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5. Statistical details are available on request.
6. The peak latency of P3 (P300), measured at Pz for the ERP's to the infrequent classes of stimuli, yielded the same main effects, and lack of interaction, as RT. The increase in the peak latency of P3 with the introduction of the mask supports the findings of G. McCarthy and E. Donchin [*Science* **211**, 77 (1981)] in an experiment that crossed two independent variables in a 2 by 2 design according to logic similar to ours. The possibility that the changes in the peak of N_A across conditions were due to alterations of motor potentials or the latency of P3 is remote because the nature of the classification task had a significant main effect on RT and P3 latency but not on N_A peak latency.
7. The average percentages of maximum amplitude of N_A and N2, derived separately for each subject and collapsed across the four choice RT conditions, were, respectively, 50 and 29 at Cz, 65 and 47 at Pz, 77 and 77 at Oz, 92 and 92 at T5, and 46 and 66 at the mastoid. By the more conservative method outlined by J. R. Jennings and C. C. Wood [*Psychophysiology* **13**, 277 (1976)], analysis of variance yielded a significant interaction between components and electrodes ($P < .004$). The topography of N_A seems to differ from the selective attention effects on visual ERP's found by S. Van Voorhis and S. Hillyard [*Percept. Psychophys.* **22**, 54 (1977)], which comprised a positive enhancement around 220 msec at O1 and a negative enhancement around 155 msec at Cz.
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9. We gratefully acknowledge the comments of N. Squires. Supported by PHS grants HD 10804, MH 06723, 1F32 AGO-5193, and core grant HD 01799.

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Impulse Conduction in the Mammalian Brain: Physiological Properties of Individual Axons Monitored for Several Months

Abstract. *Microelectrode recordings were used in conjunction with antidromic activation to monitor impulse conduction along individual mammalian cerebral axons for periods of up to 165 days. Approximately half of the axons studied showed a stable conduction velocity and stable aftereffects of impulse activity. The remaining axons showed slow and progressive increases or decreases in conduction velocity overtime. In these latter axons, changes in the magnitude of the aftereffects of impulse conduction were far less pronounced than were changes in axonal conduction velocity.*

Little is known about the long-term stability of axonal conduction properties in the central nervous system. It is not known, for example, whether the velocity of impulse conduction and the aftereffects of impulse activity in individual axons of adult mammals remain stable for long periods of time. The paucity of such information is primarily attributable to the lack of a method with which to study individual axons for more than a few hours. Such information is important for the understanding of progressive pathological influences on impulse conduction. In the study reported here, extracellular microelectrode recording methods were combined with antidromic activation of axons at one or several sites. These methods enabled the continuous study of conduction properties of individual axons in the mammalian brain for several months.

Recordings were obtained from the cell bodies of visual callosal axons in adult Dutch Belted rabbits. This axonal system consists of both nonmyelinated and small myelinated axons (*1*) which conduct impulses relatively slowly (*2–4*). The aftereffects of impulse conduction in this system have been documented (*2*, *4*, *5*). Microelectrodes were permanently implanted near the border of visual areas I and II (Fig. 1) (*6*). Stimulating electrodes activated the callosal axon near the midline (*7*) and in some cases near the terminals in the contralateral hemisphere. Recording sessions began approximately 1 week after surgery (*8*). Antidromic activation was differentiated from synaptic activation by means of collision tests and other criteria (*9*). Brain temperature was monitored (*10*) to ensure that the observed variations in conduction properties were not temperature-dependent (*11*). At the end of the experiments the animals were killed and the tissue was prepared for histological analysis (*12*).

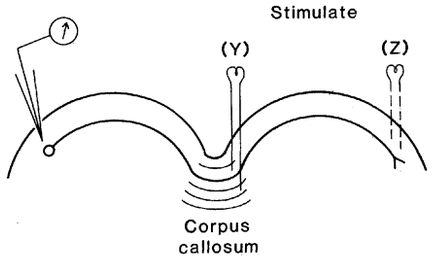

Fig. 1. Schematic illustration of the experimental setup. Microelectrodes were implanted into superficial cortical layers near the border of visual areas I and II. Banks of stimulating electrodes were implanted in the splenium of the corpus callosum (*Y*) and, in some cases, in the contralateral hemisphere (*Z*).

The present results are for 23 neurons studied for 20 to 165 days (mean, 48 days). An additional 40 neurons were studied for 5 to 19 days and provide confirmatory data. This report focuses on conduction velocity, supernormal conduction velocity, and the minimum interspike interval, since these three conduction properties were either very stable over time or showed progressive, systematic changes. Other measures studied were less stable over time (*13*).

Figure 2 shows oscilloscope tracings illustrating these measures. The records were obtained on the 60th day of recording from a cell that was studied for 165 days. Antidromic latency (25.3 msec) to a single antidromic test stimulus is shown in Fig. 2A. In Fig. 2B the supernormal conduction velocity that follows a single prior impulse is shown. The increase in conduction velocity is manifested as a decrease in antidromic latency to a test stimulus that follows a spontaneous spike or an electrically elicited spike at appropriate intervals (*14*). The minimum interspike interval (2.15 msec), shown in Fig. 2C, is the minimum interval between two conducted action potentials elicited by two stimuli presented at an interval near the absolute refractory period of the axon (1.3 msec).

Eleven of the 23 axons studied at length demonstrated a stable antidromic latency, with a variation of < 5 percent from the first to the last day of recording. In four of the remaining neurons, cumulative decreases in antidromic latency (increases in conduction velocity) of 8 to 14 percent occurred at a mean rate of 0.2 percent per day, while in eight neurons cumulative increases in antidromic latency (decreases in conduction velocity) of 6 to 81 percent occurred at a mean rate of 0.5 percent per day (*15*). Figure 3A shows data for three neurons studied for 78 days, 101 days, and 165 days. They demonstrated stable, decreasing, and increasing antidromic latencies, respectively.

The minimum interspike interval reflects the recovery processes of the entire axon between stimulating electrode and soma and is the reciprocal of the maximum firing frequency that may occur along this length of axon. Although the value of this measure varied significantly between cells (1.55 to 3.25 msec for the 23 cells studied at length), little

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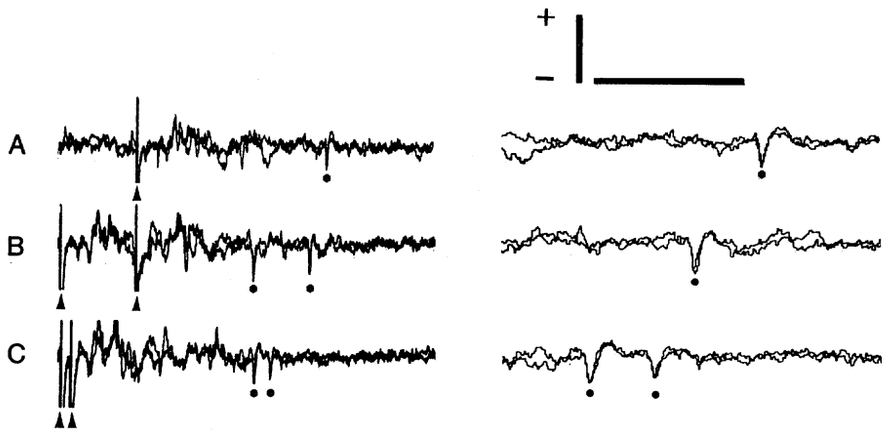

Fig. 2. Oscilloscope tracings showing three of the measures studied. Each trace consists of two superimposed traces of a digital oscilloscope (temporal resolution, 0.05 msec). These records were obtained after 60 days of recording from a cell (cell 3) that was studied for 165 days. Filled circles identify antidromic spikes. (A) Antidromic latency (25.3 msec) to a single antidromic test stimulus (arrowhead). (B) Supernormal antidromic latency. Here the antidromic test stimulus (second arrowhead from left) is preceded at an interval of 10 msec by an antidromic conditioning stimulus (first arrowhead), which also results in a spike, and the response to the test stimulus is now reduced to 23.5 msec (*15*). (C) Minimum interspike interval (2.15 msec) elicited by two stimuli presented at an interval very near the absolute refractory period of the axon (1.3 msec) (arrowheads). The right portion of the figure shows expanded sweeps of the records on the left. Calibration: 20 msec on the left and 5 msec on the right. Brain temperature: 39.2°C.

variation was seen within cells from day to day. Of the cells showing either stable or progressively decreasing antidromic latencies, the total range of fluctuations in a given cell never exceeded 13 percent (range, 4.5 to 12.7 percent; mean, 8 percent) of the mean value of this measure for the cell. However, cells that showed progressive increases in antidromic latency also showed progressive increases in the minimum interspike interval (range, 2.5 to 21.5 percent; mean, 13.4 percent). These increases, however, were not as great as those seen in latency. Figure 3B shows this measure for the three cells represented in Fig. 3A. Note the lack of overlap in this measure despite the progressive increase seen in cell 3 (which also showed a progressive increase in antidromic latency). Of all the cells with decreasing or stable antidromic latencies, cell 1 showed the widest fluctuations in the minimum interspike interval (12.7 percent).

Also showing remarkable stability over time was the supernormal conduc-

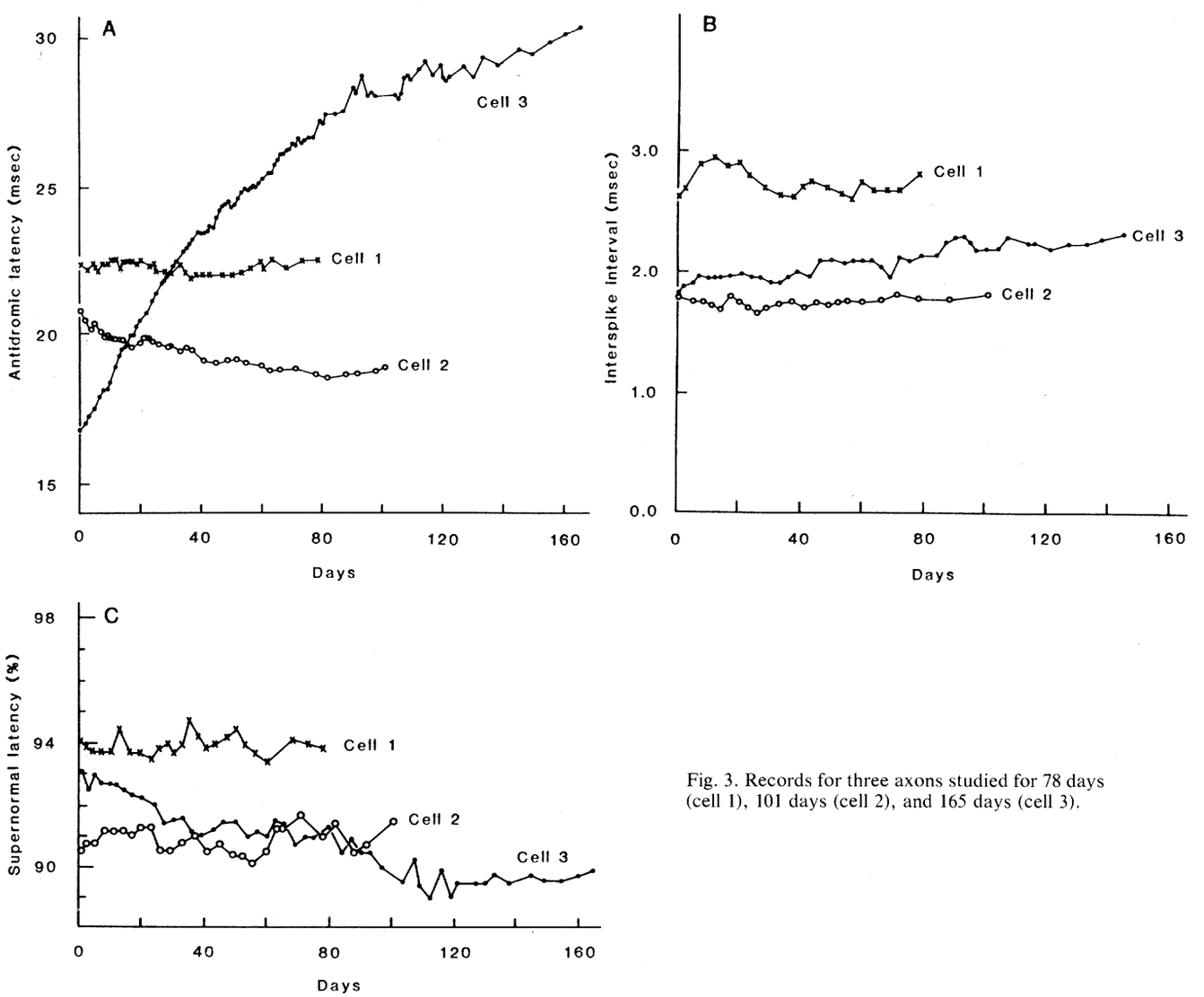

Fig. 3. Records for three axons studied for 78 days (cell 1), 101 days (cell 2), and 165 days (cell 3).

tion that follows the relative refractory period of a single impulse. While significant differences in the magnitude of this variable were seen between cells, differences within cells were slight over time. Thus, for the 23 cells the maximal decrease in antidromic latency varied from 3 to 15 percent of the control antidromic latency, but the total range of variations over time within single cells rarely exceeded 2 percent of the control antidromic latency. However, three of the eight cells that showed progressive increases in antidromic latency also showed a progressive increase in the magnitude of supernormal conduction. Figure 3C shows this measure over days for the three cells represented in Fig. 3, A and B. Note the stability of this measure in cells 1 and 2 and the progressive increase of this measure in cell 3, which also showed a progressive increase in antidromic latency and minimum interspike interval.

These experiments leave questions regarding the underlying mechanism of the slow and progressive increases and decreases in conduction velocity and other conduction properties observed in some axons. Changes in conduction velocity could result from a number of factors (for example, changes in axonal diameter or ion channel density). Such changes may occur naturally, or they may result from subtle pathological changes elicited by the recording (*12*) or stimulating electrode. It seems unlikely, however, that increases in conduction velocity would result from pathological conditions.

I have shown that the physiological properties of individual cerebral axons may be monitored over a period of several months. Three measures of impulse conduction properties (conduction velocity, supernormal conduction velocity, and the minimum interspike interval) were found to be very stable over time or to vary in a progressive and systematic fashion. Systematic changes generally occurred at a rate of less than 1 percent per day and were often not detectable for several days or even weeks. Thus, these three measures provide an unequivocal signature with which to identify a neuron if recordings are obtained at relatively short intervals (2 to 3 days). The stability of the above conduction properties and the slow, systematic nature of the changes that do occur suggest the feasibility of studying the long-term effects of pharmacological, toxicological, or other variables on the conduction properties of individual cerebral axons.

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5. J. D. Kocsis, H. A. Swadlow, S. G. Waxman, M. H. Brill, *ibid.* **65**, 230 (1979).
6. Microelectrodes were usually constructed of platinum-iridium wire (diameter, 25 μm) insulated with Teflon and cut flush. These electrodes were rigidly implanted, with no attempt made to "float" the electrode with respect to the cortical surface. In early experiments, such wires were electrolytically etched and the tips were insulated with a formfitting glass pipette [M. Salkman and M. J. Bak *IEEE Trans. Biomed. Eng.* **20**, 253 (1973)]. However, these electrodes were very difficult to produce and implant and were no more successful in gaining long-term recording stability than the electrodes described above. In each rabbit 5 to 16 microelectrodes were implanted singly or in groups of two to four, each electrode being separated by 0.3 to 0.5 mm. For each electrode (or group of electrodes) a small hole (approximately 0.5 mm in diameter or smaller) was teased into the dura, and the microelectrode was thrust through the pia into the cortex. Stimulation pulses were successively passed through the stimulating electrodes in the corpus callosum as the microelectrode was lowered. When antidromic spikes were elicited, the microelectrode was cemented into place with acrylic cement. All surgery was performed under barbiturate anesthesia.
7. Methods for implanting stimulating electrodes in the splenium of the corpus callosum have been described (*4*). The timing of stimulus pulses was controlled by four-channel digital circuitry (Digitimer).
8. Rabbits were awake and unrestrained during the recording sessions, which were usually held at intervals of 1 to 3 days. Differential signals (between neighboring microelectrodes) were preamplified and led to a digital oscilloscope for display, and the data were stored in digital form.
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10. Brain temperature was monitored (±0.1°C) by a thermocouple implanted 9 to 11 mm beneath the cortical surface into the thalamus. Recordings reported here were obtained when brain temperature was between 39.1° and 39.9°C.
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12. While most of the brains were processed for the identification of Nissl substance, in one rabbit, after 244 days of recording, a 20 percent solution of horseradish peroxidase (HRP) was infused into the contralateral hemisphere, homotopic to the recording microelectrode. As seen in the stained tissue, the microelectrodes were encapsulated in a glial sheath approximately 25 μm thick. Outside this glial sheath, however, the tissue appeared quite normal. Several HRP-labeled neurons were seen within 50 μm of the microelectrode tips and many were seen within 100 μm of the tips.
13. In addition to the measures reported, other measures of impulse conduction studied included antidromic threshold, a measure of the refractory period (arrowheads in Fig. 2C), and a measure of the period of subnormal impulse conduction which follows a burst of impulses.
14. Previous studies of the callosal system of the rabbit (*2*, *4*) and monkey [H. A. Swadlow, D. L. Rosene, S. G. Waxman, *Exp. Brain. Res.* **33**, 555 (1978)] showed that these decreases in antidromic latency are due to changes in axonal conduction velocity which occur along the length of the callosal axon. Further, these changes in conduction velocity are due to prior impulse activity in the axon under study rather than to ephaptic interactions between axons or other indirect effects of the electrical stimulation. The period of supernormal conduction velocity following a single impulse lasts 20 to 170 msec. In this experiment the interval between the conditioning and test stimuli was that which yielded the maximum supernormal conduction velocity.
15. In one cell it was demonstrated that the progressive increase in antidromic latency results from a decrease in conduction velocity distributed along the axon. The cell showed progressively increasing antidromic latency and could be antidromically activated by stimulating electrodes both at the midline and near the axon terminal. While a progressive increase in latency was seen at both stimulation sites, the magnitude of the increase in latency (in milliseconds) was significantly greater with stimulation of the axon near the terminal.

6 August 1982

Rapid and Precise Down Regulation of Fast Axonal Transport of Transmitter in an Identified Neuron

Abstract. *Within 1 day after the removal of one branch of the bifurcated axon of an identified neuron in* Aplysia, *the cell body reduced its output of transmitter storage vesicles to adjust precisely for the decreased need. This adjustment terminated the initial consequence of the removal, the transport of an inappropriately large number of vesicles to the remaining synapses. The most likely cause of the reduction of transport of transmitter is the loss of information normally provided by the disconnected axon or synapses.*

The axon and synapses of a neuron, which lack ribosomes and most nucleic acids, depend on fast axonal transport for the delivery of newly synthesized organelles from the cell body (*1*). To fully understand expressions of plasticity in axons and synapses, such as those occurring during development and regeneration, it will be important to determine how the cell body is guided to adjust its output of rapidly transported material to meet changing demands from its periphery. We are interested in understanding how the neuron regulates the transport of material destined for use at functioning synapses. Is the normal amount transported in a mature neuron influenced by informational feedback from the neuronal periphery? To address this question we studied the changes in the amount transported when a neuron suddenly had fewer synapses to supply.

For this study we used an identified serotonergic neuron, the giant cerebral neuron (GCN) in the central nervous system of the sea hare *Aplysia californica*. The axon of this monopolar neuron bifurcates within the neuropil of the ganglion, close to the cell body, into branches of similar diameter which course essentially unbranched in separate nerves to innervate separate post-

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