type B cells can at least partially explain the reduced phototaxic behavior through increased inhibition of type A photoreceptors which, via interneurons, excite motoneurons involved in phototaxis [Y. Goh and D. L. Alkon, *Soc. Neurosci. Abstr.* **8**, 825 (1982); I. Lederhendler, Y. Goh, D. L. Alkon, *ibid.*, p. 825].

12. Similar experimental approaches involving the substitution of electrical brain stimulation for the natural unconditioned stimulus have been explored in mammalian classical conditioning paradigms [F. W. Mis, I. Gormezano, J. A. Harvey, *Science* **206**, 473 (1979); G. K. Martin, T. Land, R. F. Thompson, *J. Comp. Physiol. Psychol.* **94**, 216 (1980); G. M. Powell and J. W. Moore, *Physiol. Behav.* **25**, 205 (1980)].
13. For phototactic tests, animals were initially confined to one end of a clear plastic tube (16 cm long, 5/8 inch inside diameter) filled with seawater (15°C); they underwent dark adaptation for 15 minutes. A light of 1×10^2 erg cm^{-2} sec^{-1} was then turned on and focused on the distal end of the tube. Measurements of phototactic latencies required for animals to traverse the complete distance of the tube were obtained by one or more observers with no knowledge of the experimental histories of the animals. Animals were dark-adapted again and tested for negative geotactic behavior. For both light (phototactic) and dark (negative geotactic) tests of locomotor behavior, the tubes were oriented vertically (*8*). Cut-off scores before and after training, were: light test, 15 and 120 minutes; dark test, 30 and 120 minutes.
14. Relaxation and restraint of animals were aided by the addition of 10^{-4} *M* nicotine to the seawater bath, and the nervous system was exposed and then secured on a stainless steel substage attached to a micromanipulator.
15. Input resistances were estimated from linear regression curves fitted to the current-voltage data for hyperpolarizing current injections (range, −0.2 to −0.8 nA) obtained just before and 5 minutes after training, once V_m had reached a steady state.
16. This set of stimulation parameters was based on our previous (*3*) in vitro simulation of the effects of light-rotation pairings in the isolated nervous systems, which had shown (i) five paired (but not random) presentations of 30 seconds of light and 30 seconds of electrical stimulation of the caudal hair cell (at 2-minute intervals) produced a persistent cumulative depolarization of 9 mV and (ii) the synaptic enhancement of the type B cell long-lasting depolarization was most pronounced during the 30 seconds immediately after the offset of light.
17. Animals were considered recovered only if they clung to the side of the testing tube or locomoted around the base of the tube during both post-training light tests. Decisions as to recovery were made without knowledge of the previous treatment condition. Of the 24 preparations for which we successfully conducted the paired training, 13 (54 percent) recovered. For unpaired and sham treatments, the numbers were 8 of 24 and 7 of 18 (33 and 39 percent), respectively. These differences were not statistically significant. The major factor preventing recovery in animals was the prolonged restraint necessary to accomplish the electrophysiology and apparently not exposure to the dilute nicotine. A separate group of animals dissected (but not restrained) and exposed to nicotine for comparable durations recovered fully (six of six).
18. A one-way repeated-measured analysis of variance revealed a significant main effect of treatment [$F(2, 23) = 4.75$; $P < 0.05$] and interaction of treatment with training day [$F(4, 46) = 2.83$; $P < 0.01$]. Group P differed significantly from group UP at 24 and 48 hours after training [$t(17) = 1.89$ and 2.53, respectively], as well as from group S at these same times [$t(18) = 1.92$ and 1.96]. Group UP did not differ from group S at either 24 or 48 hours [$t(11) = 0.46$ and 1.49]. Similarly, there were no significant differences among any of the groups in terms of original baseline latencies.
19. The pairing-specific differences in phototactic suppression were only partially attributable to treatment-produced differences in light-evoked behavior, since "dark" latencies were also significantly increased for all groups (means: P, 109; UP, 118; and S, 110 minutes). Analysis of the distances traveled within this time, however, revealed that animals did in fact move. There were no significant differences among treatment conditions, however. Thus, some degree of light-specificity was demonstrated.
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23. We thank L. Holmak, B. Pallotti, J. Kuzirian for typing and W. Richards for technical assistance. Partially supported by funds from Princeton University.

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Selective Recording and Stimulation of Individual Identified Neurons in Freely Behaving *Aplysia*

Abstract. *A neuroethological technique is described for selective recording and stimulation of an individual neuron in freely behaving* Aplysia *by means of a fine wire glued into the connective tissue sheath above the identified cell body. A whole-nerve cuff electrode simultaneously monitored functionally related multiunit axon activity. For biophysical analysis the soma was impaled with a microelectrode when the ganglion was subsequently exposed. The technique is illustrated for several identified neurons involved in different behaviors.*

Three criteria are currently used to establish the behavioral importance of an identified neuron (*1*). To establish that a cell is "necessary" for a given behavior, it must be selectively destroyed (or prevented from firing) in an intact animal to show that the normal behavior is abolished or severely disrupted (*2*). To establish that a cell is "sufficient," it must be selectively stimulated in an intact animal to show that the behavior is elicited. However, a cell could be both necessary and sufficient by these criteria but not fire appropriately during a normal behavior. To establish that a cell is "appropriate," its normal pattern of activity must be recorded when the behavior occurs spontaneously, which also specifies the appropriate pattern of stimulation to establish the sufficiency criterion. Thus, a

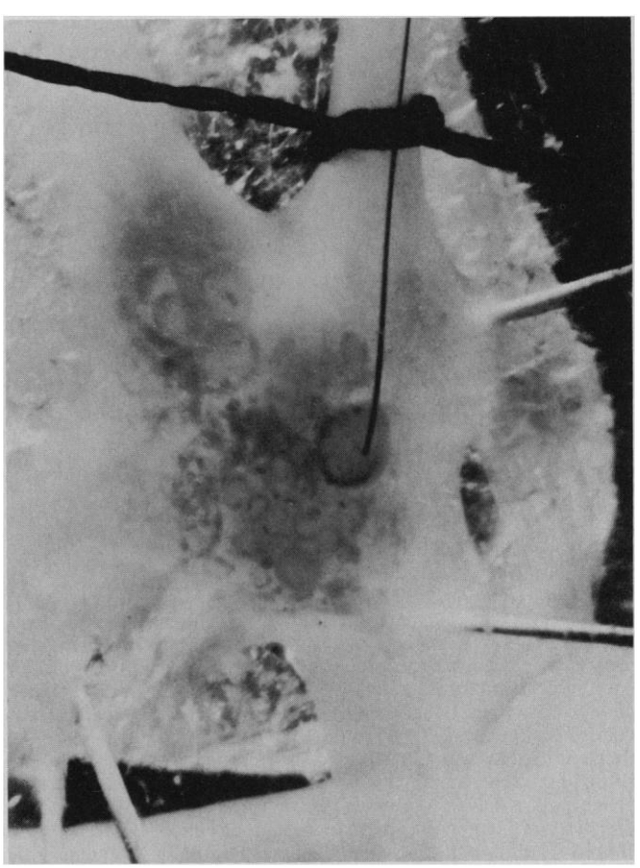

Fig. 1. Dorsal abdominal ganglion of *Aplysia californica* during surgical implantation of fine wire electrode to selectively stimulate and record from an identified neuron during normal behavior. The ganglion is pinned to a subilluminated stage. The fine wire, loosely sutured to the right connective (top right), extends caudally across the surface of the ganglion; the uninsulated tip is inserted into the connective tissue to lie directly above the large, darkly pigmented cell body of a giant neuron, cell R2 (diameter, 600 μm).

technique is required for selective stimulation and long-term monitoring of an identified neuron in freely behaving animals.

Many invertebrate neurons have a large, pigmented cell body that is visible through a connective tissue sheath on the surface of a central ganglion, making it easy to identify and impale the cell. However, intracellular recording prevents normal behavior because it requires a surgically reduced (semi-intact or isolated) preparation that is often perfused with artificial solutions. Furthermore, intracellular recordings can be obtained from only a few neurons simultaneously, whereas behavior (even in simple organisms) is typically generated by coordinated activity in large neuronal ensembles. For a more representative sample of ongoing activity, permanently implanted whole-nerve electrodes can monitor activity in large axonal populations during normal behavior (*3*). However, when many units are active simultaneously, it is difficult to recognize the activity of any single member of the population. Furthermore, it is difficult to stimulate selectively with whole-nerve electrodes. Small surface-area electrodes placed close to a target neuron can stimulate and record more selectively (*4*), as is done routinely with unidentified units in intact vertebrates (*5*). However, these techniques cannot be applied directly to invertebrate preparations that lack structures for attaching the electrodes. The fact that somas are much larger and less densely packed than their axons is an advantage in developing a selective, noninvasive way to monitor or stimulate an identified neuron during normal behavior. We now report a simple technique for implantation of a fine wire electrode in the connective tissue above an identified cell body, as illustrated by selective recording and stimulation of several identified neurons in intact *Aplysia californica*.

Under anesthetic a fine (25 μm in diameter) insulated wire of stainless steel was placed on the ganglion surface and tied loosely to a nerve. The cut tip of the wire was inserted into the connective tissue (*6*) overlying a morphologically identified target cell body. A typical electrode placement for cell R2 is shown in Fig. 1. By recording during implantation, signal-to-noise properties of the waveforms (and selectivity of stimulation) could be maximized before gluing the wire in position, and the identity of the cell could often be confirmed by its characteristic firing pattern (*7*). For simultaneous whole-nerve recordings, a cuff electrode was implanted on the ap-

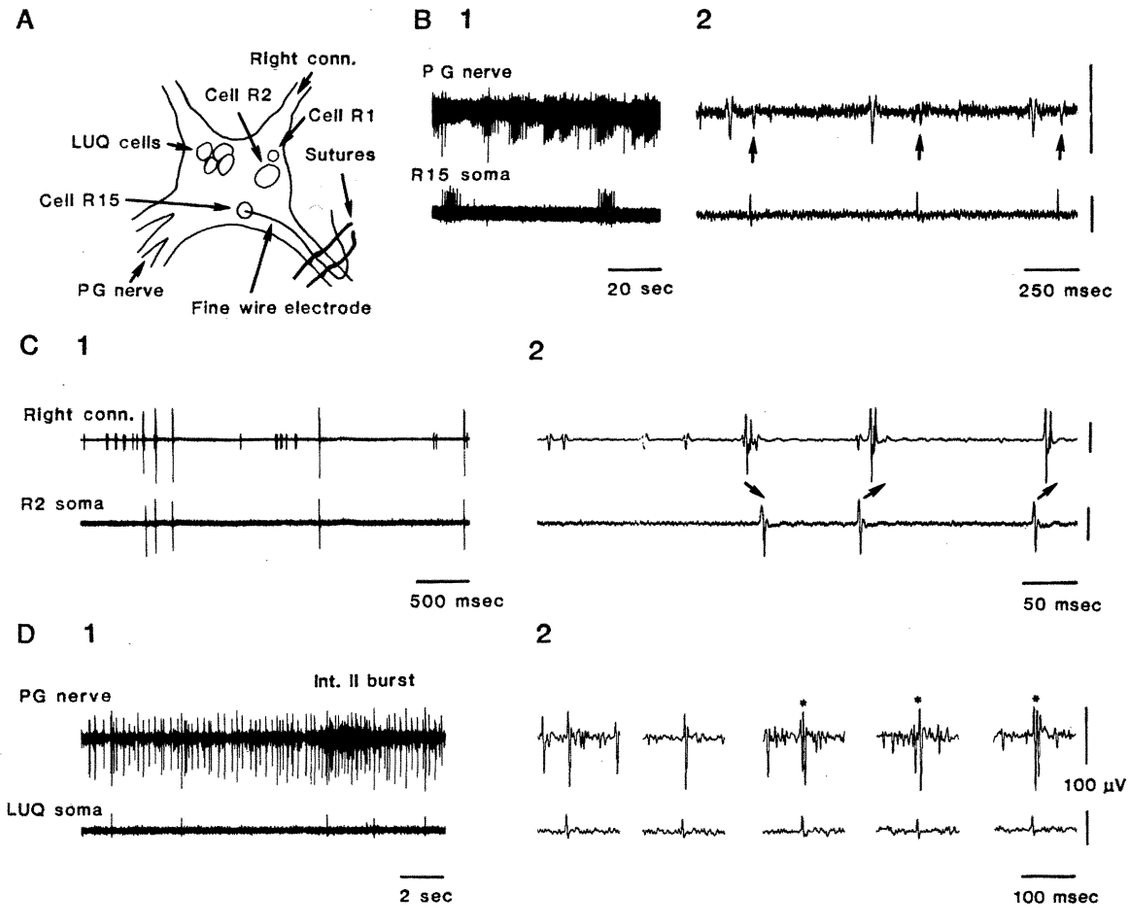

Fig. 2. Selective cell body and simultaneous whole-nerve recordings from identified abdominal ganglion neurons in intact *Aplysia*. (A) Schematic dorsal view of typical locations of recorded cells. In each animal (B to D), a fine wire was implanted in the connective tissue above the soma (bottom trace) and a single cuff electrode was implanted on the appropriate nerve or connective (top trace). Runouts are shown at slow (1) and fast (2) speeds. (B1) Spontaneous activity in the pericardial-genital (*PG*) nerve and R15 soma during implantation of the fine wire prior to gluing. Soma recording shows parabolic bursting pattern of R15. (B2) Small axon spike of R15 can be identified in the fast runout by correlating cell body and axonal spikes (arrows). (C1) Tactile body stimulation in a different intact animal elicits activity in both R1 (small unit in nerve recording) and R2 (large unit), but only the R2 spike is recorded by the soma electrode. (C2) The first R2 spike travels along the connective to R2 cell body; subsequent spikes conduct away from the R2 soma (arrows). (D1) Spontaneous activity in the pericardial-genital nerve and in the soma of a left upper quadrant (*LUQ*) bursting neuron (probably L4). The last three spikes in this LUQ cell occur during heightened activity associated with a "respiratory pumping" (interneuron II) contraction. (D2) The last three LUQ spikes in the nerve record are compound (*) due to superimposed activity of other units, whereas the soma recordings are unitary throughout.

propriate peripheral nerve or central connective, providing an additional criterion (one-to-one correspondence between soma and axonal spikes) for identifying the target neuron during surgery. If simultaneous intracellular analysis was subsequently required, a shallow ring of plastic approximately 150 μm in diameter was placed over the target cell body, and the entire assembly was secured to the ganglion with a thin film of Super Glue (*8*). The ring protected the underlying tissue from the adhesive and remained in place throughout the experiment; when the intact animal was surgically reduced and the nervous system exposed, a microelectrode was guided through the open lumen into the cell body below. Rigorous behavioral criteria (including attachment to the substrate, withdrawal reflexes, and feeding) were used to establish that postoperative behavior (and presumably neuronal activity) was normal.

Representative cell body and simultaneous whole-nerve recordings in intact animals are shown for three different identified neurons (Fig. 2A) in the abdominal ganglion. The recordings from R15 (Fig. 2B) show how activity is monitored during implantation and illustrate the good signal-to-noise properties of the selective soma spikes compared to the relatively small axonal spikes (arrows) in the highly active pericardial-genital nerve recordings. The recordings from R2 (Fig. 2C) demonstrate bidirectional conduction by comparing temporal offsets (arrows) between soma and axonal spikes. The LUQ cell sample (Fig. 2D1) illustrates the selectivity of the soma recording, which is particularly advantageous when many other functionally related units are active simultaneously. Despite the heightened activity and compound waveforms (asterisks) that developed in the multiunit axonal recordings during a spontaneous "respiratory pumping" contraction (Fig. 2D2), only the target unit was recorded with the soma electrode. We have now obtained cell body recordings with good signal-to-noise properties from single cells or small groups of cells in freely moving animals for 2 to 3 days with little or no apparent signal or behavioral degradation, suggesting that the fine wire electrodes are suitable for prolonged monitoring of normal neuronal activity. With this technique, it may be possible to record from identified cell bodies whose axons are too small for their spikes to be recorded with whole-nerve electrodes.

The fine wire electrode was also used to stimulate individual identified cells selectively in an intact animal (Fig. 3A). In this animal, both R2 and R1 were recorded with the whole-nerve electrodes, but only R2 was recorded with the soma electrode (Fig. 3A1). When the soma of R2 was stimulated via the fine wire electrode, only cell R2 was activated and R1 remained silent (Fig. 3A2). Even repetitive suprathreshold stimulation of R2's soma did not produce the inking or other signs of agitation that often accompany connective stimulation of R2's axon in the intact animal. After R2 was monitored and stimulated in the intact animal, the abdominal ganglion was exposed and the cell body of R2 was impaled through the lumen of the previously implanted plastic ring (Fig. 3B2). As suggested by the results for the intact animal (Fig. 3A2), extracellular stimulation of the soma (Fig. 3B1) was as selective as intracellular stimulation (Fig. 3B2). In contrast, nerve stimulation that elicits R2 firing is nonselective (*9*): an R1 spike was always elicited at a lower threshold (Fig. 3B3) than an R2 spike (Fig. 3B4). Furthermore, spikes that failed to invade the cell body could also be recorded with the fine wire electrode (Fig. 3, C1 to C3).

This technique should be useful in other preparations in which cell body

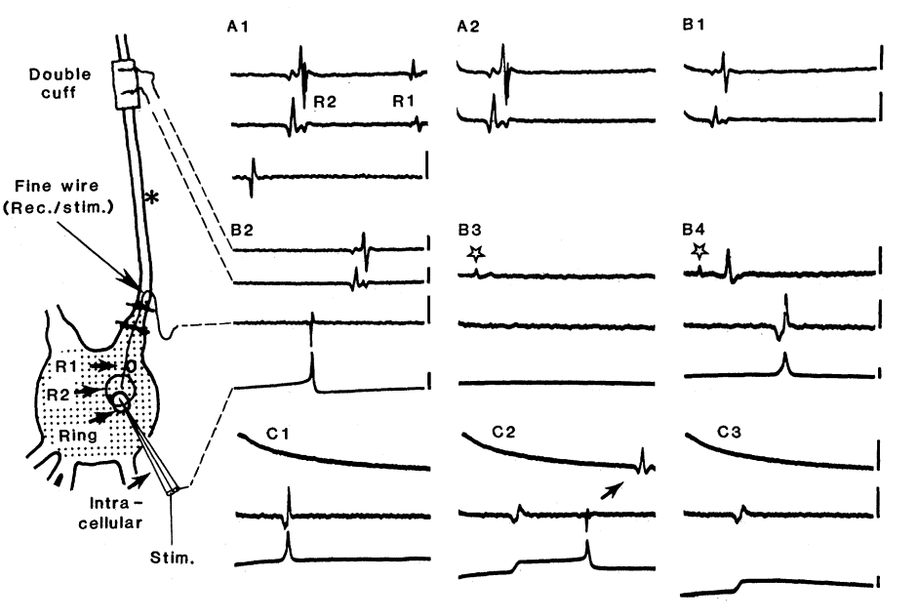

Fig. 3. Selective stimulation of cell R2 in intact and isolated preparations. A double cuff electrode (3 mm separation) was placed on the right pleurovisceral connective, and a fine wire electrode and plastic ring were glued in place over cell R2 (dotted area). When the nervous system was subsequently isolated, R2 was impaled through the ring with a two-barrel microelectrode. (A) Intact animal. (A1) Body stroke stimulation evokes both R1 and R2 axon spikes in the distal (top) and proximal (middle) cuff recordings, but only the R2 spike is seen in the fine wire recording (bottom). (A2) Soma stimulation through fine wire electrode (1 msec at 1.2 mA) elicits selective 1:1 firing of R2 (up to 9 Hz). (B) Isolated preparation (note reduction in signal amplitude). (B1) Stimulation through the fine wire electrode (1 msec at 1.3 mA) again elicits selective 1:1 firing in the R2 axon. (B2) Cell R2 was depolarized and its soma spike recorded intracellularly (bottom trace) and extracellularly (middle trace). Only the R2 axon spike is seen in the cuff electrode recordings (top two traces). (B3) A second single-cuff electrode (top trace) was placed (*) between the ganglion and the double cuff. Low-intensity nerve stimulation via the double-cuff electrode elicits a spike only in R1 (☆) that is not seen in the fine wire (middle) or intracellular (bottom) recordings. Note faster sweep speed (also in B4). (B4) To recruit R2 activity, higher intensity nerve stimulation must be used and R1 is always present (traces same as in B3). (C) Isolated preparation. Fine wire electrode records electrotonic spikes that failed to invade the cell body. Repeated nerve stimulation through distal channel of double-cuff electrode: proximal cuff channel (top trace in each part); fine wire (middle trace); and R2 intracellular (bottom trace). The stimulus elicits short-latency antidromic axon spikes in R1 and R2 (masked in the top trace by the stimulus artifact) that precede the cell body spikes. (C1) First stimulus in series elicits antidromic R2 spike that invades the cell body. (C2) With repeated stimulation, some antidromic spikes invade the cell body with a delay that is longer than the refractory period and, consequently, elicit orthodromic R2 spikes (arrow). Note the noninvading electrotonic spike in both the intracellular and fine wire recordings. (C3) When the antidromic spike fails to invade the cell body, the electrotonic spike is still recorded, but no orthodromic spike occurs. Calibration is 100 μV for all extracellular recordings, except for B2 (top two traces, 25 μV) and top trace of B3, B4, and C1 to C3 (50 μV). Calibration for all intracellular recordings, 40 mV. Time calibration is 20 msec in all cases except B3 and B4 (10 msec).

activity is correlated with normal behavior. It combines the selectivity of intracellular approaches with the noninvasiveness of extracellular approaches and provides a direct link between electrophysiological procedures used to monitor and manipulate neuronal activity in intact animals and procedures used in reduced preparations. Selective stimulation and recording provide two additional methods for assessing the behavioral function of an identified neuron in an intact animal that complement recently developed techniques (*2*) for selectively destroying individual identified nerve cells (*10*).

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6. The wire was triple-coated Stainless Steel 304 Tri-ML (CFW 19225-TMLSR-1, California Fine Wire). Usually a shallow cut was made in the surface of the connective tissue to one side of the cell body to facilitate entry of the tip. Current passing could mark the tip location.
7. General details of surgery and recovery are described in (*3*). Cell naming follows W. T. Frazier, E. R. Kandel, I. Kupfermann, R. Waziri, and R. E. Coggeshall [*J. Neurophysiol.* **30**, 1288 (1967)]. Cell R2 has the largest darkly pigmented cell body in the right hemiganglion and is thought to be involved in mucus secretion [S. Rayport and E. R. Kandel, *Soc. Neurosci. Abstr.* **5**, 249 (1979)]. Cell R15 has a large, whitish cell body located mediocaudally in the right hemiganglion and is a neurosecretory cell believed to be involved in osmoregulation [J. Stinnakre and L. Tauc, *J. Exp. Biol.* **51**, 237 (1969); I. Kupfermann and K. R. Weiss, *J. Gen. Physiol.* **67**, 113 (1976)]. The LUQ cells have large pigmented cell bodies in the left upper quadrant of the ganglion, and their function is unknown. Under anesthesia the activity patterns are similar to those described in vitro. For example, R2 is typically silent; R15 has an endogenous "parabolic" bursting pattern; and the LUQ cells are also endogenous bursters.
8. Super Glue (or a similar commercial-grade cyanoacrylate) was applied in minute amounts (on the tip of a very fine, flame-drawn, plastic pipette tip) to the area where the wire entered the connective tissue as well as to most of the remainder of the exposed, dried ganglion surface, including the suture holding the wire to the nerve (Fig. 3). If too much glue was applied, the bond strength was reduced and adjacent structures were often glued together. For a review of cyanoacrylate chemistry and action in neurological applications, see B. E. Mickey and D. Samson [*Clin. Neurosurg.* **28**, 429 (1981)]. We observed no obvious toxic effects of Super Glue on abdominal ganglion cells over a period of 5 days, but did not examine longer periods. To date we have been unable to obtain the less toxic isobutylcyanoacrylate. Plastic rings were made by cutting thin slices from flame-drawn polyethylene pipette tips.
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Auditory Intensity Discrimination at High Frequencies in the Presence of Noise

Abstract. *Over a wide range of intensities, subjects were able to detect small differences in the intensity of a high-frequency band of noise that was presented with a relatively intense, complementary band-reject noise. This indicates that neither of two possible mechanisms for peripheral intensity coding, those based on timing and on spread of excitation, is necessary for the large dynamic range of human hearing. It is shown that the information available in the firing rate of a small number of nerve fibers can account for these data.*

A striking and fundamental property of hearing is the enormous range of intensities over which the auditory system can operate. The loudness of a pure tone increases over a 130-dB range; more important, relatively small changes in intensity, approximately 1 dB, can be perceived over at least a 110-dB range (*1*). The dynamic range demonstrable in such psychophysical situations contrasts sharply with the 30- to 50-dB dynamic range of typical fibers in the auditory nerve (*2*). Clearly, usable intensity information over at least a 110-dB range is coded in the nerve; how it is coded is not known.

One general coding scheme, that based on the firing rate of a group of fibers, has been discounted primarily because it has been thought unlikely that enough unsaturated fibers are available to signal intensity changes at high intensities (*3*). A plausible alternative scheme, that based on information available in the timing of spike discharges, is examined here. It is shown that a large psychophysical dynamic range is maintained at frequencies for which a timing code is extremely unlikely. Thus timing information, specifically temporal synchrony to waveform fine structure, is not necessary for a large dynamic range. It is also shown, using a detection theory analysis of recent physiological data, that the information available in the firing rate of a very small number of fibers is, in fact, sufficient to account for the experimental results, that is, a rate-based intensity code is at least theoretically possible.

The possibility that intensity can be coded temporally over a wide intensity range is suggested by recent data on the synchronization of the firing pattern of primary fibers to the fine structure of complex waveforms (*4*). Of particular interest is the observation that the function relating degree of synchronization to intensity, which for single components has a dynamic range comparable to that of the rate-intensity function, can be rigidly shifted to higher intensities by the addition of a second component (*5*). The effect of the second component on synchronization to the first component can be approximately described as attenuative, that is, the reduction or suppression in synchrony is similar to reducing the intensity of the first component by a constant proportion, independent of its actual intensity. This characteristic of synchrony suppression is similar to the effect of adaptation in the visual system and, as in vision, may be the mechanism that permits a large dynamic range, at least for complex stimuli.

The experiment reported here examined intensity discrimination of a band of noise that was presented with a relatively intense band-reject noise whose spectral notch corresponded to the passband of the signal. The experiment is similar to previous intensity discrimination experiments with band-reject noise (*6*), with the important difference that the frequency range over which the intensity changes occur is 6 to 14 kHz and is above that for which primary fibers preserve temporal synchrony to waveform fine structure (*7*). The use of band-reject noise minimizes the possibility that intensity changes are signaled by unsaturated fibers that are activated or "recruited" by the intensity-dependent