yond any capacitive artifacts, allowing more accurate measurement. The peak inward current during the 30-mV test pulse was small after conditioning steps of low or moderate (20-, 50-, and 80-mV) voltages, indicating partial inactivation during those conditioning steps. However, the current approached the amplitude of the control current after conditioning steps of sufficient positive potential (for example, 120 mV) to retard the entry of Ca and Sr. The absence of inactivation of the inward current after large positive potentials lasting 100 msec indicates that inactivation of the Ca channel does not occur in response to depolarization. Potential-independent inactivation as displayed by the Ca channel departs qualitatively from the potential-dependent inactivation characteristic of the Na channel described in the Hodgkin-Huxley model (*10*).

The injection of the Ca chelating agent EGTA into *Paramecium* produces prolonged action potentials (*13*). In voltage clamp experiments we found that injection of EGTA retards inactivation of the Ca current so that relaxation of the early current is slowed, and a net inward current persists for hundreds of milliseconds. This suggests that it is the accumulation of free Ca^{2+} rather than the passage of Ca through its channel that leads to inactivation of the channel.

A possible blocking effect of intracellular Ca^{2+} on the Ca conductance was first indicated in a study of internally perfused barnacle muscle (*14*). A reduction of the Ca spike after elevation of intracellular EDTA-buffered Ca could not be compensated by a proportional increase in extracellular Ca. Studies on perfused molluskan neuron (*15*) and tunicate egg (*16*) also indicate that the Ca conductance can be influenced by EGTA-buffered intracellular Ca concentration. Our study on *Paramecium* indicates that entering Ca^{2+} produces physiological inactivation of the channel, and does so within a time frame suitable to act during the course of a single Ca response (that is, graded Ca action potential of *Paramecium*), and may therefore participate in the repolarization of the excited membrane.

The discovery of this new regulatory activity of the Ca ion has provided a clearer understanding of how Ca entry and accumulation may be regulated in *Paramecium*. Depolarization activates Ca channels, allowing Ca entry through the surface membrane covering the cilia. The rapid rise in Ca concentration in the minute intraciliary space results in a rapid inactivation of the Ca channels, seen as a relaxation of the inward current. However, complete inactivation under prolonged depolarization is not predicted by our findings, since a steady state must be established between Ca entry and elevation of the intraciliary Ca concentration on the one hand, and the resulting inactivation of Ca channels by elevated intraciliary Ca on the other hand. This negative feedback relation between Ca entry and Ca-dependent inactivation should result, during steady depolarization, in an elevated intraciliary Ca concentration maintained by a small steady Ca current equal to the loss of Ca from the cilium. That a sustained depolarization produces a sustained Ca current and a maintained increment in intraciliary Ca concentration has, in fact, been inferred from the behavior of the cilia under depolarization (*17*); reversed beating, which relaxes only slowly during prolonged depolarization, is a sensitive indicator of elevated intraciliary Ca concentration (*8*).

A parallel investigation, by techniques that differ in part from the ones that we used, has also indicated a Ca-dependent, voltage-insensitive inactivation of the Ca conductance in molluskan giant neurons (*18*). The presence of similar behavior in Ca systems in such phylogenetically diverse organisms suggests that Ca-dependent inactivation may be a universal characteristic of Ca channels.

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19. We thank D. Tillotson for providing ideas throughout the project, S. Hagiwara for stimulating discussions and for criticism of the manuscript, W. Moody, M. Masuda, L. Byerly, and J. Umbach for helpful comments, and R. Rivera for technical assistance. Supported by NSF grant GNS 77-19161.

13 July 1978; revised 11 October 1978

Transient Synapses in the Embryonic Primate Spinal Cord

Abstract. *Electron microscopic and tritiated thymidine autoradiographic analysis of the embryonic spinal cord in the rhesus monkey reveals considerable rearrangement of cellular and synaptic relationships in the posterior (sensory) quadrant during early developmental stages. This remodeling involves the death of an entire population of neurons that received synapses from sensory afferent axons and the possible relocation of these afferents upon subsequently generated viable substantia gelatinosa neurons.*

The complex cellular organization and synaptic circuitry of the adult central nervous system is the end result of an enormous number of changes that occur during ontogenetic development (*1*). We now report that considerable reorganization of synapses occurs in relation to the genesis of transient neurons in the region of the prospective substantia gelatinosa Rolandi of the primate spinal cord. The substantia gelatinosa, a structure that plays a role in nociception (*2*), consists of a translucent mixture of neurons and fibers forming lamina II of Rexed in the dorsal horn of the spinal cord (*3*). Analysis of the simplified spinal cord of early embryos has revealed new developmental mechanisms for elaborating the extremely complex synaptic relationships of this region.

Fifteen rhesus monkey fetuses in the first third of the 165-day gestational period were fixed either by immersion or by vascular perfusion with 1 percent glutaraldehyde and 1.25 percent paraformaldehyde. Transverse slices (0.3 mm) from the fetal cervical cord were embedded in Epon-Araldite. Thick (1-μm) sections were stained with toluidine blue or with a modified Protargol-gold

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method. The trimmed portion of the blocks containing the posterior quadrant of the cord were cut at 500 Å, mounted on Formvar-coated grids, and stained with lead citrate and uranyl acetate. Electron-micrograph montages of the entire area of the posterior quadrants were made at a power of 4600. Serial sections were made from selected sites for three-dimensional reconstructions at powers of 10,000 to 46,000. Another series of monkeys exposed to [^{3}H]thymidine at various embryonic ages and killed after birth were processed for autoradiography (*4*). This experimental series was used to determine the time of origin of neurons of the posterior horn.

Synaptic junctions were first found in the posterior quadrant of the cervical cord at embryonic day 31 (E31). This is 2 weeks before they first appear in the ventral (motor) horn of the cord (*5*). The earliest synapses are located in the oval bundle of His (*6*), a part of the marginal zone (Fig. 1A) containing primary sensory axons originating from the dorsal root ganglion (DRG). These axons enter the cord, bifurcate, and direct a branch rostrally and caudally (*7*). With the addition of fibers, the oval bundle enlarges and eventually becomes the dorsal funiculus of the adult cord (Fig. 1C).

Two morphologically distinct synaptic types can be recognized in the oval bundle by approximately E30. One type involves the small dendritic appendages of borderline cells (so called because they are situated at the inner aspect of the oval bundle where it borders the mantle zone) (Fig. 1A). The presynaptic axons run longitudinally and have characteristic lightly stained axoplasm (Figs. 1A and 2A). The synaptic gaps are approximately 300 Å wide. The apposed presynaptic and postsynaptic membrane thickenings are asymmetrical and small; clear, round synaptic vesicles cluster at the presynaptic sites.

The second type of synapse is similar ultrastructurally and it also involves lightly stained afferent axons. It differs in that contact is with the leading process of the borderline cells or of cells lying deeper in the mantle zone (Figs. 1A and 2B). Autoradiographic analysis establishes that the permanent neurons of the posterior horn, including the substantia gelatinosa cells, have not yet been generated at this age and thus are not engaged in the initial period of spinal cord synaptogenesis.

By E40 the borderline cells are surrounded by newly arrived axons and lie fully within the oval bundle (Fig. 1B). Additional new synapses have appeared in the mantle layer of His (*6*) in the region of the prospective dorsal horn. These synapses occur within acellular clefts or bisecting interfaces, which become prominent at this age (Figs. 1B and 2C). These names reflect their clear appearance in Nissl-stained sections, which is attributable to an abundance of fibers. These clefts are oriented dorsoventrally, perpendicular to the mediolateral pathway of migrating neurons (Figs. 1B and 2C). They appear to be formed in part as a consequence of the invasion of darkly stained axons originating from ascending or descending fibers of the oval bundle. After entering the clefts, these axons form synapses with the beaded leading processes of presumptive posterior horn cells (Fig. 1B). The leading processes are initially oriented mediolaterally; they make a sharp right angle turn as they enter a cleft and usually follow the debris of some degenerating cells. Electron-microscopic analysis at subsequent developmental stages (Fig. 2D) establishes that synapses within the clefts are the forerunners of one class of adult substantia gelatinosa synapses (*8*). In support of this conclusion, [^{3}H]thymidine autoradiographic analysis shows that genesis of neurons of the substantia gelatinosa begins in monkeys a few days before the appearance of these synapses, at approximately E38 (Fig. 2E).

By E50, primary afferents with lightly stained axons enter the acellular clefts where they join the dark axons in forming synapses with cells of the prospective posterior horn. This region now begins to acquire the characteristics of lamina II of Rexed. All seven types of synaptic junctions characteristic of the adult monkey (*8*) can be recognized (Figs. 1C and 2D). These involve both dark and light afferents and dendrites, leading processes and somata of the substantia gelatinosa neurons.

At E50, most borderline cells show ul-

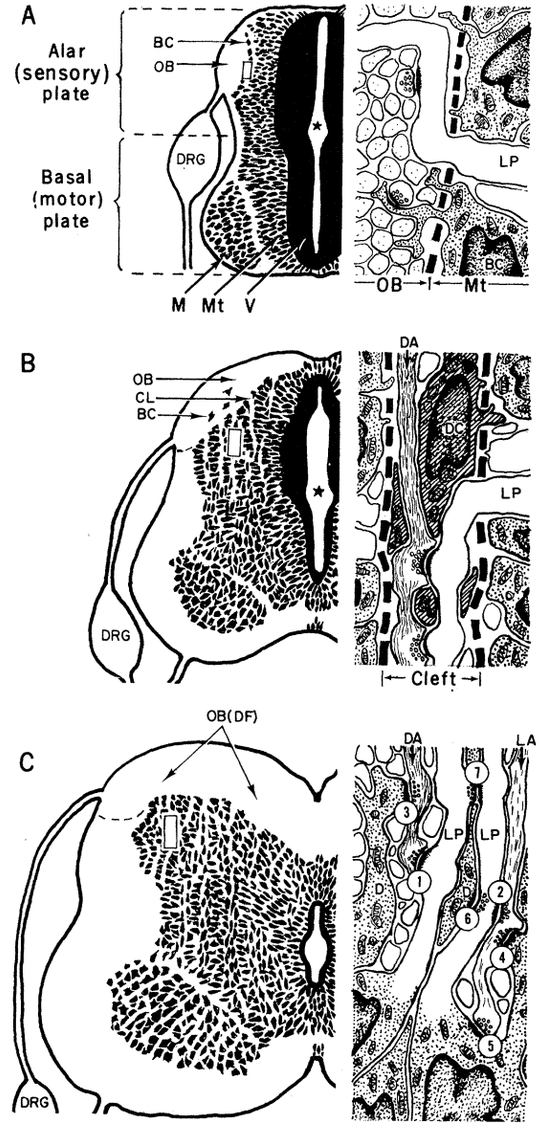

Fig. 1. Semischematic illustration of the cross section of one half of the spinal cord (left) and typical synaptic contacts (right) in the rhesus monkey at early embryonic stages. (A) Spinal cord of an embryo between E30 and E32 with well-defined ventricular (*V*) and mantle (*Mt*) zones and a thin marginal (*M*) zone that enlarges at the level of the alar plate into the oval bundle (*OB*). Asterisk indicates sulcus limitans that delineates alar and basal plates. Area outlined by the rectangle is enlarged on the right to illustrate temporary synapses that light afferent (*LA*) axons establish with the leading processes (*LP*) and dendrites of borderline cells (*BC*) that penetrate the interface (dashed line) between mantle zone and oval bundle. (B) In embryos between age E38 and E42, the mantle zone of the alar plate transforms into the prospective dorsal horn and develops acellular vertical clefts (*Cl*). One cleft from the area marked with a rectangle is enlarged to display synapses between dark afferent (*DA*) axons and bulbous dilatations of a leading process (*LP*) originating from a substantia gelatinosa neuron. (C) Spinal cord between E48 and E53 with a well-developed posterior horn. Area within the rectangle contains seven "permanent" types of synapses (1 through 7) between light and dark afferents with leading processes, dendrites, and somas of the substantia gelatinosa neurons. Additional abbreviations: *DC*, degenerating cell; *DF*, dorsal funiculus; and *DRG*, dorsal-root ganglion.

trastructural evidence of degeneration—shrinkage of perikaryon, dark vacuolated cytoplasm, swollen mitochondria, dilated Golgi apparatus, and rough endoplasmic reticulum (Fig. 2F). Occasionally synapses may persist on the surface of degenerating cells. In the course of subsequent development, all borderline cells disappear as the oval bundle shifts medially and forms the dorsal funiculus (Fig. 1C).

The fate of axons that terminated on degenerating borderline cells is uncertain. Some may degenerate, as evidenced by the large numbers of bipolar neurons that die during normal development in peripheral ganglia (*9*). Nevertheless, we have observed degeneration of borderline cells but not of their presynaptic element. It is possible, therefore, that after the death of primary target cells some axons may enter the posterior horn and form new synapses with the later-generated neurons of the substantia gelatinosa.

As no temporary synaptic relationships with transient neurons have so far been described in ultrastructural analyses of avian (*10*) and rodent (*11*) alar plate of the spinal cord at comparably early embryonic ages, such relationships may be unique for primates. However, other neuronal circuits within the central nervous system seem to develop by way of transient synaptic arrangements (*1*, *12*). Thus, in the developing superior colliculus, a certain number of synapses form on neurons that eventually degenerate (*13*). Some of the axons distributed in the lamina dissecans, a transient fiber layer of the embryonic cerebellum, form synapses before becoming redistributed to establish permanent contacts (*1*, *14*). Temporary synapses occur also in the subcortical strata of the monkey fetal subplate layer (*15*) in which thalamocortical axons "wait" several weeks before entering the cortical plate (*16*).

The synaptic remodeling in the posterior quadrant of the embryonic spinal cord reported here appears to be brought about by the death of an entire population of neurons, the borderline cells. These transient synapses may provide an

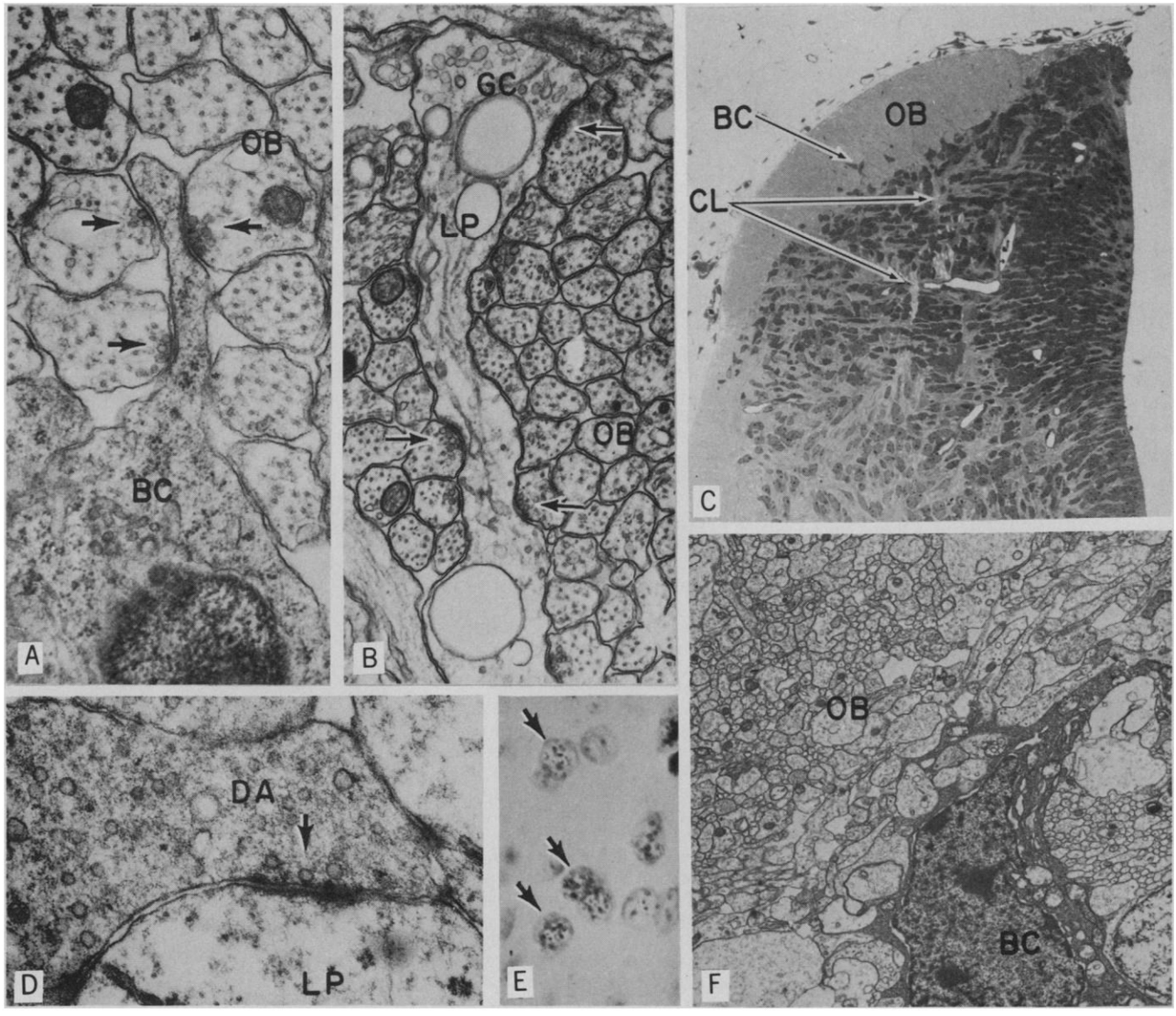

Fig. 2. (A) Electron micrograph of a borderline cell (*BC*) at E31. Its small dendritic appendages penetrate the oval bundle (*OB*) and form synapses (arrows) with ascending and descending light afferent axons (×32,000). (B) Synaptic junctions (arrows) between light afferents of the oval bundle and leading process (*LP*) in the same specimen (×25,000). (C) Light microscopic photograph of the posterior quadrant of the spinal cord in an E42 specimen displaying the oval bundle in which borderline cells become embedded and acellular bisecting clefts (*Cl*) in the area of prospective dorsal horn (×160). (D) Electron micrograph of synaptic junction (arrow) between a dark afferent (*DA*) axon and a leading process in the bisecting cleft at E53 (×50,000). (E) Light microscopic photograph of radioactive neurons (arrows) in the substantia gelatinosa in 3-month-old monkey that had been exposed to [^{3}H]thymidine at E38. (F) Electron micrograph showing degenerating borderline cell surrounded by axons of the oval bundle in an E53 specimen (×9000).

essential step in the formation of the permanent and more complex nociceptive neuronal system in the primate spinal cord.

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17. Supported by PHS grants NS14841 and NS12200. Monkeys with timed pregnancies were obtained from the New England Regional Primate Research Center, Southborough, Mass. The research was begun in P.R.'s laboratory in the Department of Neuropathology, Harvard Medical School. E.K. and B.C. were on leave from the University of Szeged.

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8 August 1978

Intraventricular Alloxan Eliminates Feeding Elicited by 2-Deoxyglucose

Abstract. *Evidence suggests that alloxan reacts with membrane-bound glucoreceptors and that it competes with glucose molecules for these sites. We therefore administered small quantities of alloxan into the cerebrospinal fluid of rats to determine what effect this might have on their ability to react to changes of glucose concentration. Rats treated in this manner did not eat as much as controls in response to the intraperitoneal administration of 2-deoxyglucose or to a 24-hour fast, and they became hypoglycemic significantly sooner than controls when fasted. The data suggest that the function of brain glucoreceptors is to protect the body from sudden decreases of glucose and that these glucoreceptors play little if any role in the normal regulation or maintenance of feeding, body weight, or blood glucose concentrations.*

The presence of glucoreceptive neurons within the central nervous system (CNS) has been suggested by physiological and behavioral experiments (*1*, *2*). Several functions have been attributed to these glucoreceptors, including control of normal feeding (*3*), body weight regulation (*4*), and protection against changes of blood glucose concentrations which could compromise brain functioning (*5*). Because glucose is a primary source of energy for most brain cells, it has frequently been assumed that CNS glucoreceptor cells are responsive to changes of energy availability as reflected by changes in the rate of glucose utilization (*6*, *7*).

However, a second mechanism has been postulated to exist in some cells for the recognition of changes of glucose levels. Research on the endocrine pancreas suggests that the insulin-secreting B (beta) cells initially respond to glucose molecules interacting with cell membrane receptors and only later respond to the change of intracellular metabolic activity generated by the transported glucose (*8*). There is also evidence for a membrane-bound glucoreceptor that influences secretion of glucagon from the A (alpha) cell of the pancreas (*9*). The experiments described herein suggest that the CNS glucoreceptors are similar and that their normal functions include initiation of reflexes that protect against glucoprivic challenges (during glucose deprivation) but do not include the control of normal eating or body weight regulation.

Alloxan is a toxic drug frequently used to elicit experimental diabetes mellitus in animals because large doses given systemically destroy B cells and hence limit the capacity of the organism to secrete insulin (*10*). The B cells can be protected from alloxan by the prior application of D-glucose (*11*), suggesting that alloxan molecules compete for the same receptor sites as glucose molecules (*12*). The observation that the protection against alloxan is influenced by the anomeric specificity of the glucose molecules (*13*) implies that alloxan is acting at a membrane-bound receptor [see (*8*)]. Alloxan has also been reported to reduce the capacity of the taste buds to respond to glucose, and prior treatment of the tongue with glucose protects against this response (*14*). Analogously, taste bud glucoreceptors have also been reported to be sensitive to the anomeric specificity of glucose (*15*). This suggests that membrane-bound glucoreceptors located in different organs may have similar properties.

Since there was considerable evidence for the presence of glucoreceptors in the brain (*1*-*5*), we administered small amounts of alloxan into the CNS by way of the ventricular system in an effort to determine if any responses attributed to these receptors were altered. We used female Wistar rats that were about 120 days old (375 g) at the beginning of the experiment. They were housed individually in standard hanging cages in a room with constant temperature and regular lighting conditions (12 hours of light and 12 hours of darkness; lights on at 7 a.m.) and had continuous access to water and food (Purina pellets).

Experimental rats received an injection of alloxan (40 μg in 2 μl of 0.9 percent saline) into the left lateral cerebral ventricle while they were anesthetized with Equithesin (3 ml/kg) and held in a stereotaxic instrument. The coordinates and procedures of the injection have been reported (*16*). Various control groups received either 2 μl of saline without alloxan injected into the left lateral cerebral ventricle, an intraperitoneal injection of 40 μg of alloxan, or no treatment at all. No reliable differences were observed among any of the control groups on any of the dependent variables monitored, and their data were pooled for the subsequent analyses.

Daily food intake and body weight were unaffected by the intraventricular (IVT) injection of alloxan for at least several months. These rats ate the same amount of food and gained weight at the same rate as rats in the combined control groups. Furthermore, preliminary observations indicated that the patterning of meals was also unaffected. This suggests that normal or spontaneous feeding does not rely on the integrity of whatever cells were affected by the treatment. Since no

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