*Proc. Natl. Acad. Sci. USA*
Vol. 89, pp. 440–443, January 1992
Neurobiology

# Long-term potentiation of inhibitory circuits and synapses in the central nervous system

**(glycinergic synapses/Mauthner cell)**

HENRI KORN*, YOICHI ODA*, AND DONALD S. FABER†

*Institut National de la Santé et de la Recherche Médicale U261, Laboratory of Cellular Neurobiology, Institut Pasteur, 25, rue du Dr-Roux 75724 Paris Cedex 15, France; and †Division of Neurobiology, Department of Physiology, State University of New York, 313 Cary Hall, Buffalo, NY 14214

*Communicated by Richard F. Thompson, October 14, 1991*

ABSTRACT **Glycinergic inhibition evoked disynaptically in the teleost Mauthner cell by stimulation of the contralateral eighth nerve exhibits long-term potentiation following classical tetanization of that pathway. This enhancement occurs at the synapses between primary afferents onto second-order interneurons and the connections between these inhibitory cells and the Mauthner neuron. The evidence for modifications of glycinergic transmission is that the slope of the relation between the presynaptic volley and the synaptic conductance can be greater after the tetanus. This increase in gain is still manifest after pharmacological block of potentiation at the excitatory synapse with glutamate antagonists. Inhibitory long-term potentiation is induced by tetani weaker than those required for enhancement of the monosynaptic excitation of the other (ipsilateral) Mauthner cell. Thus, *in vivo* learning can alter the balance between excitation and inhibition within a network by modifying one or both of them.**

Long-term potentiation (LTP) of inhibition has not been reported, although the stimuli used for testing and inducing excitatory LTP generally also activate feed-forward and/or feedback inhibitory circuits. Conflicting results have been obtained, particularly in the hippocampal slice, where, when observed, enhanced inhibition during excitatory LTP has been attributed to increased excitation of the inhibitory interneurons and pathways (1–3). Conversely, decreased GABAergic transmission (GABA = γ-aminobutyric acid; refs. 4, 5) was postulated to occur at the inhibitory synapses (1, 6–8), although no modifications of the synaptic chloride conductance were detected (9). These contradictory findings may be related to difficulties in activating inhibitory responses alone.

The Mauthner cell (M cell) system of teleosts allows this question to be addressed because inhibition of this neuron can be evoked without introducing parallel excitation. As shown in Fig. 1, the posterior branch of the eighth nerve produces disynaptic inhibition of the contralateral M cell, relayed through crossed second-order vestibular interneurons (11). Their terminals, which are grouped on the soma (12), are glycinergic (11, 13), and there is evidence that their synapses are modifiable (14, 15).

Although this is a reticulospinal neuron that processes sensory information, excitatory (electrotonic and chemical) synapses onto the ipsilateral M cell exhibit a typical LTP (10). We report here that the crossed inhibitory pathway also undergoes LTP at both connections in the network. Thus, in the intact brain, conditions favoring complementary or selective modifications produced by a common drive can be studied.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

## MATERIAL AND METHODS

Recordings were obtained from the M cell soma of goldfish (*Carassius auratus*) anesthetized with MS 222 and immobilized with *d*-tubocurarine, using micropipettes (2–5 MΩ) filled with KOAc (4 M) or KCl (2.7 M). Single-electrode voltage clamp techniques were similar to those used for analysis of inhibitory synaptic currents (16).

Test and conditioning disynaptic inhibitions were evoked by activating the posterior branch of the contralateral eighth nerve (VIII n) through microstimulating bipolar steel electrodes with tip diameters and separations of about 10 and 50 μm, respectively. Test stimuli, applied every 2 sec, were typically below the threshold for firing the other M cell. Tetanizations were with short trains (12–20 pulses) at 300–500 Hz, repeated one every 2–4 sec for 1–3 min, using intensities that were not always equal to that of the test.

Changes in inhibitory responses were quantified on averaged sweeps ($n = 4$–20) using two methods. The first involved comparisons of peak inhibitory postsynaptic potential (IPSP) or inhibitory postsynaptic current (IPSC) magnitudes. The second was based on measurements of the reduction in the antidromic spike height due to the inhibitory shunt. Since this action potential propagates passively into the soma, any conductance change can be calculated as $r' = (V/V') - 1$, where $V$ and $V'$ are spike amplitudes in the absence and presence of inhibition, respectively. This expression represents the ratio (or fractional conductance) $G_{ipsp}/G_m$, the two terms being the inhibitory and resting conductances (17).

The specificity of the tetanization effects and stability of chloride loading were assessed by monitoring the recurrent collateral inhibition, which involves a second set of interneurons and is produced by antidromic activation of the M axon in the spinal cord. The constancy of $G_m$ was also verified by measuring the antidromic spike, or in voltage clamp, of responses to command pulses. Results reported are from cells in which these parameters and resting potential (−70 to −90 mV) varied by no more than 10%.

In some experiments, (*i*) the calcium chelator 1,2-bis(*o*-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid in KOAc electrodes (3–10 mM) was continuously injected iontophoretically or (*ii*) the excitatory amino acid receptor antagonists 6-cyano-7-nitroquinoxaline-2,3-dione (50 μM) and D,L-2-amino-5-phosphonovaleric acid (100 μM) were applied by superfusion in saline.

## RESULTS

In five of six chloride-loaded cells, inhibition was enhanced whereas the collateral IPSP was unchanged, with the mean increase being 64%—i.e., to 164% of control at 10 min after

Abbreviations: M cell, Mauthner cell; IPSP and IPSC, inhibitory postsynaptic potential and conductance; CNS, central nervous system; M axon, Mauthner axon; LTP, long-term potentiation.

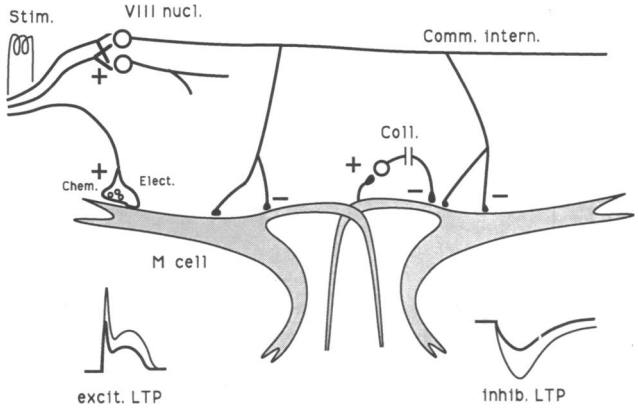


FIG. 1. Diagram of M cell-related networks that manifest long-term changes in synaptic strength. Both M cells are inhibited disynaptically following eighth nerve stimulation (Stim.), by second-order vestibular commissural interneurons (Comm. intern.) whose cell bodies are in the vestibular nuclei (VIII nucl.). Eighth nerve fibers excite only the ipsilateral M cell dendrite by way of mixed electrotonic (Elect.) and chemical (Chem.) synapses, both of which exhibit LTP after classical tetanic stimulations (10). As shown here, the same paradigm potentiates the crossed inhibitory pathway, whereas the non-tetanized recurrent collateral inhibition (Coll.) is unaffected. The symbols + and − refer to the corresponding synaptic function.

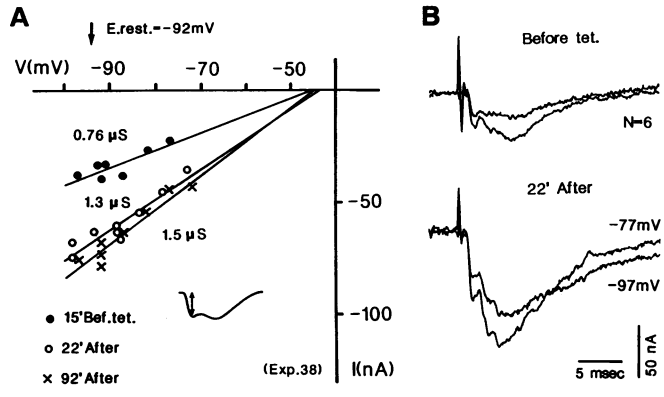


FIG. 2. Increased synaptic conductance during composite inhibitory LTP. (*A*) Current–voltage relationships of the first component (*Inset*) of a test IPSC recorded in a single-electrode voltage clamp experiment (chopping frequency, 16.0 kHz) from a chloride-loaded M cell. Data were obtained at the indicated times before and after tetanization of the contralateral eighth nerve [trains of 20 pulses at 500 Hz every 2 sec during 90 sec, intensity ($i$) = 10× test strength]. Note that following the tetanus, the conductance almost doubled and remained elevated throughout the recording session, while the reversal potential was stable. The regression lines were fit with least square statistics. E.rest., resting potential. (*B*) Sample averaged recordings collected at two membrane potentials (−77 and −97 mV), illustrating the enhancement of both IPSC components and the characteristic accelerated decay at the more hyperpolarized level.

onset of conditioning. In another seven cells, facilitation, although present, could not be quantified due to shifts in $Cl^-$ loading. More reliable information was given by the other methods.

**Voltage Clamp Analysis.** The eighth nerve-evoked IPSC often has two monosynaptic components, and both were enhanced in three of five cells studied, the average increase in the inhibitory conductance being 75% ± 25% (±SEM). No changes occurred in the other two experiments. The reversal potential for all components was the same as that of the collateral current and remained relatively constant. Thus LTP was not associated with a novel current. The resting conductance, $G_m$, which ranged from 5.6 to 13.7 μS, was also unaffected. Such results are illustrated in Fig. 2*A*. The synaptic conductance increased by 71% at 22 min and 97% at 92 min after tetanization in this cell, which was held for >2 hr; there was a comparable facilitation of the second component (Fig. 2*B*), without obvious alterations in the kinetics or voltage dependence of these IPSCs.

**Assessment of LTP with Fractional Conductance.** Measurements of inhibitory shunts, which are independent of $Cl^-$ loading (17), allowed us to follow LTP continuously. In the case of Fig. 3, the ratio of inhibitory to resting conductance, $r'$, was constant for 30 min before induction of LTP, increased immediately by about 200%, and remained at that level until the penetration was lost (Fig. 3*A*). In these experiments, the peak of the antidromic spike occurred about 3 msec after that of the presynaptic inhibitory volley, which corresponds to the early part of the IPSP's falling phase (18). It can also be noted in Fig. 3 that the enhancement was specific to the tetanized pathway as $r'$ Coll. remained stationary (Fig. 3 $B_1$ and $B_2$), varying by no more than 10% about a mean of 1.16.

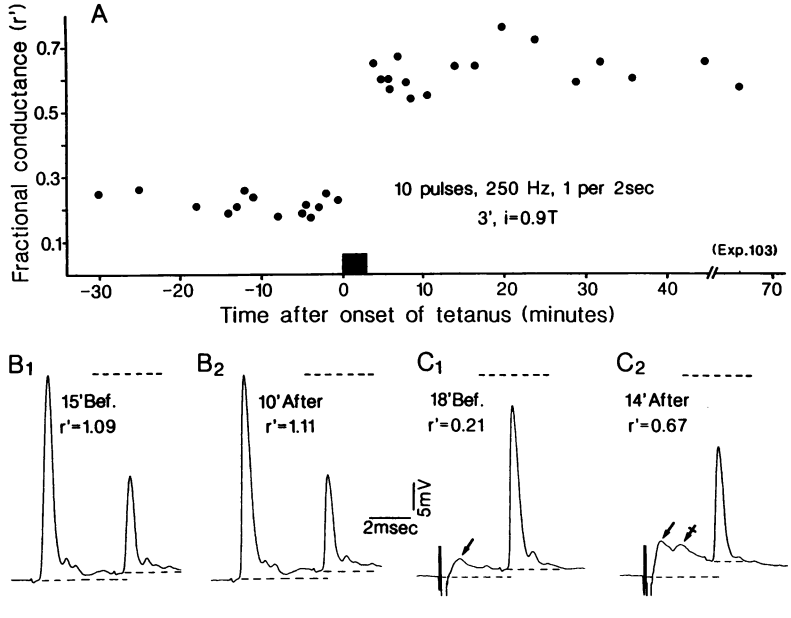


FIG. 3. Overall characteristics of inhibitory LTP. (*A*) Plot of the fractional conductance increase ($r'$), after contralateral eighth nerve stimulation with indicated parameters, versus time. $r'$ was calculated as indicated in the text. ($B_1$–$C_2$) Presynaptic component of LTP and synapse specificity. ($B_1$ and $B_2$) Lack of an effect of eighth nerve tetanization on collateral inhibition (uppermost horizontal dashed lines indicate control action potential amplitudes, and lower ones are continuations of baselines used for measuring the related potentials). ($C_1$ and $C_2$) Increased eighth nerve-evoked inhibition after tetanization. The responses consist of the intracellularly recorded presynaptic volley (arrows) followed by a small depolarizing IPSP (crossed arrow in $C_2$), the latter producing the shunt. LTP, which appears as an increased IPSP and fractional conductance ($r'$), is associated with an enhanced volley.

The average enhancement of fractional conductance was 119.6% ± 11.7% (±SEM; $n$ = 17; range, 63–233%). This potentiation, which occurred in every experiment, is somewhat larger than that obtained in voltage clamp with a more restricted sample. Initial values for $r'$ were from 0.04 to 0.45, and they were not correlated with the magnitude of the LTP. Similarly, there was no relation between the latter and the conditioning intensity, which varied from 1 to 10 times the test strength.

**Loci of Involved Sites.** A major advantage of the M cell system is that the inhibitory volley can be recorded extracellularly close to the terminals as the extrinsic hyperpolarizing potential (19). It is also observed with intracellular recordings as an early positivity, whose amplitude and time course parallel those of the extrinsic hyperpolarizing potential. Thus, measurements of the volley allowed us to distinguish between an increased excitation of inhibitory cells and changes at the inhibitory synapses (see Fig. 1).

*Potentiated interneuronal excitation.* Since the M cell $Cl^-$ equilibrium potential is near the resting level, the IPSP following the volley is small (Fig. 3 $C_1$ and $C_2$) or not apparent (Fig. 4 *Upper*) when recording with KOAc electrodes. In this optimal condition for measuring the volley, it increased during LTP in 10 of 13 experiments (83% ± 15.6%, mean ± SEM), while $r'$ was augmented by 155% ± 31%. Such is the case in Fig. 4, where the volley and the depolarizing IPSP went up.

Such data suggested a facilitated transmission at excitatory synapses between primary afferents and the commissural interneurons. This was confirmed by extracellular recordings of the typical eighth nerve-evoked field potential (20) in the ipsilateral vestibular nucleus. The early and late postsynaptic components of this field showed LTP ($n$ = 4) for the duration of the experiments.

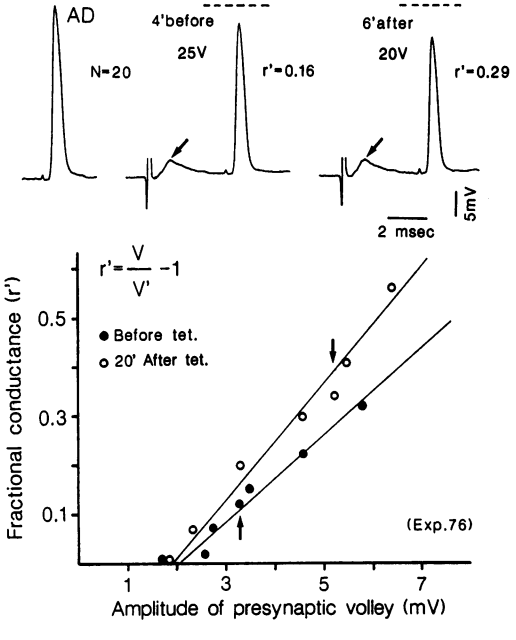


FIG. 4. LTP at the level of inhibitory synapses. (*Upper*) Evidence for an increased synaptic gain. From left to right, control antidromic spike (AD) and its shunt before and after conditioning, with the test stimulus being reduced from 25 to 20 V, in order to match the presynaptic volleys (arrows). The fractional conductance ($r'$) was nevertheless increased by 81%. (*Lower*) Plots of $r'$ (calculated 36 min after onset of trains with the indicated formula) versus the peak amplitude of the corresponding afferent volley obtained by varying stimulus strength. The arrows designate data points produced by the same control test stimulus (25 V), its displacement up and to the right illustrating the degree of network involvement. The slopes of the linear regression lines were 0.09 (●, $r^2$ = 0.95) and 0.12 (○, $r^2$ = 0.98)—that is, synaptic efficacy was enhanced by 33% (KOAc electrode; trains of 20 pulses at 500 Hz, 1 per 2 sec, for 90 sec with an intensity 1.2 times that of the test).

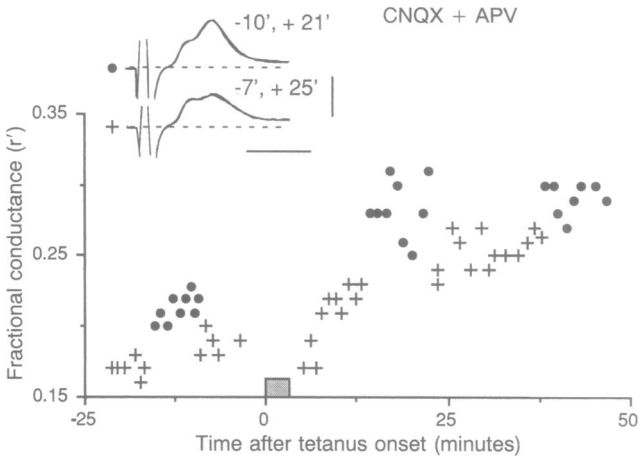


FIG. 5. Isolation of LTP to the inhibitory synapse. Plot of fractional conductance ($r'$) versus time, from a preparation treated with 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and D,L-2-amino-5-phosphonovaleric acid (APV). Two test strengths were used for eighth nerve stimulations (+, ●), and their responses were both potentiated (conditioning trains of 15 pulses at 500 Hz every 4.5 sec, for 2½ min; intensity = higher test strength). (*Inset*) Stability of presynaptic volleys recorded intracellularly. Superimposed averaged responses obtained before ($n$ = 10) and during ($n$ = 10) LTP, at the indicated times. For the stronger (above) and lower (below) intensities, volleys remained unaffected. Calibrations: 5 mV, 1 msec.

*LTP at inhibitory synapses.* In the three experiments above in which the presynaptic volley did not change, there was nevertheless an elevated shunt, of 52%, 80%, and 150%. Therefore, in another six experiments we compared the input–output relations of the inhibitory synapses by varying stimulus intensity and plotting $r'$ against the volley amplitude. This procedure is exemplified by Fig. 4, where LTP increased both parameters. Yet, when they match that of the control (Fig. 4 *Upper*), $r'$ still was enhanced significantly. The steeper $r'$–volley plot (Fig. 4 *Lower*) indicated a greater overall synaptic efficacy. Such shifts were found in four of these experiments, during periods when LTP was stabilized—i.e., 10, 20, 30, and 36 min after its induction; the corresponding maximum increases in gain were 108%, 63%, 97%, and 33%.

Transmission at the first-order synapses is mixed—i.e., electrotonic and chemical—the first postsynaptic potential alone being sufficient to fire the inhibitory interneurons (20, 21). To block potentiation at this level, 6-cyano-7-nitroquinoxaline-2,3-dione and D,L-2-amino-5-phosphonovaleric acid were both applied throughout in five experiments. Their effectiveness was demonstrated by the absence of changes in the volleys after tetanization. Yet, as shown in Fig. 5, there still was an inhibitory LTP, which in this case was stable from 20 min on, the enhancement averaging then 42% ($n$ = 13) and 37% ($n$ = 8) for low and high test intensities, respectively. Treating these two strengths separately, the mean potentiation at 15–50 min after conditioning was 41.2% ± 2.8% (± SEM, $n$ = 6), with a range of 36–57%.

## DISCUSSION

The augmented volley reflects potentiation of primary excitatory afferent synapses, with more interneurons being fired by a fixed stimulus. Given the restricted terminal field of these cells (12), and the electrical properties of the extracellular space (19, 21), the extrinsic hyperpolarizing potential and its intracellular correlate reliably signal their activation,

although a presynaptic increase in $Ca^{2+}$ current cannot be eliminated. But the most interesting finding of this work concerns the persistent facilitation of the inhibitory connections themselves. This effect occurred in the majority of the experiments designed to detect it.

**Involvement of Inhibitory Connections.** The magnitude of LTP at inhibitory junctions in the absence of treatment appears greater (75% average increased gain, $n = 4$) than with excitatory amino acid blockers (41%), suggesting there may normally be two components to this potentiation. One may involve glycine interactions with glutamate receptors, including those for *N*-methyl-D-aspartate, which are present on the M cell (22), as is the case in cortex (23). This notion is favored by the observation that conditioning trains are followed by a slowly (200–300 msec) decaying depolarizing tail (only a fraction being $Cl^-$ dependent) about 3 mV in magnitude. This tail was reduced by the blockers (unpublished observations with M. Titmus).

Evidence that changes in postsynaptic calcium may be involved in the inhibitory LTP was obtained in a separate series by injecting the calcium chelator 1,2-bis(*o*-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid in the M cell ($n = 5$). In every case, the volley increased but the input–output curves were not displaced (potentiation being then restricted to the first relay). This suggests induction of LTP requires a postsynaptic elevation in $Ca^{2+}$, either by an influx or internal release. Both may be consequences of a glycine interaction with the M cell membrane similar to that of γ-aminobutyric acid with cerebellar granule cells (24). Increased $Ca^{2+}$ is presumably not related to $Cl^-$ movements, given the small driving force in the KOAc experiments. Alternatively, there could be a modulation of a voltage-dependent $Ca^{2+}$ channel not yet found in the M cell. In any case, enhancement of M cell glycinergic responses mediated by cAMP (15) is one possible intracellular pathway (see also ref. 25).

As with classical LTP, that of inhibition is synapse specific, and it can be expressed when a few cells are active. This conclusion was reached by assessing the number of interneurons fired, by the test stimulus, since the typical cell produces an $r'$ of about 0.2 (17, 26, 27). In nine KOAc experiments in which conditioning and test strengths were comparable, LTP resulted from the coactivation of only 2–12 interneurons (mean ± SEM = 6.6 ± 1), and in five of these cases a component of the facilitation was at the inhibitory synapses.

**Implications of a Two-Stage Process.** LTP at terminal synapses will reinforce inhibition when the parent interneurons are excited by any of the numerous convergent inputs (21), even if the latter were not involved in its induction. Upstream, LTP of excitatory synapses onto these cells may be important for selectively enhancing feed-forward inhibition, which is a common feature of central nervous system networks. For example, in a previous study of ipsilateral M cell excitatory LTP, the tetanizing strength had to be above threshold (T) for orthodromic activation of that neuron (10). In contrast, the contralateral inhibition was potentiated by weaker stimuli, ranging from 0.5 to 0.9 T ($n = 10$). This preferential process, which has been predicted for the hippocampus as well (28), is in part the consequence of the network design, with any weak input being more potent when activating high-resistance interneurons (13).

Thus, inhibition not only regulates the plasticity of excitation—for instance, by setting the threshold for its modification (29)—it plays an active role in the maintenance of associative learning (30), and its prolonged reinforcement at either stage may underlie habituation of some behavioral responses, such as that of the M cell-mediated escape response.

We thank N. Ankri for help with computer programing. This work was supported by Grant 90–069 from the Direction des Researches, Etudes et Techniques.

1. Misgeld, U., Sarvey, J. M. & Klee, M. R. (1979) *Exp. Brain Res.* **37**, 217–229.
2. Abraham, W. C., Gustafsson, B. & Wigström, H. (1987) *J. Physiol. (London)* **394**, 367–380.
3. Chavez-Noriega, L. E., Bliss, T. V. P. & Halliwell, J. V. (1989) *Neurosci. Lett.* **104**, 58–64.
4. Yamamoto, C. & Chujo, T. (1978) *Exp. Neurol.* **58**, 242–250.
5. Haas, H. L. & Rose, G. (1982) *J. Physiol. (London)* **329**, 541–552.
6. Scharfman, H. E. & Sarvey, J. M. (1985) *Neuroscience* **15**, 695–702.
7. Stelzer, A., Slater, N. T. & ten Bruggencate, G. (1987) *Nature (London)* **326**, 698–701.
8. Maru, E., Ashida, H. & Tatsuno, J. (1989) *Brain Res.* **478**, 112–120.
9. Griffith, W. H., Brown, T. H. & Johnston, D. (1986) *J. Neurophysiol.* **55**, 767–775.
10. Yang, X.-D., Korn, H. & Faber, D. S. (1990) *Nature (London)* **348**, 542–545.
11. Korn, H., Faber, D. S. & Triller, A. (1990) in *Handbook of Chemical Neuroanatomy, Vol. 8: Analysis of Neuronal Microcircuits & Synaptic Integration*, eds. Björklund, A. & Hökfelt, T. (Elsevier, Amsterdam), pp. 403–480.
12. Triller, A. & Korn, H. (1986) *J. Comp. Neurol.* **253**, 500–513.
13. Faber, D. S. & Korn, H. (1988) *J. Neurophysiol.* **60**, 1982–1999.
14. Mintz, I., Gotow, T., Triller, A. & Korn, H. (1989) *Science* **245**, 190–192.
15. Wolszon, L. R. & Faber, D. S. (1989) *J. Neurosci.* **9**, 784–797.
16. Faber, D. S. & Korn, H. (1987) *J. Neurosci.* **7**, 807–811.
17. Faber, D. S. & Korn, H. (1982) *J. Neurophysiol.* **48**, 654–678.
18. Korn, H. & Faber, D. S. (1976) *Science* **194**, 1166–1169.
19. Furukawa, T. & Furshpan, E. J. (1963) *J. Neurophysiol.* **26**, 140–176.
20. Korn, H., Sotelo, C. & Bennett, M. V. L. (1977) *Neuroscience* **2**, 851–884.
21. Faber, D. S. & Korn, H. (1978) in *Neurobiology of the Mauthner Cell* (Raven, New York), 271–279.
22. Wolszon, L. R. & Faber, D. S. (1988) *Soc. Neurosci. Abstr.* **14**, 939.
23. Thomson, A. M. (1990) *Neuroscience* **39**, 69–79.
24. Connor, J. A., Tseng, H.-Y. & Hockberger, P. E. (1987) *J. Neurosci.* **7**, 1384–1400.
25. Song, Y. & Huang, L.-Y. M. (1990) *Nature (London)* **348**, 242–245.
26. Korn, H., Mallet, A., Triller, A. & Faber, D. S. (1982) *J. Neurophysiol.* **48**, 679–707.
27. Faber, D. S. & Korn, H. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 8708–8712.
28. Buzsaki, G. & Eidelberg, E. (1982) *J. Neurophysiol.* **48**, 597–607.
29. Marr, D. (1971) *Philos. R. Soc. London Ser. B.* **262**, 23–81.
30. Mamounas, L. A., Thompson, R. F. & Madden J., IV (1987) *Proc. Natl. Acad. Sci. USA* **84**, 2101–2105.