agents, including steroid hormones. Steroid hormones alter the state of phosphorylation of the regulatory subunit (R_2) of the type 2 cyclic AMP–dependent protein kinase specifically in nervous and nonnervous target tissues (*27*). Thus, it appears that steroid hormones may modulate protein phosphorylation systems both at the level of protein kinases and at the level of specific substrate proteins for these protein kinases.

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13. Female CD 1 rats (180 to 250 g; Charles River) were adrenalectomized and ovariectomized at least 1 week before treatment. Estradiol-treated rats received a subcutaneous injection of 10 μg of estradiol benzoate in 50 μl of sesame oil on two successive days. Corticosterone-treated rats received a 100-mg solid pellet of corticosterone, implanted subcutaneously for periods from 1 hour to 14 days. The pellets maintain a physiological serum concentration of corticosterone for at least 14 days (*17*). Dexamethasone-treated rats received a subcutaneous pellet of 25 percent dexamethasone in cholesterol, which remained there for 7 days. Control rats received no treatment, injections of sesame oil, or pellets containing cholesterol. Two days after the second estradiol injection, various periods of time after the implantation of corticosterone pellets, and 7 days after the implantation of dexamethasone pellets, the preoptic area, medial basal hypothalamus, hippocampus, and portions of the parietal cerebral cortex were removed from the rat brains as previously described [V. N. Luine, R. J. Khylchevskaya, B. S. McEwen, *J. Neurochem.* **23**, 925 (1974)]. The regions, quickly frozen on dry ice, were analyzed within 1 day after being homogenized in 1 ml of 1 percent sodium dodecyl sulfate (SDS) (4°C) per 5 to 10 mg of tissue. The SDS homogenates were then diluted 40-fold in a buffer containing (final concentrations) 200 m*M* NaCl, 10 m*M* EDTA, 10 m*M* NaH_2PO_4 (*p*H 7.4), 0.5 percent NP-40, 0.1 percent SDS. The concentration of protein 1 in each homogenate was determined in triplicate by radioimmunoassay [S. E. Goelz, E. J. Nestler, B. Chehrazi, P. Greengard, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 2130 (1981)]. This assay is detergent-based, competitive, and nonsolid phase in which SDS and NP-40 solubilize protein 1 and minimize its nonspecific interactions. The assay was performed in three steps: (i) rabbit antiserum to highly purified protein 1 from bovine brain was added to homogenates or to standard purified protein 1 from bovine or rat brain (protein 1 from these two species was immunochemically indistinguishable) and incubated for 20 minutes; (ii) ^{125}I-labeled purified protein 1 from bovine brain was then added and incubated for 1 to 8 hours; and (iii) protein A–bearing *Staphylococcus aureus* cells were added to precipitate the bound ^{125}I-labeled protein 1 and incubated for 25 minutes. The assay was linear over at least a 25-fold concentration range of standard protein 1 or homogenate and as little as 2 fmole of protein 1 could be accurately measured. The total protein concentration in each SDS homogenate was determined according to the method of O. H. Lowry *et al.* [*J. Biol. Chem.* **193**, 265 (1951)] with bovine serum albumin used as the standard. Freezing and storing the homogenates for up to 3 days did not significantly affect either the protein 1 or total protein determinations. No difference was observed in protein 1 content of the brain regions examined in the three types of control animals. Therefore, the data obtained for the various control groups in each experiment were combined.
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Optical Recording of Calcium Action Potentials from Growth Cones of Cultured Neurons with a Laser Microbeam

Abstract. *Simultaneous recordings of calcium action potentials directly from growth cones and from somata of neuroblastoma cells indicated that they could be generated in the neurites at or near growth cones. Growth cone responses were measured with a fluorescent voltage-sensitive dye and a 5-milliwatt helium-neon laser microbeam as a monitoring light source.*

Cell bodies and neurites often differ in electrical properties. Examples are found for both invertebrate (*1*) and vertebrate (*2–5*) preparations. Knowledge of the electrical properties of processes and growth cones may be important in understanding the role of a particular ion in regulating the growth of neurites (*5*), the regrowth of regenerating neurites (*6*), the formation and maintenance of intercellular connections (where usually only the postsynaptic properties are studied) (*7*), and the integrative characteristics of the basic computation elements, the dendrites (*5, 8*).

Even in vitro, studies of the electrical properties of processes or growth cones are difficult with current techniques. Extracellular recording from processes of cultured neurons has been reported (*9*). However, the extracellular technique is inadequate when studying graded potentials or regional variations in the form of action potentials. By using intracellular recording from the relatively large soma and extracellular stimulation of the neurite, it may be possible to study the electrical properties of neurites (*2, 4*); however, only propagated events can be detected, and frequently the results are susceptible to ambiguous interpretations.

Optical methods (*10, 11*) for monitoring membrane potential offer an alternative tool. These methods are simple in principle: voltage-sensitive dyes bind to external sites on the membrane, where they serve as molecular transducers, transforming changes in membrane potential into optical signals caused by the change in the optical properties of the stained membrane. Optical recordings have the same time course as intracellular electrical recording, but the magnitude of the potential change is not readily determined in the optical measurements. To study the electrical properties of neurites and growth cones, we devel-

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oped optical methods of monitoring activity from multiple sites along the processes of nerve cells in culture (*12*).

It was reported that processes of mature amphibian neurons (*2, 3*) and chick dorsal root neurons (*4*) cannot propagate Ca^{2+} action potentials. In other preparations it appears that Ca^{2+} action potentials arise locally and are not propagated actively (*5*). Conducted Ca^{2+} spikes have also been reported (*1*). In some cases the Ca^{2+} channels in the processes seem to be functional only at an early stage of development (*2, 3*). Therefore it was important to find out whether functional Ca^{2+} channels exist at the growing tip of differentiated cells that already have well-developed Na^+ action potentials. We started with neuroblastoma cells (*13–15*) because of their large size. This report describes simultaneous recordings of calcium action potentials directly from a distant growth cone (optically) and from the soma (electrically). In addition, simplifications in the apparatus are described.

We found that a 5-mW Hughes H3025 He-Ne laser (the size of a flashlight) provides sufficiently stable illumination (632.8 nm); fluctuations of light intensity were only 10^{-3} of the resting intensity (bandwidth, 10 Hz to 1 kHz). This laser is ~20 times more stable than a 50-mW Spectra Physics laser (*12*) and 20 times less expensive. The laser microbeam was formed by adding a ×10 objective and a lens with a 5-cm focal length in front of the epi-illumination system of the microscope (*12*). By adjusting the lens, the diameter of the microbeam could be varied from 1.5 to 120 μm. Additional details, including the correction of the fluorescence records for dye bleaching, have been described (*12*).

Optical signals obtained from cell bodies, a growth cone, and a process are shown in Fig. 1. The cell body was first hyperpolarized and then depolarized by passing current through a stimulating electrode. The optical recording (noisy line) closely follows the potential change, indicating the linearity of the dye response, over a large range of membrane potential (Fig. 1A).

Moolenaar and Spector (*14*) used voltage-clamp experiments to show that in these cells tetrodotoxin (TTX) blocks the Na^+ channels, that there are no fast inward currents in Na^+-free tris saline, and that the slow action potential is due to inward Ca^{2+} currents. Furthermore, Ca^{2+} currents may be blocked by Co^{2+} ions. In the experiments described below we used the same type of cells and some of the same pharmacological tools to obtain Ca^{2+} action potentials in the soma or along the process and record the response directly from the growth cone.

Figure 1B illustrates an optical recording of a Ca^{2+} action potential. The improved signal-to-noise ratio in the optical recording was obtained by filtering the high-frequency component of the optical noise (shot noise). In Fig. 1B both the electrode and the fluorescence signals were passed through identical resistance-capacitance filters having a time constant of 5 msec. Figure 1C shows the simultaneous recording of an action potential from the soma (with an electrode) and from a 600-μm distant growth cone (under the microbeam). The optical recording from a 4-μm process is shown in Fig. 1D. Somatic electrical activity passively spreads over a long distance in the processes of these cells, which have relatively large space constants [up to 1000 μm (*12*)].

Three types of observations suggest that the Ca^{2+} action potentials recorded from growth cones do not merely reflect passive spread of the action potential from a soma. (i) In three experiments where the somata were stimulated with an intracellular electrode, the rapidly rising phase of the action potential in the soma was slower than that in the growth cone (for example, Fig. 1C). Passive spread from the soma should give the opposite result. (ii) In five experiments the delays between the fastest portion of the rising phase of the action potentials in the soma and the growth cone were less than 1 to 2 msec. Passive spread of soma responses would give rise to a

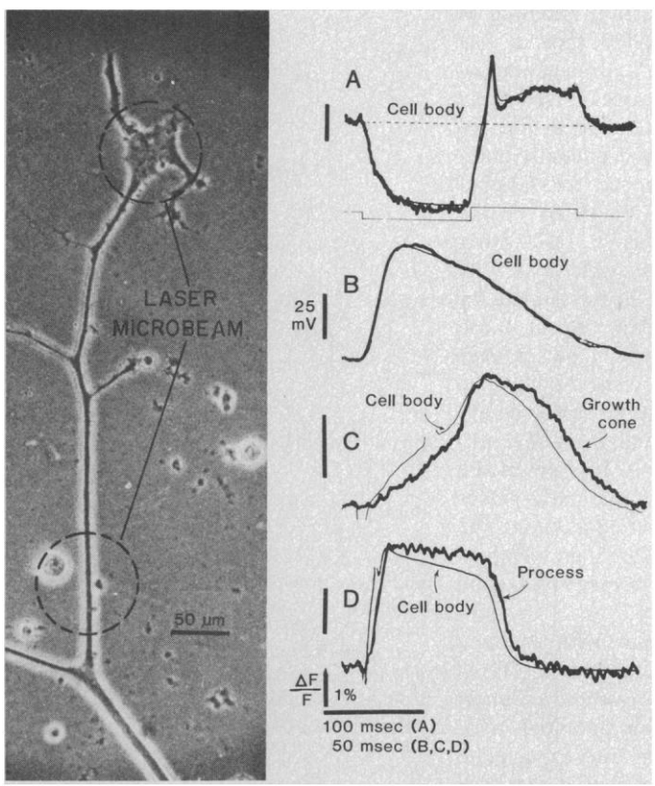

Fig. 1. Electrical and optical recordings from somata, a process, and a growth cone. Tissue culture dishes were mounted in a temperature-controlled (37°C) attachment on the stage of a Zeiss Universal microscope. Cells were impaled with 50- to 90-megohm microelectrodes filled with 3*M* KCl. Microelectrodes were controlled by M-103 Narishigi hydraulic manipulators mounted on the movable stage. To permit accurate comparison of electrical and optical recordings from the soma (A and B), cells were impaled with two microelectrodes, one for passing current and the other for recording the voltage response. To record from a given point, the cell could be moved by means of the microscope stage, with respect to the fixed microbeam, while maintaining a stable electrode penetration. Neurons were stained with saline containing 10 μ*M* oxonol dye (WW802) (*16*). The dye was left in the bath during the measurements. The microbeam size was 30 to 40 μm. Excitation light was removed from the fluorescence with an RG-665 cutoff filter; an EMI 9658R photomultiplier was used. (A) Comparison of electrical (thin trace) and fluorescence (noisy trace) recordings from the soma. Four trials were averaged. Saline composition: 120 m*M* Na^+, 5.4 m*M* K^+, 4 m*M* Ca^{2+}, 0.8 m*M* Mg^{2+}, 10 m*M* glucose, and 10 m*M* Hepes buffer adjusted to *p*H 7.4. Osmolarity was adjusted to 340 milliosmoles per liter with sucrose. The optical signal was filtered through a resistance-capacitance filter with a time constant of 0.5 msec. (B) Calcium action potential from another cell, obtained in medium containing 20 m*M* Ca^{2+}, no Na^+, and 15 m*M* TEA, and 120 m*M* tris ions. One trial was recorded. (C) Comparison of the time course of Ca^{2+} action potential in the soma and in the growth cone recorded from a third cell in normal medium to which 10^{-6}*M* TTX and 15 m*M* TEA were added. One trial was recorded; the process length was 600 μm, growth cone diameter 50 to 60 μm, and neurite diameter 5 μm. The fastest portion of the rising phase of the action potential in the growth cone is faster than the corresponding rising phase in the soma. (D) Same as (C) except that the recording was made from a segment of a 4-μm process. Four sweeps were averaged. The photograph shows a typical growth cone of a N1E-115 neuroblastoma cell.

delay of 4 to 15 msec under similar conditions (example in Fig. 2C). (iii) In one experiment we measured the amplitude of the growth cone response to an 80-mV Ca^{2+} action potential evoked in the soma. After the Ca^{2+} action potential was blocked by 10 m*M* Co^{2+}, the growth cone response to depolarization of the soma by 70 mV (by passing current) was 2.3 times smaller. It is clear that in the presence of Ca^{2+} there was active depolarization somewhere along the process.

In order to locate active Ca^{2+} channels along the neurite, we stimulated the neurite extracellularly close to the growth cone [600 to 1000 μm, or 0.7 to 1.5 space constants (*12*), away from the soma]. In more than 20 experiments we observed graded subthreshold soma responses rather than all-or-none responses (*4*), whereas direct stimulation of the soma resulted in an action potential in the soma; this suggested that it was impossible to generate a propagating action potential in the process. However, since Ca^{2+} action potentials in the somata of these cells were frequently graded as well, our evidence for lack of active Ca^{2+} channels in some of the processes is not conclusive. In 25 other experiments it was possible to evoke Ca^{2+} action potentials in the somata by stimulating the neurites. In all these cases, when hyperpolarizing pulses were applied to block the somatic spike, we still failed to detect (in the soma) an all-or-none action potential from the process [compare with (*4*)]. In view of the long space constants (*12*), such pulses could block action potentials along the process as well. Therefore it is still possible that some of the processes propagate Ca^{2+} action potentials.

In three experiments with medium containing tetraethylammonium (TEA) and TTX we obtained indirect support for localized Ca^{2+} action potentials at or near growth cones. In one experiment (Fig. 2A) the action potential recorded from the growth cone preceded the one recorded from the stimulated soma, suggesting a lower threshold for the Ca^{2+} action potential at or near the growth cone. In another experiment subthreshold stimulation of the soma elicited a Ca^{2+} action potential that was recorded from the growth cone (Fig. 2B). A hyperpolarizing pulse passively spread from the soma and was detected in this remote growth cone (neurite length, 850 nm). The depolarization and hyperpolarization of the soma were equal but the depolarization of the growth cone was twice the hyperpolarization (Fig. 2, B and C), indicating additional depolarization somewhere along the process. The long delay (20 msec from peak to peak) in this experiment and the existence and timing of the inflection point on the growth cone action potential are consistent with the occurrence of a localized action potential at or near the growth cone. In another experiment (Fig. 2D), where the neurite was stimulated next to the growth cone, a delay of ~ 30 msec was observed between the peaks of the action potentials in the growth cone and the peak of the soma response. This large delay and the very slow initial rise of the soma response suggest that the process did not conduct a regenerative action potential.

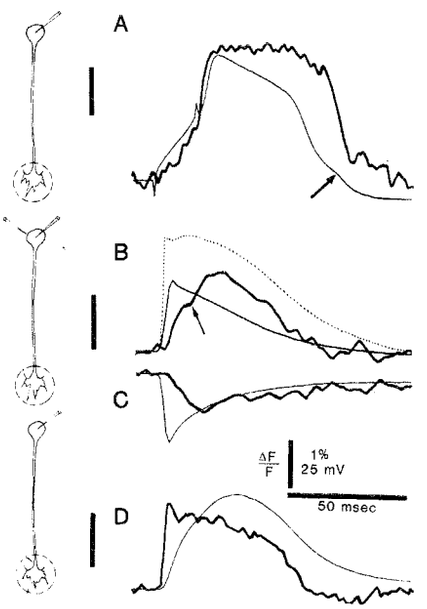

Fig. 2. Evidence for Ca^{2+} action potentials at or near growth cones. The normal saline contained 15 m*M* TEA and 10^{-6}*M* TTX. The thick traces are fluorescence recording from the growth cone. (A) Comparison of the time course of Ca^{2+} action potential in the soma and in the growth cone. The amplitudes were arbitrarily normalized to the same height. One trial was recorded; the process length was 700 μm, growth cone diameter 40 to 60 μm, and neurite diameter 5 μm. The soma was intracellularly stimulated with a 120-msec current pulse. The rapidly rising phase of the growth cone response preceded the action potential in the soma. The shoulder (arrow) in the falling phase of the somatic action potential may be due to passive spread from the growth cone. (B) Suprathreshold (dotted line) and subthreshold responses to extracellular stimulation. The fluorescence recording from the growth cone shows its response to the subthreshold depolarization of the soma. The arrow shows the inflection point on the rising phase of the growth cone response. Since the data are noisy, two trials were averaged. The inflection point was clearly evident in each of them. (C) Hyperpolarizing pulse in the soma and fluorescence recording from the growth cone; note the large peak-to-peak delay for passive spread. Two trials were averaged. (D) A 500-μm neurite of another cell was stimulated next to its growth cone. Note the slow initial rise of the soma response and the long peak-to-peak delay.

The results presented here provide strong evidence for the existence of Ca^{2+} action potentials in the processes of neuroblastoma cells at or near their growth cones. They raise the possibility that while some of the neurites conduct Ca^{2+} spikes, other processes fail to conduct actively, although Ca^{2+} action potentials may arise locally at or near their growth cones.

As demonstrated here, more systematic studies of the development of excitability and other electrical events in the growth cone and of regional variations in electrical properties of nerve cells should be possible with a combined electrophysiological and optical approach. Better spatial resolution may be achieved by simultaneously recording from several sites or by using patch electrodes or localized microperfusion techniques together with optical measurements. Greater sensitivity may also be obtained (*16, 17*). We recently studied thin (1 to 2 μm) processes and small growth cones of dorsal root ganglion cells (*18*). The minilaser microbeam may also prove useful for the study of presynaptic electrical events during synaptogenesis and of localized presynaptic chemosensitivity in remote segments of the axon or dendrites.

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16. The dye WW802, [1,3-dipentylbarbituric acid-(5)]-[1-*p*-sulfophenyl-3-methyl-5-pyrazolone-(4)]-pentamethinoxonol, was our best choice (*12*), yet it is still a poor probe because photodynamic damage and dye bleaching limit the duration of the experiments and make them difficult. However, photodynamic damage was reduced at least 100-fold by removing oxygen from the saline (*11*). We think that by alternately perfusing oxygen-free and oxygen-enriched solutions, much longer experiments, employing signal averaging for better resolution, would be feasible without anoxia.
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Intravenous Self-Administration of Nomifensine in Rats: Implications for Abuse Potential in Humans

Abstract. *Rats acquired and maintained intravenous self-administration of nomifensine, a new antidepressant compound. Additional experiments implicated dopamine-containing neurons in this behavior. These findings, along with the marked pharmacological similarities between nomifensine and such drugs as cocaine and methylphenidate, indicate a potential for nomifensine abuse by humans.*

The tetrahydroisoquinoline nomifensine is a member of a new class of compounds that have significant antidepressant properties in man (*1*). The compound is being used clinically in Europe and is being considered for use in North America. The pharmacological and biochemical properties of nomifensine differ somewhat from the classic tricyclic antidepressants. The compound shares with tricyclic compounds the common property of inhibiting the uptake of norepinephrine into brain nerve endings (synaptosomes), but differs in that it is also a potent inhibitor of dopamine (DA) uptake by synaptosomes obtained from brain regions that are rich in DA (*2, 3*). Furthermore, unlike many tricyclic compounds, nomifensine is a weak inhibitor of synaptosomal uptake of serotonin (*3, 4*).

In rats nomifensine has been found to increase locomotor activity and, at higher doses, to produce stereotyped behavior (*5*). These effects are commonly induced by drugs known to increase the activity of central DA systems and are consistent with the effect of nomifensine on DA uptake (*6, 7*). The fact that 6-hydroxydopamine lesions of the ascending DA systems or reserpine treatment abolishes nomifensine-induced stereotypy confirms that nomifensine is an indirectly acting DA agonist (*8*). Apart from motor stimulation and stereotypy, another universal property of such indirectly acting DA agonists as cocaine, amphetamine, and methylphenidate is that they can be self-administered intravenously by animals (*9*) and have the potential for abuse by humans (*10*). Because the pharmacological profile of nomifensine appears to be very similar to that of cocaine and methylphenidate, we sought to investigate its potential for abuse by determining whether the compound would support self-administration behavior in the rat.

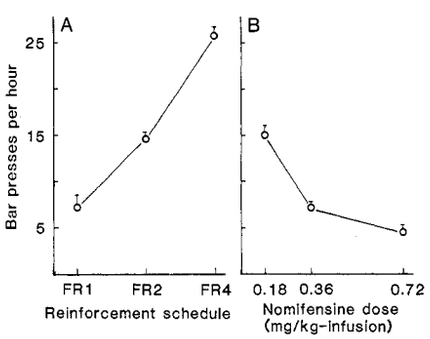

Fig. 1. (A) Number of bar presses per hour to obtain nomifensine (0.36 mg/kg per infusion), as a function of reinforcement schedule. On the FR1 schedule every bar press resulted in an intravenous infusion of nomifensine, whereas on the FR2 and FR4 schedules every second and fourth bar press produced an infusion. (B) Number of bar presses per hour (on an FR1 schedule) to obtain nomifensine, as a function of dose per infusion. Each data point represents the mean ± standard error for seven animals. Six rats with no history of drug self-administration bar-pressed for saline (operant level) at the rate of 0.15 ± 0.01 presses per hour.

Male Wistar rats weighing 300 to 320 g at the time of surgery were implanted with a Silastic jugular catheter under pentobarbital anesthesia (*11*). One or two days later, they were given access for 4 to 6 hours per day to a lever mounted on one wall of the cage. Each depression of the lever produced a 4-second infusion of 0.2 ml of nomifensine hydrochloride (0.6 mg/ml) solution through the catheter. In the first experiment, self-administration of cocaine (1.25 mg/ml) was established (*12*). After 4 to 5 days of stable responding, the animals were transferred to nomifensine to determine whether the compound would cause them to maintain bar pressing. In subsequent experiments, naïve animals were given immediate access to nomifensine to determine whether they would initiate and maintain bar pressing to obtain the drug.

Nomifensine caused maintained self-administration behavior in the animals that had previously acquired the behavior with cocaine reinforcement. More important, nomifensine was effective in causing the initiation and maintenance of bar pressing in the naïve animals. Typically, the rate of bar pressing was somewhat erratic for the first few days and then stabilized within 3 to 5 days. To establish that the animals were responding to maintain a relatively constant blood level of nomifensine, several additional experiments were conducted. First, the schedule of reinforcement was varied so that the animals had to press either once (FR1), twice (FR2), or four times (FR4) for each infusion (*13*). Second, the amount of nomifensine per infusion was varied.

The results of these experiments are shown in Fig. 1. The rate of bar pressing varied significantly ($P < .001$, analysis of variance) as a function of the operant schedule. This relation appeared to be linear, with the rate on the FR4 schedule being approximately four times greater than that on the FR1 schedule (Fig. 1A). The rate of responding also varied significantly ($P < .001$) as a function of the dose per infusion (Fig. 1B). Both experiments demonstrate that intravenous nomifensine is reinforcing and that animals will work to maintain relatively constant serum (and presumably brain) concentrations of the drug. At the end of the experiments with nomifensine, the animals were transferred to solutions containing imipramine (0.5 mg/ml). In accordance with the results of experiments on monkeys (*14*), imipramine did not cause

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