Granular Cells of the Mormyrid Electrosensory Lobe and Postsynaptic Control Over Presynaptic Spike Occurrence and Amplitude Through an Electrical Synapse

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Submitted 1 December 2006; accepted in final form 9 January 2007

Zhang J, Han VZ, Meek J, Bell CC. Granular cells of the mormyrid electrosensory lobe and postsynaptic control over presynaptic spike occurrence and amplitude through an electrical synapse. J Neurophysiol 97: 2191–2203, 2007. First published January 17, 2007; doi:10.1152/jn.01262.2006. Primary afferent fibers from the electroreceptors of mormyrid electric fish use a latency code to signal the intensity of electrical current evoked by the fish’s own electric organ discharge (EOD). The afferent fibers terminate centrally in the deep and superficial granular layers of the electrosensory lobe with morphologically mixed chemical–electrical synapses. The granular cells in these layers seem to decode afferent latency through an interaction between primary afferent input and a corollary discharge input associated with the EOD motor command. We studied the physiology of deep and superficial granular cells in a slice preparation with whole cell patch recording and electrical stimulation of afferent fibers. Afferent stimulation evoked large all-or-none electrical excitatory postsynaptic potentials (EPSPs) and large or none GABAergic inhibitory postsynaptic potentials (IPSPs) in both superficial and deep granular cells. The amplitudes of the electrical EPSPs depended on postsynaptic membrane potential, with maximum conductances in its near environment by measuring small inhibitory postsynaptic potentials (IPSPs) from electroreceptors at a greater distance, and EPSPs of central origin driven by the motor command that drives the electric organ discharge [electric organ corollary discharge (EOCD)]. The synaptic potentials recorded inside primary afferents are considered to reflect synaptic inputs to granular cells that are observed inside the presynaptic primary afferents through electrical synapses. Electron microscopy shows that the primary afferents contact granular cells with mixed chemical-electrical synapses (Bell et al. 1989).

The electrical current generated by the fish’s electric organ discharge (EOD) passes through the skin. The fish senses the conductances in its near environment by measuring small variations in the pattern of EOD-induced current flow. In mormyrids, the electroreceptors in the skin that measure the pattern of current flow are known as mormyromasts. The afferent fibers from mormyromast electroreceptors encode the intensity of local EOD current flow by the latency of the first spike in the EOD evoked afferent burst (Bell 1990b; Sawtell et al. 2006; Szabo and Hagiwara 1967). Behavioral experiments show that the fish measures post-EOD afferent latency by comparing the timing of the afferent spike with that of a centrally originating EOCD signal (Hall et al. 1995). The EOCD-evoked EPSP considered to be an input to granular cells shows minimal variation and is hypothesized to be the centrally originating timing signal. Under this hypothesis, the granular cells transform the relative timing of afferent and EOCD inputs into a nontemporal code such as the number of spikes in a burst. One goal of this study was to determine if the cellular properties of ELL granular cells are consistent with such measurement of relative timing. Such cellular properties might include the following: fast rise times of the EPSPs involved; and higher-order ELL cells (Bell and Grant 1992; Bell et al. 1997; Mohr et al. 2003), almost nothing is known about the granular cells that transfer information between primary afferents and higher-order cells.

Some inferences can be made, however, about the inputs to ELL granular cells. Intra-axonal recordings from primary afferent fibers near their central terminals reveal synaptic potentials in addition to axon spikes from a peripheral electroreceptor (Bell 1990a). These synaptic potentials include excitatory postsynaptic potentials (EPSPs) from electroreceptors close to the receptor of origin, inhibitory postsynaptic potentials (IPSPs) from electroreceptors at a greater distance, and EPSPs of central origin driven by the motor command that drives the electric organ discharge [electric organ corollary discharge (EOCD)]. The synaptic potentials recorded inside primary afferents are considered to reflect synaptic inputs to granular cells that are observed inside the presynaptic primary afferents through electrical synapses. Electron microscopy shows that the primary afferents contact granular cells with mixed chemical-electrical synapses (Bell et al. 1989).

INTRODUCTION

This study examines the first central synapses of the electrosensory system in electric fish of the family Mormyridae. Primary afferent fibers from electroreceptors in the skin terminate in the deep and superficial granular layers of the electrosensory lobe (ELL). The small granular cells of the two sublayers are distinct in their morphology and immunocytochemistry (Bell et al. 2005). The granular cells of both sublayers relay electrosensory information to Purkinje-like cells and other higher order cells of ELL. Although much is known about the physiology of both afferent fibers (Bell 1990b; Bennett 1965; Sawtell et al. 2006; Szabo and Hagiwara 1967) and higher-order ELL cells (Bell and Grant 1992; Bell et al. 1997; Mohr et al. 2003), almost nothing is known about the granular cells that transfer information between primary afferents and higher-order cells. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
nonlinear interactions among synaptic inputs; a capacity to
generate spike bursts; and minimal convergence. Minimal
convergence would be important to maintain good spatial
resolution and because the capacity to measure afferent latency
precisely would be reduced if a large number of afferents with
different latencies converged onto the same cell. Some of these
features were indeed observed in our recordings from ELL
granular cells.

ELL granular cells from both the deep and superficial
granular layers were recorded with the whole cell patch tech-
nique in vitro slices. Electrical activation of primary affer-
ents evoked electrical EPSPs and chemical IPSPs as expected
from previous morphological studies and intra-axonal record-
nings from primary afferents. Surprisingly, the amplitude and
even the occurrence of the electrical EPSPs depended on the
membrane potential of the granular cells. We suggest that this
dependency on the postsynaptic membrane potential is caused
by tight coupling of the potential in the postsynaptic cell and
the potential in the presynaptic terminal through a high con-
ductance electrical synapse.

METHODS

General

We recorded ELL granular cells from in vitro slices of mormyrid
fish of the species Gnathonemus petersii. A total of 60 fish were used.
The fish were wild caught and obtained from fish importers so their
ages could not be determined. We examined responses to stimulation
of primary afferent fibers from electrorceptors and responses to
intracellular current injection. Recorded cells were injected with
biocytin for morphological identification. Local extracellular injec-
tions of biocytin were also made in some slices to examine granular
fiber morphology. Finally, dye coupling between primary afferent
fibers and granular cells was examined by placing neurobiotin (Vec-
tor) on the cut end of an electrosensory nerve and allowing the fish to
survive for 1 wk before being perfused.

Slice preparation

The fish were deeply anesthetized with cold tricaine methane
sulfonate (MS 222) at a concentration of 100 mg/L. The skull was
opened, and the brain was irrigated with ice-cold artificial cerebro-
spinal fluid (ACSF). A vertical cut was made in the transverse plane
immediately rostral to the ELL, and the spinal cord was sectioned
immediately caudal to the ELL. The block of brain containing the
ELL was removed and transferred to ice-cold ACSF for 60 s to harden
it a little. The rostral cut surface of the brain was glued to a microtome
blade. A U-shaped wall of gelatin (12.5%) was glued
facing the blade. A 100 m diameter) or with bipolar tungsten
electrodes. Electrodes were placed 20–200 μm away from the re-
corded cell in the intermediate layer where the afferents enter ELL
the dorsolateral zone. Recordings were made from the small cells of
the deep and superficial granular layers. PClamp 8 software (Axon
Instruments, Foster City, CA) was used for recording and analyzing
the data. The Student’s t-test was used to compare measurements
made in deep and superficial granular cells. Statistics are given as the
mean ± SE.

The electrodes used for whole cell patch recording had resistances
of 10–15 MΩ after filling with an integral solution that contained 1%
biocytin in the pipette tip. The composition of the internal solution was as
follows (in mM): 122 K gluconate, 5 Na2ATP, 2.5 MgCl2, 0.0003
CaCl2, 5.6 Mg-glucuronate, 5 K-HEPES, 5 H-HEPES, and KOH for
compensation of pH (pH, 7.2–7.4; mOsm, 275 ± 5). The calculated
liquid junction potential was 10 mV; this was compensated for using
the pipette offset feature on the amplifier. Cells were visualized under
infrared Nomarski optics with a ×40 water immersion objective.

The recordings were performed under both voltage- and current-clamp
modes using a Multiclamp 700A amplifier (Axon Instruments). Elec-

trode capacity compensation in current clamp was done automatically
by the amplifier. Cells were usually held at −70 mV with hyperpo-
larizing current (−10 pa) during current-clamp recording unless
otherwise stated, and the holding potential under voltage clamp was
also −70 mV.

Primary afferent fibers were stimulated electrically with a micropi-
pette filled with ACSF (10 μm diameter) or with bipolar tungsten
electrodes. Electrodes were placed 20–200 μm away from the re-
corded cell in the intermediate layer where the afferents enter ELL
cortex. Current pulses were 0.1 ms in duration and ranged between 0.1
and 1.0 mA.

Biocytin was injected into the cells by inontophoresis after studying
them electrophysiologically (tip positive current pulses 400 ms on and
600 ms off, 0.3–0.5 nA for 5–10 min). Slices were fixed in 4%
paraformaldehyde in 0.1 M phosphate buffer within 20 min after
the injection of biocytin. Slices were kept in the same fixative overnight.

The biocytin injected into cells was revealed by incubating the
slices with streptavidin conjugated to Texas Red for 4–6 h (543 nm,
Molecular Probes, 1:200 in 0.1 M phosphate buffer). A green fluo-
rescent Nissl stain was used to reveal cell layers (488 nm, Molecular
Probes). After incubation, the slices were washed in phosphate buffer
and mounted on slides using fluorescent mounting medium Vecta-
shield (Vector Laboratories, Burlingame, CA).

Pharmacology

The following pharmacological agents were used: the glutamate
AMPA receptor antagonist, 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-ben-
zo[f]quinoxaline-7-sulfonamide (NBQX, 10 μM); the glutamate N-
methyl-D-aspartate (NMDA) receptor antagonist, d(-)-2-amino-5-
phosphonopentanoic acid (AP-5, 35 μM); the GABA_A receptor
antagonist, bicuculline (35 μM); the sodium channel antagonist, TTX (3

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**Extracellular biocytin injections**

Biocytin injections were made iontophoretically under direct visual control into the granular layer of 400-μm-thick in vitro slices to determine the morphology of superficial granular cells. Electrodes were filled with 2% biocytin in 0.5 M NaCl, and small deposits were made by passing 1-μA, 30-ms pulses at 3 Hz for 5 min with the electrode tip positive. After fixations in a mixture of 0.5% glutaraldehyde and 4% paraformaldehyde in 0.1 M phosphate buffer, the slices were resectioned at 40 μm on a Vibratome. The biocytin was revealed with the DAB method as described elsewhere (Meek et al. 2001). Labeled granular cells near the site of injection were drawn with the aid of a camera lucida.

**Dye coupling**

Dye coupling between primary afferents and granular cells was shown in two fish. The fish were anesthetized (MS-222, 1:25,000), and the dorsal branch of the posterior lateral line nerve, an electrosensory nerve (Szabo and Hagiwara 1967), was exposed on both sides. Neurobiotin (Vector) was placed on the cut end of the nerve on one side and biotinylated dextran amine (BDA, Molecular Probes, 3,000 MW) was placed on the cut end of the nerve on the other side. Neurobiotin is a small molecule that is known to pass through gap junctions, but BDA is a large molecule that does not. The fish survived for 1 week before they were reanesthetized with cold tricaine methane sulfonate at a concentration of 100 mg/l and perfused. One fish was perfused with 4% paraformaldehyde in 0.1 M phosphate buffer, and Neurobiotin was revealed using the DAB method as described previously (Meek et al. 2001).

**Confocal Imaging**

We used a confocal microscope to examine intracellularly filled cells and dye coupling after labeling with fluorescent markers (Zeiss, LSM-510 META). Reconstruction from serial optical sections was done with the Zeiss VISART software.
RESULTS

Morphology of ELL granular cells

Primary afferent fibers from mormyromast electrorceptors terminate in the deep and superficial granular layers of the medial and dorsolateral zones of ELL (Fig. 1, A–C). The superficial granular layer is thinner than the deep granular layer and contains cells that are smaller (3–5 μm) and more densely packed than those in the deep granular layer (5–7 μm).

Cells in the two granular layers are morphologically different and have different immunocytochemistry. Antibodies to calretinin and calbindin stain cells of the deep granular layer strongly but do not stain cells of the superficial granular layer (Bell et al. 2005). No antibody has been found that stains the superficial granular cells selectively, and our morphological description is based mainly on local extracellular injections in slices maintained in vitro that were made as part of this paper and as described in METHODS (Fig. 2A). Superficial granular layer cells have basilar dendrites that descend into the deep granular layer and may branch quite extensively. The axons ascend and may branch as they do so. The axons of superficial granular cells often pass through the ganglion layer to end in the lower molecular layer.

Deep granular cells are selectively stained with antibodies to calbindin and calretinin. The drawings of Fig. 2C are taken from sections stained with an antibody to calbindin (adapted from Fig. 8B of Bell et al. 2005). Such staining shows that the deep granular cells have round cell bodies and two to four short, unbranched dendrites of approximately equal length extending out in all directions from the cell body. The dendrites end in claws that are similar to the claws at the ends of cerebellar granule cell dendrites. The axons of deep granular cells ascend, passing through the superficial granular layer to terminate with tight clumps of synaptic swellings in the plexiform and ganglion layers of ELL. The axons do not branch in traversing the granular layers and do not cross the ganglion layer into the molecular layer. The axons of deep granular cells are extremely thin as they leave the soma and become abruptly thicker at 10–20 μm from the soma. This thin initial segment was not present in the axons of superficial granular cells (see Physiology of deep granular cells for how this morphological difference may explain a difference in spike size for the 2 types of granular cells).

The morphology of the filled cells was sufficiently clear to establish that the recorded cell was a superficial or deep...
granular cell based on branching axons, branching dendrites, thin initial axons, or axonal termination in the molecular layer (Fig. 2, B and D). Determination of the layer in which such cells were located provided a confirmatory indication of their identity.

Dye coupling

Both terminals and superficial granular cells showed clear and strong labeling on the side of ELL in which the neurobiotin-filled nerve terminated (Fig. 3A). In contrast, only the terminals of primary afferents, and not the postsynaptic cells, were labeled on the side of ELL in which the BDA-filled nerve terminated (data not shown).

Confocal imaging showed that the postsynaptic cells labeled with neurobiotin on one side of ELL were contacted on their somas by even more strongly labeled primary afferent fibers (Fig. 3B). Such dye coupling between pre- and postsynaptic elements is strongly correlated with the presence of electrical synapses. Dye coupling was much less marked in the deep granular layer, where only a few cells were lightly labeled. Interestingly, cells of the nucleus of ELL that receive primary afferent input from knollenorgan electroreceptors were not labeled, even though electron microscopy shows that these synapses have gap junctions (Szabo et al. 1983), and physiology shows that the synapses are electrical (Bell and Grant 1989). Although the lack of postsynaptic labeling in cells of the nucleus of ELL could be due to diffusion of the label into a somewhat large cell body, differences in gap junctional proteins might also account for these differences in dye coupling at different electrical synapses.

Physiology of superficial granular cells

Properties of superficial granular cells and electrical EPSPs. Nineteen of the recorded cells were identified as superficial granular cells based on the morphology of the biocytoin-labeled cells. An additional 30 cells were recorded that had physiological properties similar to the morphologically identified cells. Resting membrane potentials ranged from –37 to –58 mV (–43.3 ± 1.7 mV, n = 18). Input impedances were between 1 and 3 GΩ (1.9 ± 0.1 GΩ; n = 22). (We usually maintained cells at –70 mV with hyperpolarizing current and did not always remove the current to measure the cell’s resting membrane potential. Nor did we measure every cell’s input impedance. The above numbers include all of the cells for which resting potential and input impedance measurements were made.)

Afferent stimulation evoked large, all or none responses with short latencies and fast rise times (Fig. 4A), which were taken to be electrical EPSPs. The EPSP amplitudes, measured at –70 mV, were between 5 and 30 mV (19 ± 1.7 mV, n = 27). The latencies were 0.2–1.0 ms (0.47 ± 0.04 ms, n = 27), and the rise times (10–90%) were 0.3–2.0 ms (0.8 ± 0.06 ms, n = 27). Half widths ranged between 28 and 88 ms (41.3 ± 4.8 ms, n = 27). The currents underlying the responses (Fig. 4E), as recorded in voltage clamp, had durations of 1–3 ms (1.5 ± 0.1 ms, n = 10) and amplitudes of 0.2–0.8 nA (0.35 ± 0.07 nA, n = 10). The EPSPs could follow repetitive stimulation to 333/s, the highest frequency tested in superficial granular cells (Fig. 4G).

Our stimulus electrodes were between 20 and 200 μm from the recorded cell, and we wished to show that the large all or none responses were indeed EPSPs caused by activation of presynaptic fibers and not the result of direct activation of all-or-none responses in the dendrites, axons, or somas of the recorded cell by the extracellular stimulus. We eliminated the latter possibility by comparing the EPSPs with the responses to a graded series of brief (1 ms) current injected into the same cells as the ones from which EPSPs were recorded. The responses to brief intracellular currents were similar in waveform, with the same long time constant as the responses to extracellular stimuli, but these responses were smoothly graded with increasing intensity and never included a large all or none response like the EPSP. Figure 4B shows the responses to current pulse recorded in the same cell as the EPSPs in Fig. 4A. We conclude that the large all-or-none responses evoked by afferent stimulation are EPSPs. The EPSPs and the more intense current pulses could evoke slow, graded, subthreshold, regenerative responses (arrows in Fig. 4, A and B) and spikes.

In most cases, only a single all-or-none response was evoked as stimulus strength was gradually increased (Fig. 4A), but in five cells, a gradual increase in stimulus intensity evoked two to four discrete levels of response as shown for voltage re-

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sponses in Fig. 4C and current responses in Fig. 4E. Responses for the cells shown in Fig. 4, C and E, are shown plotted against stimulus intensity as stimulus intensity was gradually increased in Fig. 4, D and F, respectively. Note the step-like changes in EPSP amplitude as stimulus intensity was increased. The size of the shock artifact was used as an indicator of stimulus intensity for these plots, because intensity was changed in a smoothly graded manner during the procedure and was not measured directly. The finding that gradual increases in extracellular stimulus strength sometimes resulted in a series of two to four step-like responses is consistent with the convergence of a few different afferent fibers onto a single cell. The finding of a series of step-like responses also provides an additional indication that the responses are EPSPs rather than voltage responses of the recorded cell caused by direct activation by the extracellular stimulus, because a series of step-like voltage responses of the postsynaptic cell is unlikely and was not observed with current injection.

The short latencies, fast rise times, and high following frequency indicate that the EPSPs were electrically rather than chemically mediated. Pharmacological tests were also consistent with an electrical rather than a chemical EPSP. The gap junction antagonist, carbenoxolone (200 μM), reduced the responses by an average of 50% (n = 5; Fig. 5A and B), whereas blocking chemical transmission with the calcium channel blocker, cadmium (200 μM; n = 5; Fig. 5C), or with a combination of NMDA (APV, 35 μM) and AMPA (NBQX, 10 μM) glutamate receptor antagonists had no discernible effect on the EPSPs or EPSCs (n = 5; Fig. 5D). As expected, the sodium channel antagonist, TTX, blocked the response completely (Fig. 5E). We conclude that the large all or none responses evoked by afferent stimulation are electrical EPSPs.

POSTSYNAPTIC CONTROL OVER THE EFFICACY OF ELECTRICAL SYMPHAPSES IN SUPERFICIAL GRANULAR CELLS The large electrical EPSPs indicate strong electrical coupling between the presynaptic fiber and the postsynaptic cell. Assuming a presynaptic spike of 100 mV, a 30 mV EPSP yields a large coupling coefficient of 0.3 (Bennett and Zukin 2004). Strong

**FIG. 4.** Primary afferent stimulation evokes electrical excitatory postsynaptic potentials (EPSPs) and currents (EPSCs) in superficial granular cells. A: all-or-none electrical EPSP. Responses to an extracellular stimulus that was gradually increased from below threshold to above threshold for the EPSP. EPSP sometimes elicited low-amplitude voltage responses (arrow). Spikes can arise from EPSPs or low-amplitude voltage responses. B: responses to gradually increasing intracellular current pulses. Pulses were 1 ms in duration. More intense current pulses evoked low-amplitude voltage responses (arrow) or spikes. Same cell as in A. C: step-like changes in EPSP amplitudes to a gradually increasing extracellular stimulus. Highest level of response evoked a burst of 3 spikes. Different cell from that shown in A. D: plot of EPSP amplitudes against stimulus intensity. Same cell as in C. EPSP was measured at delay indicated by dotted line shown in C. Size of artifact was used as an indicator of stimulus intensity. E: step-like changes in EPSP amplitude. Extracellular stimulus was gradually increased from below threshold. Initial all-or-none response was followed by a 2nd, smaller step-like change in current. F: plot of EPSP amplitude against stimulus intensity. Same cell as in E. EPSP was measured at delay indicated by dotted line in E. Size of artifact was used as an indicator of stimulus intensity. G: electrical EPSP follows high-frequency extracellular stimulation. Note that amplitudes of electrical EPSPs decrease when they occur on top of depolarizations caused by previous EPSPs.

**FIG. 5.** Pharmacology of electrical EPSPs and EPSCs evoked by primary afferent stimulation in superficial granular cells. A: reduction of electrical EPSP by gap junction antagonist carbenoxolone (200 μM). EPSP in the presence of carbenoxolone was measured 15 min after start of drug application. B: reduction of electrical EPSP by gap junction antagonist carbenoxolone (200 μM). Different cell from that shown in A. EPSP in the presence of carbenoxolone was measured 15 min after start of drug application. C: lack of effect of calcium channel blocker, cadmium (200 μM), on electrical EPSP. EPSP in presence of cadmium was measured 15 min after start of drug application. D: lack of effect of glutamate receptor antagonists, APV (35 μM) and NBQX (10 μM), on electrical EPSP. EPSPs in the presence of glutamate blockers was measured 15 min after start of drug application. E: suppression of electrical EPSP by sodium channel antagonist TTX (3 μM). EPSP was blocked by TTX within 3 min.
coupling in the orthodromic direction suggests the possibility of coupling in the antidromic direction, from postsynaptic cell to presynaptic fiber, as indicated also by the presence of excitatory postsynaptic potentials as large as 5 mV in intra-axonal recordings from primary afferent fibers near their central terminals in vivo (Bell 1990a). The synaptic potentials are thought to reflect synaptic inputs to postsynaptic granular cells that are observed inside the presynaptic primary afferents through electrical synapses. We tested such antidromic effects further by examining the influence of postsynaptic membrane potential on the electrical EPSP.

We found that the membrane potential of the recorded cell determined the amplitude and even the occurrence of the electrical EPSP (Fig. 6). The EPSP was absent at membrane potentials more depolarized than a certain level, the precise level ranging between −55 and −40 mV for different cells. The EPSP increased in an approximately linear manner with hyperpolarization of the membrane above this level (region indicated by black brackets in Fig. 6, B, D, and F), reaching a maximum at membrane potentials between −65 and −110 mV (−84 ± 6 mV, n = 25). In most cells (19/25), additional hyperpolarization, beyond the membrane potential yielding a maximum, resulted in the abrupt disappearance of the electrical EPSP (Fig. 6, A and B). In other cells (2/25), the additional hyperpolarization resulted in an abrupt decrease in EPSP amplitude, and further hyperpolarization caused an abrupt disappearance of the smaller EPSP (Fig. 6, C and D). Finally, in a few cells (4/25), the EPSP became progressively smaller, in an approximately linear manner, as the membrane was hyperpolarized further beyond the level yielding a maximum (Fig. 6, E and F).

At membrane potentials more hyperpolarized than the potential yielding a maximum (regions indicated by red brackets in Fig. 6, B, D, and F), depolarization by another synaptic input can be hypothesized to cause either a sharp increase from no response to a maximal EPSP, a step increase in EPSP amplitude, or a graded increase in EPSP size, depending on the fiber. The last possibility is shown in Fig. 6G for an EPSP similar to the one plotted in Fig. 6F. The membrane potential for this cell was held at a potential slightly more hyperpolarized than the potential at which the EPSP was maximal. An electrical EPSP was evoked at different delays after the onset of an artificial EPSP induced by current injection. Note that the amplitude of the electrical EPSP was larger when it occurred at the peak of the artificial EPSP (electrical EPSP amplitudes indicated by blue vertical lines in Fig. 6G). In addition, the larger EPSP plus the underlying artificial EPSP evoked spikes.

We hypothesize that the effect of postsynaptic membrane potential on EPSP size is caused by close linkage of the membrane potentials of the postsynaptic cell and the presynaptic terminal through a high conductance gap junction. Postsynaptically induced changes in presynaptic membrane potential will in turn affect the amplitude and occurrence of the presynaptic spike that generates the electrical EPSP (see Discussion).

ALL-OR-NONE IPSPS IN SUPERFICIAL GRANULAR CELLS Stimulation in the region of the afferents often evoked large all-or-none IPSPs (Fig. 7A). We assume that the IPSPs were sometimes evoked disynaptically through activation of afferents and...
around – 80 mV. Different cell from that shown in membrane potential on IPSP amplitude. IPSP reversed at membrane potential was observed within 20 min after removing the bicuculline in GRANULAR CELLS. Current injection evoked spikes with amplitudes between 10 and 40 mV (29.1 mV; n = 7) and latencies were between 0.6 and 1.2 ms (0.9 ± 0.2 ms, n = 8).

Sometimes monosynaptically through direct stimulation of inhibitory cells (Fig. 1C). The IPSPs had latencies of 0.8–2.7 ms (1.2 ± 0.05 ms, n = 10) and reversed at membrane potentials between –50 and –85 mV (–66 ± 1.5 mV, n = 7; Fig. 7, C and D). We cannot fully explain the wide range of IPSP reversal potentials in our recordings. Factors that might have contributed to this variability include errors in membrane potential measurement and different intracellular chloride concentrations. Small variations in chloride leakage from outside the cell or in the chloride concentration in the recording electrodes could have marked effects on chloride concentration in these small cells. The IPSPs were blocked by bicuculline (35 μM; n = 6; Fig. 7B), indicating that they are mediated by GABA and GABA\textsubscript{A} receptors. Partial recovery of the IPSP was observed within 20 min after removing the bicuculline in three cells. As expected, the IPSPs were blocked by cadmium (200 μM; n = 4).

In seven cells, both all-or-none IPSPs and all-or-none EPSPs were recorded in the same cell. For example, the cell shown in Fig. 8, A–D, showed an electrical EPSP when stimulated at site 1 (Fig. 8, A and B) but showed an IPSP when stimulated at site 2, which in this case was about 50 μm away (Fig. 8, C and D).

Responses to current injection and I-V curves in superficial granular cells. Current injection evoked spikes with amplitudes between 10 and 40 mV (29.1 ± 3.6 mV; n = 8; Fig. 9A). The spikes usually had pronounced afterhyperpolarizations. The spikes evoked by current injection or electrical EPSPs are only a few milliseconds in duration, much briefer than the electrical EPSPs evoked by afferent stimulation. In addition, the spikes remained unchanged after carbamoxolone had reduced the electrical EPSPs by 50% or more (data not shown). Thus the spikes are occurring in the postsynaptic cell rather than in the presynaptic terminal.

I-V curves were constructed under voltage clamp at 300 ms after the onset of the voltage steps, when spike-like activity had ceased and the current trace was constant (Fig. 9, B and C). Superficial granular cells showed marked outward rectification with a sharp transition at about –40 mV (n = 8) between a region of low conductance at more hyperpolarized potentials and a region of high conductance at more depolarized levels (Fig. 9C).

**Physiology of deep granular cells**

Properties of deep granular cells and electrical EPSPs. Six of the recorded cells were identified as deep granular cells based on the morphology of the biocytin labeled cells. An additional 10 cells were recorded that had physiological properties similar to the morphologically identified cells. Resting membrane potentials ranged from –38 to –57 mV (–46.0 ± 2.1 mV, n = 7), and input impedances were between 1 and 3 GΩ (2.0 ± 0.2 GΩ; n = 8). (As with superficial granular cells, we did not measure resting membrane potential and input impedance in all cells. The above numbers represent all of the cells in which these measurements were made.)

Deep granular cells also responded to stimulation in the region of the primary afferents with all-or-none electrical EPSPs (Fig. 10A). These EPSPs, measured at a membrane potential of –70 mV, were significantly smaller in amplitude (7.9 ± 1.5 mV, n = 8) than those of the superficial granular cells (19 ± 1.7 mV, n = 27; t-test, P < 0.001) and had slower rise times (10–90%, 2.0 ± 0.3 ms, n = 8). EPSP half widths ranged between 20 and 50 ms (26.6 ± 3.3 ms, n = 8), and latencies were between 0.6 and 1.2 ms (0.9 ± 0.2 ms, n = 8).
granular cells (10 of 49 recorded cells). The activation was observed in deep granular cells (12 of 16 recorded cells) than in superficial granular cells (Fig. 11). These IPSPs were more tonic evoked large all-or-none IPSPs in deep granular cells, as in superficial granular cells. Afferent stimulation at one site evoked an IPSP and stimulation at another site evoked an EPSP.

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**FIG. 9.** Responses to current injection and I-V curves of superficial granular cells. A: voltage transients obtained in response to steps of current injected through the electrode. Single action potential is generated at lower level of depolarizing current and 3 action potentials are generated at the higher current level. Holding potential was –70 mV. B: current transients in response to steps of voltage from a holding potential of –70 mV to membrane potentials between –100 and +10 mV. C: I-V curves constructed under voltage clamp after 300 ms after onset of voltage steps. Marked outward rectification was observed with a sharp transition at about –40 mV between a region of low conductance, at more hyperpolarized potentials, and a region of high conductance at more depolarized levels. Circles show average current, and bars show SE for 8 different cells.

As with superficial granular cells, the responses could be shown to be EPSPs and not caused by direct activation of the recorded cells by noting that brief pulses of injected current never evoked all-or-none responses like the EPSPs (Fig. 10, A and B). The EPSPs in deep granular cells followed stimulus frequencies as high as 500 Hz (Fig. 10E).

**FIG. 10.** Primary afferent stimulation evokes electrical EPSPs in deep granular cells. A: all-or-none electrical EPSP. Responses to an extracellular stimulus that was gradually increased from below threshold to above threshold for EPSP. Holding potential = –80 mV. Same cell shown in A–D. B: responses to intracellular current pulses of gradually increasing amplitude. Waveforms were similar to electrical EPSP evoked by extracellular stimulation, but responses were smoothly graded in amplitude, and no all-or-none response was evoked. Pulses were 1 ms in duration. C: electrical EPSPs evoked at different membrane potentials. D: plot of electrical EPSPs as a function of amplitude. EPSP falls abruptly at membrane potentials more depolarized than –90 mV. E: electrical EPSPs follow high-frequency extracellular stimulation ≤500 Hz. Different cell from that in A–D. Note that amplitudes of electrical EPSPs decrease when they occur on top of depolarization caused by previous EPSPs. Holding potential = –70 mV.

**POSTSYNAPTIC CONTROL OVER THE EFFICACY OF ELECTRICAL SYNAPSES IN DEEP GRANULAR CELLS** Changes in membrane potential affected EPSP amplitude, as in superficial granular cells (Fig. 10, C and D). EPSP amplitudes were at a maximum at membrane potentials between –60 and –90 mV (–78.3 ± 4.3 mV, n = 6). The amplitudes decreased in a roughly linear manner with depolarization, falling to zero between –55 and –40 mV. The EPSPs fell abruptly to zero with hyperpolarization beyond the potential at which the EPSP was maximal (Fig. 10, C and D). The step-like and gradual reductions in amplitude with hyperpolarization beyond the potential at which the EPSP was maximal that were present in some superficial granular cells were not observed in any of the deep granular cells.

**ALL-OR-NONE IPSPS IN DEEP GRANULAR CELLS** Afferent stimulation evoked large all-or-none IPSPs in deep granular cells, as in superficial granular cells (Fig. 11). These IPSPs were more common in deep granular cells (12 of 16 recorded cells) than in superficial granular cells (10 of 49 recorded cells). The latencies were 0.9–1.6 ms (1.2 ± 0.1 ms, n = 12). Reversal potentials were between –40 and –75 mV (–64.6 ± 1.8, n = 8). IPSPs were blocked by bicuculline (35 μM, n = 5; Fig. 11B) and by cadmium (200 μM; n = 3). In two cells, stimulation at one site evoked an IPSP and stimulation at another site evoked an EPSP.

**FIG. 11.** Afferent stimulation evokes electrical EPSPs in deep granular cells. Afferent stimulation at one site evoked an IPSP and stimulation at another site evoked an EPSP.

**FIG. 12.** Responses to intracellular current pulses of gradually increasing amplitude. Waveforms were similar to electrical EPSP evoked by extracellular stimulation, but responses were smoothly graded in amplitude, and no all-or-none response was evoked. Pulses were 1 ms in duration. C: electrical EPSPs evoked at different membrane potentials. D: plot of electrical EPSPs as a function of amplitude. EPSP falls abruptly at membrane potentials more depolarized than –90 mV. E: electrical EPSPs follow high-frequency extracellular stimulation ≤500 Hz. Different cell from that in A–D. Note that amplitudes of electrical EPSPs decrease when they occur on top of depolarization caused by previous EPSPs. Holding potential = –70 mV.
large, all-or-none, GABAergic IPSPs that we observed in both the intermediate layer of ELL (Meek et al. 2001) (Fig. 1). The morphology of inhibitory synapses that cover one third or more of the cell body (Bell et al. 1989). A subsequent study showed that these terminals are GABAergic and arise from the den- rrites and axons of large multipolar interneurons (LMI cells) of that these terminals are GABAergic and arise from the den- 

The previous morphological study found that the granular cells on which primary afferent fibers terminate with gap junctions are also contacted by large terminals with the morphology of chemical synapses. How- ever, our physiological study found no effect of cadmium or glutamate receptor blockers on the EPSPs, suggesting that chemical transmission may not be functional at this synapse. Synapses that are morphologically mixed but that show only electrical transmission physiologically have also been identified elsewhere (Bell and Grant 1989; Bennett and Zukin 2004; Lin and Faber 1988; Szabo et al. 1983).

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DISCUSSION

Correlations with morphology

A previous morphological study showed that primary affer- ent fibers from electroreceptors terminate on the cell bodies of ELL granular cells with gap junctions (Bell et al. 1989). That paper did not distinguish between deep and superficial granular layers but did describe the gap junctions as being more prominent on smaller cells in the more superficial part of the granular layer. Such a location is consistent with our results, which showed stronger dye coupling and larger electrical EPSPs in the superficial granular cells than in the deep granular cells. The sharp rise times of the electrical EPSPs are consistent with the morphological finding of termination on the cell body.

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deep and superficial granular cells are the likely physiological correlate of the morphologically described inhibitory terminals. The morphological finding that only one inhibitory terminal was usually present on these cells is consistent with the all-or-none character of the IPSPs we observed.

Postsynaptic control of electrical EPSPs

The most striking finding of this study was that the ampli- tude and even the occurrence of the electrical EPSP depended on the membrane potential of the postsynaptic cell. We hypothesize that the dependence of EPSP size on postsynaptic membrane potential is caused by close coupling between the membrane potentials of pre- and postsynaptic elements. Our hypothesis is that when the postsynaptic membrane is strongly depolarized, the presynaptic membrane is also depolarized and sodium channels in the presynaptic terminal are inactivated. Such presynaptic inactivation is removed with hyperpolariza- tion, and the presynaptic spike becomes larger with increasing hyperpolarization, resulting in a larger EPSP. (The range of membrane potentials within which increasing hyperpolariza- 

FIG. 11. Primary afferent stimulation evokes all-or-none IPSPs in deep granular cells. A: all-or-none IPSP. Responses to an extracellular stimulus that was gradually increased from below threshold to above threshold for IPSP. IPSP was depolarizing at holding potential of ~70 mV. B: suppression of IPSP by GABA_A receptor antagonist bicuculline (35 μM). Same cell as in A. C: effect of membrane potential on IPSP amplitude. IPSP reversed at a membrane potential of about ~45 mV. D and E are from the same cell, and different from the cell in A and B. D: effect of membrane potential on IPSP amplitude. IPSP reversed at a membrane potential of about ~45 mV. C and D are from the same cell, and different from the cell in A and B. D: plot of IPSP amplitude as a function of membrane potential.

FIG. 12. Responses to current injection and I-V curves in deep granular cells. A: voltage transients obtained in response to current steps injected through the electrode. B: current transients in response to steps of voltage from a holding potential of ~70 mV. C: suppression of broad voltage response by calcium channel blocker cadmium (200 μM). D: I-V curves constructed under voltage clamp at 300 ms after onset of voltage steps for both deep and superficial granular cells. SE bars for deep granular cells were too small to be clearly visible.
Our hypothesis is supported by a modeling study that simulated an ELL granular cell and a presynaptic terminal with a conductance-based model using realistic parameters for membrane properties, gap junction resistance, and sodium channel density in the terminal (Iancu et al. 2006). The model showed the same dependence of EPSP size on postsynaptic membrane potential that we observed physiologically—absence of the EPSP at strongly depolarized membrane potentials, gradual increase of EPSP size to a maximum with hyperpolarization, and abrupt disappearance of the EPSP with additional hyperpolarization beyond that yielding a maximum in EPSP size. The magnitude of the spike-associated sodium conductance in the presynaptic terminal showed the same dependency.

Some of the EPSPs we recorded decreased abruptly in amplitude when the cell was hyperpolarized beyond the potential yielding a maximum EPSP (Fig. 6, C and D). We suggest that, in these cases, the spike was blocked at the terminal but was still evoked at a more distal node of Ranvier, although another explanation is also possible, namely that two different afferent fibers were activated and that postsynaptic hyperpolarization blocked one before the other. Finally, other EPSPs decreased linearly in amplitude with hyperpolarization beyond the membrane potential yielding a maximum EPSP. We suggest that in these latter cases the presynaptic terminal may be unmyelinated for some distance distal to the terminal. Morphological work indicates that myelination extends right up to the terminal in many, but not all, of the fine branches of the mormyromast terminal arbor (J Meek, personal communication). Increasing hyperpolarization in an unmyelinated presynaptic terminal axon could push the occurrence of the spike further and further away from the terminal. The passively propagated spike at the terminal (and the electrical EPSP in the postsynaptic cell) will be attenuated in proportion to the distance between the spike location and the terminal.

Membrane potential–dependent changes in the postsynaptic cell conductance would also cause changes in EPSP amplitude and might be proposed as an alternative explanation for our observations. However, such conductance changes cannot explain the effects of membrane potential on EPSP amplitude that we observed. To explain our observations, membrane conductance would have to be at a minimum between −65 and −110 mV when EPSP amplitude is at a maximum. Conductance would have to increase either abruptly or gradually with further hyperpolarization and increase in a graded manner with depolarization, reaching a maximum between −50 and −40 mV. However, the effect of membrane potential on conductance was completely different from this. I-V curves showed that conductance was low and constant for all membrane potentials more hyperpolarized than −40 mV (Figs. 9C and 12D). More directly, we observed that the voltage deflection caused by a brief current pulse (1 ms, 0.5 nA) was constant between membrane potentials of −110 and −40 mV (n = 3; data not shown).

Simultaneous recordings from pre- and postsynaptic elements have been obtained at some electrical synapses where both the terminals and the postsynaptic cells are large (Herberholz et al. 2002; Pereda et al. 1995). These recordings show more directly how postsynaptic membrane potential can affect the occurrence of presynaptic spikes. In crayfish, primary afferent fibers terminate on the lateral giant interneurons with electrical synapses (Herberholz et al. 2002). The presynaptic fibers are also coupled together electrically so that stimulation of a subset of afferent fibers causes an EPSP in fibers that are not themselves excited by the stimulus. Depolarization of the postsynaptic cell can, however, bring such EPSPs above threshold for a presynaptic spike. Moreover, hyperpolarization of the postsynaptic cell can block the occurrence of a presynaptic spike in a manner comparable with that hypothesized here for the primary afferent-granular cell synapse in the mormyrid ELL. Similar phenomena are observed with simultaneous presynaptic and postsynaptic recording at the synapse between eighth nerve afferents and the Mauthner cell of goldfish (Pereda et al. 1995). In this case, presynaptic spikes could be elicited in unstimulated afferents by means of EPSPs that were evoked in the Mauthner cell by stimulated afferents and relayed in the antidromic direction through the electrical synapse to the unstimulated terminal.

Our observations extend these previous results by showing that changes in postsynaptic membrane potential cannot only affect the occurrence of a presynaptic spike and the associated electrical EPSP but can also cause fine gradations in the efficacy of such an EPSP. We also show that the effect of membrane potential changes can be different within different ranges of membrane potential (regions indicated by red and black brackets in Fig. 6, B, D, and F).

The effect of small changes in membrane potential on the size and occurrence of the electrical EPSP suggests the possibility of various forms of nonlinear interactions between the electrical EPSP and the cell’s other synaptic inputs. ELL granular cells receive hyperpolarizing inhibitory input from ELL interneurons and depolarizing excitatory input from central sources linked to the EOD motor command, in addition to their primary afferent input (Bell 1990a). One type of nonlinear interaction was described in the results and is shown in Fig. 6G for an EPSP like the one shown in Fig. 6F. In this case, when the postsynaptic membrane potential is within the range indicated by a red bracket in Fig. 6F, an electrical EPSP occurring during the depolarization evoked by a second EPSP will be larger than the same EPSP occurring during the absence of the second EPSP. Such an interaction is similar to that described for NMDA-mediated synaptic responses (Mayer and Westbrook 1987).

Other types of nonlinear interactions are also possible. When the membrane potential is at a level that is more depolarized than the potential yielding a maximum EPSP (region indicated by black brackets in Fig. 6, B, D, and F), hyperpolarization by an IPSP will cause an increase in the electrical EPSP and depolarization by another EPSP will cause a decrease in the electrical EPSP. In contrast, when the membrane potential is at the potential yielding a maximum, further hyperpolarization by an IPSP will cause either an abrupt disappearance of the EPSP (Fig. 6B) or a reduction in EPSP size (Fig. 6, D and F). These effects of membrane potential are in addition to the nonlinear interactions caused by shunting inhibition during the conductance increase associated with an IPSP.

The resting membrane potentials recorded in our cells were low: −43.3 ± 1.7 mV for superficial granular cells and −46.0 ± 2.1 mV for deep granular cells. Electrical EPSPs at these membrane potentials would be quite small (Figs. 6 and 10). However, patching onto these small cells with their high input impedance may introduce a shunt that reduces the recorded membrane potential below the potential of intact cells.
Seal resistances before breaking the membrane may be taken as a rough estimate of the shunt resistance introduced by the recording. Our seal resistances were on the order of 4 GΩ (conductance of 0.25 nanosiemens). Shunt resistances of this magnitude would have little effect on large cells with low input impedances but could have a significant effect on small cells like ours with measured input impedances of 2 GΩ (conductance of 0.5 nanosiemens). The measured input conductance represents the sum of the shunt conductance and membrane conductance. The membrane conductance of the cell is thus 0.25 nanosiemens (0.5–0.25), roughly equal to the conductance of the shunt. The shunt is not ion selective and reverses at 0 mV, resulting in a reduced membrane potential. In addition, primary afferent fibers will be more active in the intact fish and may elicit inhibitory input that further hyperpolarizes the cells. Thus the actual membrane potential of these cells may be higher than what we recorded in our in vitro patch recordings.

The membrane potential of a postsynaptic cell will have a significant effect on the membrane potential of a presynaptic terminal at an electrical synapse if the resistance of the gap junction is low in relation to the input resistance of the presynaptic terminal. The large size of the electrical EPSPs and the striking dye coupling suggest that the resistance of the gap junction is low in ELL granular cells. Whether such a relationship between junctional resistance and presynaptic input resistance is present at other sites with electrical synapses remains to be determined.

Comparison of superficial and deep granular cells

The two types of granular cells were physiologically rather similar. Both types showed all-or-none electrical EPSPs with similar dependancies on postsynaptic membrane potential, and both types showed large all-or-none GABA_A-mediated IPSPs. Both cell types gave bursts of spikes during long depolarizations. The I-V curves were also roughly similar with both cell types showing a sharp increase in conductance at about −40 mV, although the conductance of superficial granular cells at depolarized membrane potentials was much greater than that of deep granular cells.

Some additional differences between the two cell types were also apparent. The EPSPs of superficial granular cells were significantly larger and had faster rise times than the deep granular cells, related perhaps to the more marked dye coupling in superficial cells. The IPSPs were of similar size in the two regions, but IPSPs were more common in the deep granular cells. Deep granular cells showed a broad depolarizing response in response to injection of depolarizing current steps with spikes superimposed on the broad response (Fig. 12A), but superficial granular cells did not show such a response. Finally, the spikes of deep granular cells were much smaller in size than the spikes of superficial granular cells. This smaller spike size could be a consequence of the characteristic long, thin initial segment in deep granular cells (Fig. 2, C and D) and location of the spike initiation site at the distal end of the initial segment. Cells in the nucleus of ELL have a similarly long, thin initial segment, as well as small somatically recorded spikes (Bell and Grant 1989).

Functional implications for the electrosensory system

The large size and all-or-none quality of IPSPs and EPSPs evoked in both types of granular cells suggest that most granular cells are inhibited by only a small number of inhibitory interneurons and excited by only a small number of primary afferent fibers. With regard to EPSPs, most cells showed a single all-or-none response, and only a few cells showed a series of two to four step-like changes in amplitude, indicating convergence of more than one primary afferent onto the same granular cell. This method underestimates the degree of convergence, because only a few stimulation sites could be tested near a recorded cell. Nevertheless, the number of fibers converging onto any one granular cell is probably quite small. In a previous study, two to seven EPSPs were recorded intraaxonally from individual primary afferent fibers near their central terminals, each EPSP arising from an electrorceptor close to the receptor giving rise to the recorded fiber (Bell 1990a). The small number of such EPSPs indicates a small degree of convergence. Similarly, field potentials recorded in vitro in the granular layer in response to afferent stimulation show no more than four to six discrete amplitude changes as stimulus intensity is increased, indicating that only a small number of afferent terminals overlap at a given point in the granular layer (Grant et al. 1998). The minimal convergence of primary afferent fibers onto granular cells means that the granular cells accurately relay primary afferent information about the spatial distribution of stimulus intensities to higher order cells of ELL.

As described in the Introduction, in vivo studies using field potentials and intra-axonal recordings show that granular cells receive excitatory input from two main sources—primary afferent fibers from the periphery and EOCD signals associated with the EOD motor command. The EOCD signals arise from the medial juxtalobar nucleus and are time locked with great precision to the EOD motor command (Bell and von der Emde 1995). The intensity of local EOD evoked current is encoded in the latency of primary afferent spikes (Bell 1990b; Szabo and Hagiwara 1967). Behavioral studies show that the fish measures the latency of EOD-evoked afferent input by the timing of such input with regard to an EOCD signal and that the fish is sensitive to latency changes as small as 0.1 ms (Hall et al. 1995). Our hypothesis is that the decoding of afferent latency by an EOCD signal occurs in the granular cells of ELL.

Several features of ELL granular cells are consistent with a role in decoding afferent latency. The sharp rise times and fixed latencies of the electrical EPSPs evoked by afferent stimulation are well suited to the transmission of precise timing information from primary afferents to granular cells. The capacity of granular cells to fire bursts of spikes in response to current injection or EPSPs (Fig. 4B) suggests that the granular cells could transform a latency code for stimulus intensity into a burst code, as suggested also by a few in vivo extracellular recordings from the ELL granular layer (Bell and Grant 1992). Finally, nonlinear interactions such as the one shown in Fig. 6G, in which the electrical EPSP is larger when delivered at the peak of an artificial EPSP, might enhance the cell’s sensitivity to small variations in the relative timing of two EPSPs. Supra-linear interactions between corollary discharge and peripheral EPSPs were in fact observed in intra-axonal recordings from primary afferents near their central terminals (Bell 1990a).
This nonlinearity was ascribed to voltage-sensitive channels in the postsynaptic cells, and we observed voltage responses below the threshold for spikes in these cells. However, it is also possible that the interaction described here between electrical EPSPs and membrane potential also plays a role. However, the significance of type of interaction for measuring relative timing remains uncertain, because it only occurs at relatively hyperpolarized membrane potentials.

Understanding the functional implications of the cellular properties of granular cells described in this study and of the differences between deep and superficial granular cells will require in vivo intracellular recording. Such recording will make it possible to examine directly the interaction between peripherally evoked and corollary discharge EPSPs.

ACKNOWLEDGMENTS

We thank M. Frerking, D. Iancu, P. Roberts, N. Sawtell, R. Taylor, L. Trussell, and H. von Gersdorff for helpful discussion and comments.

GRANTS

This work was supported by National Institute of Mental Health Grant MH-49792 to C. C. Bell.

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