th}‡	E_{ipsp}§
		mv	*per cent*‖	*mv*	*per cent*‖	*mv*	*per cent*‖	*mv*	*mv*
1	K-citrate	70	62	—	71	0	72	—	—
2	K-citrate	73	79	—	64	0	64	—	—
3	K-citrate	76	50	—	38	0	32	—	—
4	K-citrate	67	60	—	75	0	62	—	—
5	K-citrate	—	64	—	50	0	58	—	—
6	K-citrate	—	58	—	50	−0.5	53	—	—
7	KCl	80	88	2.5	126	+9.0	63	18	24
8	KCl	75	63	11.7	166	+13.0	60	20	32
9	KCl	—	64	8.5	150	+10.0	53	15	21
10	KCl	78	60	15.3	228	+15.0	78	23	68
11	KCl	—	66	10.0	254	+16.0	40	19	27

Nos. 5, 6, 10, and 11, experiments carried out with two independent intracellular electrodes.
Figs. 2 and 3 were taken from No. 3, and Figs. 4 and 5 from No. 8.
* Calculated from equation (5).
‡ Calculated from equation (3).
§ Calculated from equation (6).
‖ Per cent of the control.

respectively. Then, the change in the excitability, *i.e.*, the ratio of I_{th} to I'_{th} is expressed by:

$$I_{th}/I'_{th} = V_{th}/(V_{th} - V_{ipsp}) \times (R'_m/R_m) \tag{3}.$$

In the simpler case in which $V_{ipsp} = 0$, the equation is reduced to:

$$I_{th}/I'_{th} = R'_m/R_m \tag{4}.$$

This relation should apply to cases in which K-citrate–filled microelectrodes were used. In the experiment of Fig. 3, curves C and D (dotted line) represent

approximately the time course of R'_m/R_m and I_{th}/I'_{th} respectively. Equation (4) seems to fit these observations to the extent that the time course of these two curves is quite similar.

More generally, V_{ipsp} is not zero, the IPSP occurring in either the de- or hyperpolarizing direction. From equation (3), it should be possible to calculate values of I_{th}/I'_{th} if V_{ipsp} and R'_m/R_m are known and any fixed value is assumed for V_{th}. This was tried in the case of Fig. 5 where a large depolarizing IPSP was observed and the results were drawn with continuous lines in Fig. 6 A. Values of V_{th} used in the calculation are 23, 20, and 17 mv for upper, middle, and lower curves respectively. The time courses of V_{ipsp} and R'_m/R_m
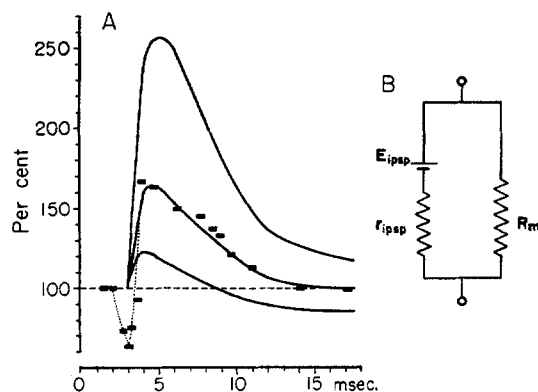

FIGURE 6.   A, excitability of M cell during collateral IPSP. Three curves were drawn according to equation (3) for three different values of V_{th} (23, 20, and 17 mv from upper to lower curves). Horizontal rectangles show excitability experimentally determined (cf. Fig. 5 D). Early dip in the excitability is attributable to the effect of EHP which was not counted in equation (3). See text for detail. B, The EMF underlying the IPSP. E_{ipsp}, EMF; r_{ipsp}, resistance of inhibitory postsynaptic membrane during action; R_m, input resistance of the cell.

were obtained from Fig. 5 B and C respectively. Horizontal rectangles in the same figure show, as in Fig. 5 D, excitability, i.e. I_{th}/I'_{th}, experimentally determined. As shown, the middle curve ($V_{th} = 20$ mv) agrees very well with the experimentally determined values over nearly the entire time course of collateral IPSP. The discrepancy in the earlier part must be neglected, for it is attributable to the effect of the EHP which was not counted in the present calculation. A good coincidence such as that observed in Fig. 6 A seems to indicate the correctness of the initial assumption that the critical depolarization for the firing of the cell (V_{th}) remains unchanged during collateral IPSP.

Similar plots tried on four other cases listed in Table I (Nos. 7, 9, 10, and 11) gave satisfactory results. Values of V_{th} used there are shown in column 9 of Table I. They were calculated from equation (3) by using experimentally

determined values of I_{th}/I'_{th}, V_{ipsp}, and R'_m/R_m for the peak time of collateral IPSP. Their values (15 to 23 mv, mean: 19 mv) are in the same range as the firing level of the cell previously reported (15 to 25 mv, reference 12).

A slight modification of equation (3) leads to an equation which describes the excitability change during the EHP,

$$I_{th}/I'_{th} = V_{th}/(V_{th} + V_{ehp})$$ (5),

where V_{ehp} stands for the EHP size. This equation takes a simpler form than equation (3) because the EHP reaches its peak before the collateral IPSP starts (hence, $V_{ipsp} = 0$ and $R'_m/R_m = 1$). Equation (5) can be used to calculate the value of V_{ehp} when I_{th}/I'_{th} and V_{th} are known. V_{ehp} thus obtained might as well be called effective size of the EHP. The results of such calculation, shown in column 5 of Table I, are in a very reasonable range of values (2.5 to 15.3 mv) as compared to the EHP size in the previous report (5 to 15 mv, reference 15). In the present experiment, however, no effort was made to find the maximum EHP size experimentally. To do so would require many insertions of the recording electrode in the vicinity of the axon hillock, thereby increasing the chances of cell damage.

Fig. 6 B is a simplified electrical circuit diagram of the inhibitory synapses in M cells. E_{ipsp} and r_{ipsp} in this diagram represent the equilibrium potential of the IPSP and the resistance of the inhibitory channel respectively. Based on this diagram the E_{ipsp} can be expressed by the following equation:

$$E_{ipsp} = V_{ipsp} \times (R_m + r_{ipsp})/R_m$$
$$= V_{ipsp}/(1 - R'_m/R_m)$$ (6).

Values of E_{ipsp} listed in column 10 of Table I were calculated from equation (6) by inserting values in columns 7 and 8 of the same table as V_{ipsp} and R'_m/R_m respectively. E_{ipsp} ranges from 21 to 68 mv. It is to be noted here that these values of E_{ipsp} are larger than V_{th} in all instances shown. This is a necessary condition for the enhancement of the excitability during the IPSP.

When the IPSP occurs in a depolarizing direction, its effect as a potential change counteracts the shunting effect of the inhibitory postsynaptic action. Under certain conditions, therefore, these opposing effects of the IPSP can cancel each other, resulting in no change of the excitability. The condition under which this can occur is easily obtained from equations (3) and (6).

$$V_{th} = E_{ipsp}$$ (7).

A very good agreement of experimental results with the equations as described above could in part be coincidental. There seem to be two major sources of error: (*a*) An increased membrane conductance during collateral IPSP would make the membrane time constant shorter resulting in a faster

rise of inside potential at the onset of the stimulating pulse. However, it is not likely that this factor would lead to any serious error, because, as will be discussed later, the membrane time constant of the M cell is shorter than the duration of stimulating pulse. (b) There is probably some error when the change in the input resistance of the cell is estimated from the reduction of the antidromic spike size. In fact, it is known that the action current of the M cell increases slightly during the IPSP (15). Apparent excitability would be increased by the first factor, while the second factor would make the reduction of antidromic spike size smaller.

Excitability Change in the M Cell during Collateral Inhibition As Tested by Block of Antidromic Impulses

Furukawa and Furshpan (15) found that the invasion of antidromic impulses into the M cell could be facilitated by passing pulses of cathodal current and suppressed by anodic current through an extracellular microelectrode in the axon cap. They showed that a much smaller anodic current was required for the block during the EHP even when the latter was not large enough to produce a block. Similar experiments were repeated in the present study, but the main objective here was to find out how the invasion of the antidromic spike is influenced during the IPSP.

A microelectrode was positioned with its tip in the axon cap; a recording microelectrode was also inserted into the cap or into the M cell itself, to monitor the spike. In order to eliminate the refractory effects, the strength of the conditioning spinal cord shock was adjusted in such a way as to excite only the contralateral M cell. A test antidromic shock, much stronger than the conditioning stimulus, was delivered at various times after that. A current pulse of about 9 msec. duration was passed through the microelectrode, its strength being adjusted so that the artificial extrinsic potential, together with collateral inhibition, was just adequate to elicit blocking.

About half the experiments were carried out with both current and recording electrodes positioned outside the M cell. These results (6 cells in all) all showed that the invasion of antidromic impulses into the M cell was neither facilitated nor suppressed during the collateral IPSP, while the invasion was strongly suppressed during the EHP. This finding seems to suggest that the invasion of antidromic impulses is not influenced by the conductance change that takes place in the soma membrane. To make this point clear, later experiments were carried out with the recording electrode placed inside the soma of the M cell to monitor the intracellular potential. It was found that the invasion of the antidromic spike was facilitated during the collateral IPSP in three instances in which a KCl-filled electrode was used for recording intracellular potential. This facilitory effect seems to be attributable to the presence of a depolarizing IPSP caused by the use of a KCl-filled intracellular

electrode. No such facilitation was observed in two instances in which a K-citrate–filled electrode was used and also in one instance using a KCl electrode. In the former case, as expected, no potential change was observed during collateral IPSP and in the latter the IPSP was very small though it occurred in a depolarizing direction. These findings seem to support the idea that conductance change across the soma membrane does not suppress the invasion of antidromic impulses.

Electrical Constants of Mauthner Cell Membrane

Rectangular electric pulses were passed through the microelectrode placed inside the soma of M cells and the change in membrane potential was measured through the same microelectrode with the bridge method or through a second microelectrode that impaled the same M cell. Fig. 7 shows a result of measurements carried out by using two independent electrodes inserted into a single M cell. A shows sample records in which upper traces represent changes in the membrane potential and lower traces monitor the current. A depolarizing current was passed in A 1 while hyperpolarizing currents of increasing strengths were passed in A 2–4. Although obscured by transients at the start and cessation of the current, it will be clear that the membrane time constant is very short. B plots changes in membrane potential at plateau level against current strengths used. All the points came on a straight line, indicating that no rectification was produced at least within the range tested. Results of measurements on nine cells are shown in Table II. As shown in column 3, the input resistance ranged from 70,000 to 200,000 ohms (mean: 130,000 ohms). These values of input resistance coincide well with the values reported by Furshpan and Furukawa (12). Specific membrane resistance (column 4), obtained by dividing values of input resistance by total surface area of the cell (5×10^{-4} cm², reference 12), ranged from 35 to 100 ohms cm² (mean: 65 ohms cm²). The membrane time constant (column 7) was obtained by measuring the time of rise of the membrane potential to 66 per cent of terminal value. It ranged from 0.25 to 0.5 msec. (mean: 0.39 msec.). The membrane capacitance (columns 5 and 6) was then calculated from the values of input resistance and time constant. Specific membrane capacitance thus obtained ranged from 3.3 to 10.3 μf/cm² (mean: 6.5 μf/cm²). Membrane constants of motoneurons of the cat are reported to be 600 ohms cm², 3.1 msec., and 5 μf/cm² for specific membrane resistance, membrane time constant, and specific membrane capacitance respectively (7). When compared with these values, it is clear that the membrane of M cells is characterized by its low membrane resistance. This results in a very short membrane time constant as compared with that of spinal motoneurons. On the other hand, the membrane capacitance is in about the same range as motoneurons. Probably the value of the surface area of the cell used here for the calculation (5×10^{-4} cm²) is a
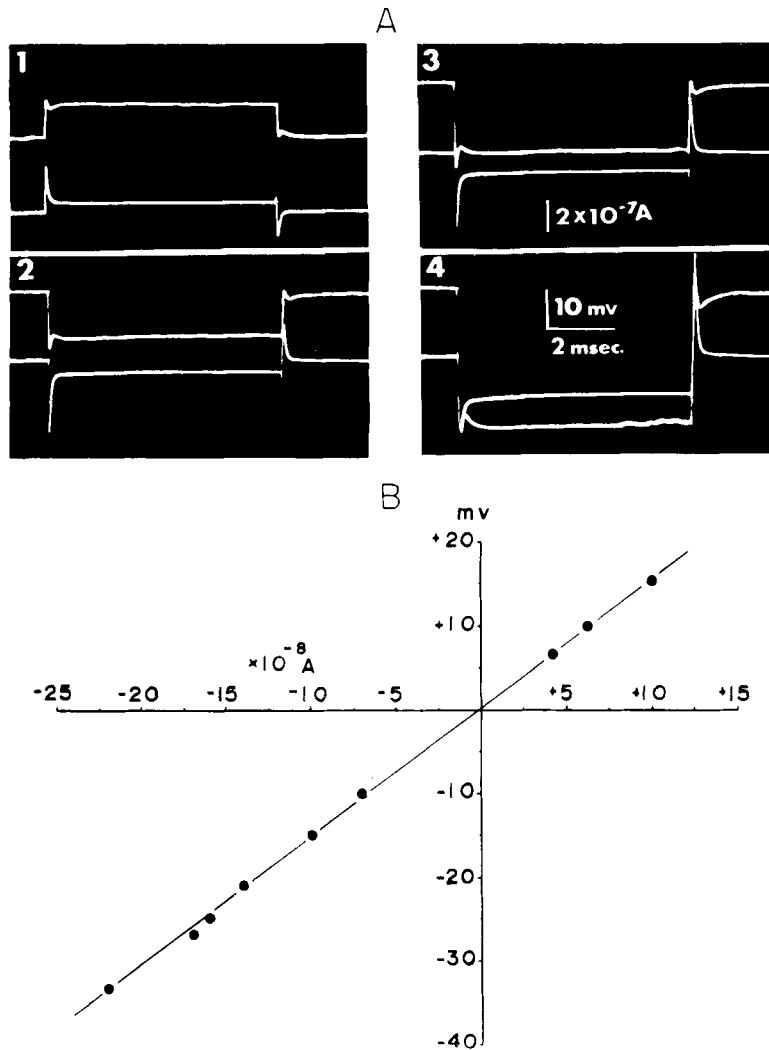
FIGURE 7.   Measurement of electrical constants of M cell. A, changes in the membrane potential (upper trace) produced by polarizing current (lower trace); two independent microelectrodes were used for potential recording and current passing. B, plot of the membrane potential change against strength of polarizing current.

little too small (12). This tends to make the estimate of specific membrane resistance too low and that of specific membrane capacitance too large. Another source of error is in the measurement of the membrane time constant. It is very difficult to carry out the measurement of the time constant of the M cell accurately, for it is very small as compared with that of other neurons.

Through stray capacitance electric current would leak across the wall of the current electrode at the start of the rectangular pulses. This would make the slope of the potential rise more slowly, resulting in a time constant a little larger than the true value. Therefore, the true value of specific membrane capacitance would be a little smaller than the values shown in Table II.

DISCUSSION

In the present study the excitability of the M cell was measured by stimulating the cell directly with depolarizing pulses applied through an intracellular

TABLE II

ELECTRICAL CONSTANTS OF MAUTHNER CELL MEMBRANE

1	2	3	4	5	6	7
No.	R.P.	Input resistance	Specific membrane resistance	Total membrane capacitance	Specific membrane capacitance	Membrane time constant
	mv	$K\ ohms$	$ohms\ cm^2$	$\mu\mu f$	$\mu f/cm^2$	$msec.$
1	80	70	35	5150	10.3	0.36
2	73	78	39	5130	10.3	0.40
3	68	115	58	4350	8.7	0.50
4	69	180	90	2330	4.7	0.42
5	64	200	100	2500	5.0	0.50
6	62	100	50	3500	7.0	0.35
7	78	107	54	3180	6.4	0.34
8	78	172	86	2040	4.1	0.35
9	64	150	74	1670	3.3	0.25
Mean		130	65	3317	6.5	0.39

Nos. 7 to 9, experiments carried out with two independent intracellular electrodes.
No. 9, shown in Fig. 7.

electrode. This method of testing was apparently much more suited for evaluating the excitability change during collateral inhibition than was orthodromic stimulation. There are several drawbacks in using orthodromic stimulation for testing the postsynaptic inhibition in the M cell: (a) Results of orthodromic stimulation are modified greatly by an inhibitory mechanism acting on the presynaptic side (13). (b) Excitatory inflow to the M cell produced by an orthodromic stimulus is often complicated and long lasting, and it is not correlated linearly with stimulus strength. This makes it difficult to express the excitability change in terms of change in the threshold. (c) An orthodromic stimulus produces, in addition to an EPSP, a positive extracellular potential change, called the extracellular orthodromic response (EOR), in the vicinity

of the M cell axon hillock (13). The EOR presumably modifies the excitatory effect of the EPSP and complicates the situation.

Direct stimulation with brief depolarizing pulses applied through the intracellular microelectrode is apparently free from these obstacles. As shown under Results, experiments carried out with K-citrate–filled microelectrodes revealed a suppression of excitability of the M cell during collateral inhibition. The suppression occurred in two phases corresponding to the presence of two types of postsynaptic inhibition. It is to be noted that the suppression during the EHP was very marked, being comparable to that during the collateral IPSP, although its duration was brief (Fig. 3). It is thus clearly shown that the EHP, as discussed by Furukawa and Furshpan (15), results in a considerable shortening of the latency of collateral inhibition. It is worth mentioning here that the M cell is supplied with electrically transmitted excitatory synapses on its lateral dendrite which may fire the cell with a very short latency (11). This fact must be taken into account in considering the functional significance of the short latency inhibition brought about by the EHP.

Excitability changes in the M cell during the collateral IPSP depend on changes in the membrane conductance as well as in the membrane potential. This constituted the basic idea underlying the analysis carried out in the present investigation. Some simple formulae were thereupon advanced to relate the excitability change to change in membrane conductance and potential, and it was shown that these equations coincided fairly well with the experimental observations.

Under normal conditions the potential change does not play an important role in the inhibition in the M cell since the equilibrium potential of the IPSP lies near the resting potential. It is only due to a great increase in the membrane conductance that a strong inhibitory action is exerted without concomitant hyperpolarization. When the IPSP is turned into a depolarizing potential change under the influence of intracellularly injected Cl ions, the potential change as such should produce an enhancement of the excitability while the increased conductance tends to a suppression as before. Therefore the excitability may be enhanced or suppressed depending on which factor dominates. If the IPSP attains a critical level of depolarization, it generates a spike potential, a phenomenon which also has been observed with the IPSP of cat spinal motoneurons (8) and with the IPSP of crustacean receptor cells (17).

The fact that the membrane time constant of the M cell is negligibly small mostly explains why the excitability changes during the IPSP could be described by simple equations such as those used in the present study. It is clear that the treatment is greatly simplified if there is no need to take the membrane capacitance into consideration. A membrane time constant ranging from 0.25 to 0.5 msec. (mean: 0.39 msec. in nine measurements) was ob-

tained in the present study. But there are some grounds for believing that the actual value could be smaller than that indicated in these measurements.

In the cat's spinal motoneurons in which the membrane time constant is much greater than in the M cell (about 3 msec.), the inhibitory synaptic current (therefore the conductance change) which underlies the IPSP takes a much shorter time course than does the latter (3, 9). Araki, Eccles, and Ito (1), who studied the excitability change during the IPSP of motoneurons by measuring it directly with depolarizing pulses, observed that the excitability was suppressed more strongly at the initial part of the IPSP than at its peak. A similar tendency, although less marked, was observed by Kubota and Brookhart with postsynaptic inhibition in the frog motoneurons (16).

We are fairly clear now about the fact that inhibitory synapses are distributed not only over the soma but also on the base of the lateral dendrite of the M cell (13, 14). In the present study, however, we did not pay any special attention to the presence of dendritic inhibition and this does not seem to have caused any serious error. This is because the conductance change that takes place in the dendrite has only a slight effect on the input resistance of the cell as measured in the present study (14). Also the dendritic IPSP does not seem to have been changed to depolarization when a KCl electrode was used, because a change in the inside concentration of Cl ions produced by the diffusion from the electrode was mostly limited to the vicinity of the electrode insertion (*i.e.*, the soma in the present study).

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