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Neocortical transplants in the rat brain: an ultrastructural study

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Summary. Electron microscopic analysis of neocortical transplants in the cerebellum of the host animals showed that the nerve cells, glial cells, and neuropil of the transplants were normal. These transplants showed anatomical integration with the host brain through various regions of interface. Neuropil interfaces were found to have a high density of synaptic profiles, and medullary interfaces had a very small number of synaptic profiles.

Various studies, in recent years, have shown that embryonic neural tissues can be transplanted into the brain of the laboratory mammals. The success of neural transplantation has been demonstrated by using thymidine-H^3 autoradiography[2], neuroanatomical staining methods[3,4], and histochemical techniques[5]. However, except for findings on connectivity of the transplants[6,7], there is no adequate information available on the ultrastructural characteristics of the transplants. This study is aimed at providing this information, and presenting characteristics of different types of interfaces between neural transplants and the host brain.

Materials and methods. Laboratory-bred Long-Evans hooded rats were used in this study. The donor embryos of 16-, 17- and 18-day stages were used to obtain neocortical tissue for transplantation. The host animals were 15-day-old on the day of surgery, and received transplants in midvermis of cerebellum via cisterna magna. Each host animal received only 1 transplant, and it was 3.0 mm^3 in volume. The techniques of obtaining embryos, dissecting them, preparing neural tissues, and transplanting them were identical to those described earlier[2,8].

The host animals were sacrificed 3.5 months after transplantation, during which time the transplants had grown and become fully differentiated. The animals were deeply anesthetized and perfused transcardially with a fixative composed of 4% glutaraldehyde and 2% paraformaldehyde in a 0.2 M cacodylate buffer (pH 7.4). After removing the brains, 1-mm-thick blocks from the transplants and from various regions of interface were prepared. They were washed in 0.1 M cacodylate buffer containing 0.1 M sucrose, postfixed in 2% osmium tetroxide with 0.1 M cacodylate buffer and 0.2 M sucrose, block-stained in 2% uranyl acetate for 2 h, and dehydrated and embedded in Epon-Araldite. At first thick sections were obtained, which aided in selecting specific structures for ultrastructural analysis. Regions from the middle of the transplants, cellular interface, medullary interface and neuropil interface were selected for further sectioning. The sections were studied under a Philips-300 electron microscope.

Results. General comments. In the brains of the host animals the neocortical transplants had not only grown large but also were anatomically integrated with the surrounding cerebellar parenchyma. There was no evidence of any pathological reaction in the transplants or the host brain, or of any glial scar formation between them. Three different types of interfaces between the transplants and the host brain could be identified, and they were: cellular interfaces, neuropil interfaces and medullary interfaces. The last 2 interfaces showed many striking differences, and therefore they were selected for a detailed analysis. Further, material prepared for light microscopy, available in the labaratory, that was processed for Nissl staining, Bodian-protargol staining and Golgi-Cox impregnation, was also studied. It provided a valuable perspective for the interpretation of ultrastructural observations. Since findings based upon light microscopic preparations have been presented elsewhere[4,9-11], they are not included in this report.

Electron microscopic observations. In the neocortical transplants large pyramidal cells (fig. 1A), stellate cells (fig. 1B) and various glial elements (fig. 1C) could be identified. The pyramidal and stellate cells had large pale nuclei located centrally in the somata. The cytoplasm surrounding these nuclei was relatively light, and contained various organelles such as Golgi apparatus, rough endoplasmic reticulum and mitochondria. In the case of the pyramidal cells the rough endoplasmic reticulum was more abundantly found. From the somata of these neurons dendrites were seen to emerge, and they were characterized by the presence of mitochondria, smooth endoplasmic reticulum and clusters of ribosomes. Generally, the transition zone between the cytoplasm and the trunk of a dendrite could be readily identi-

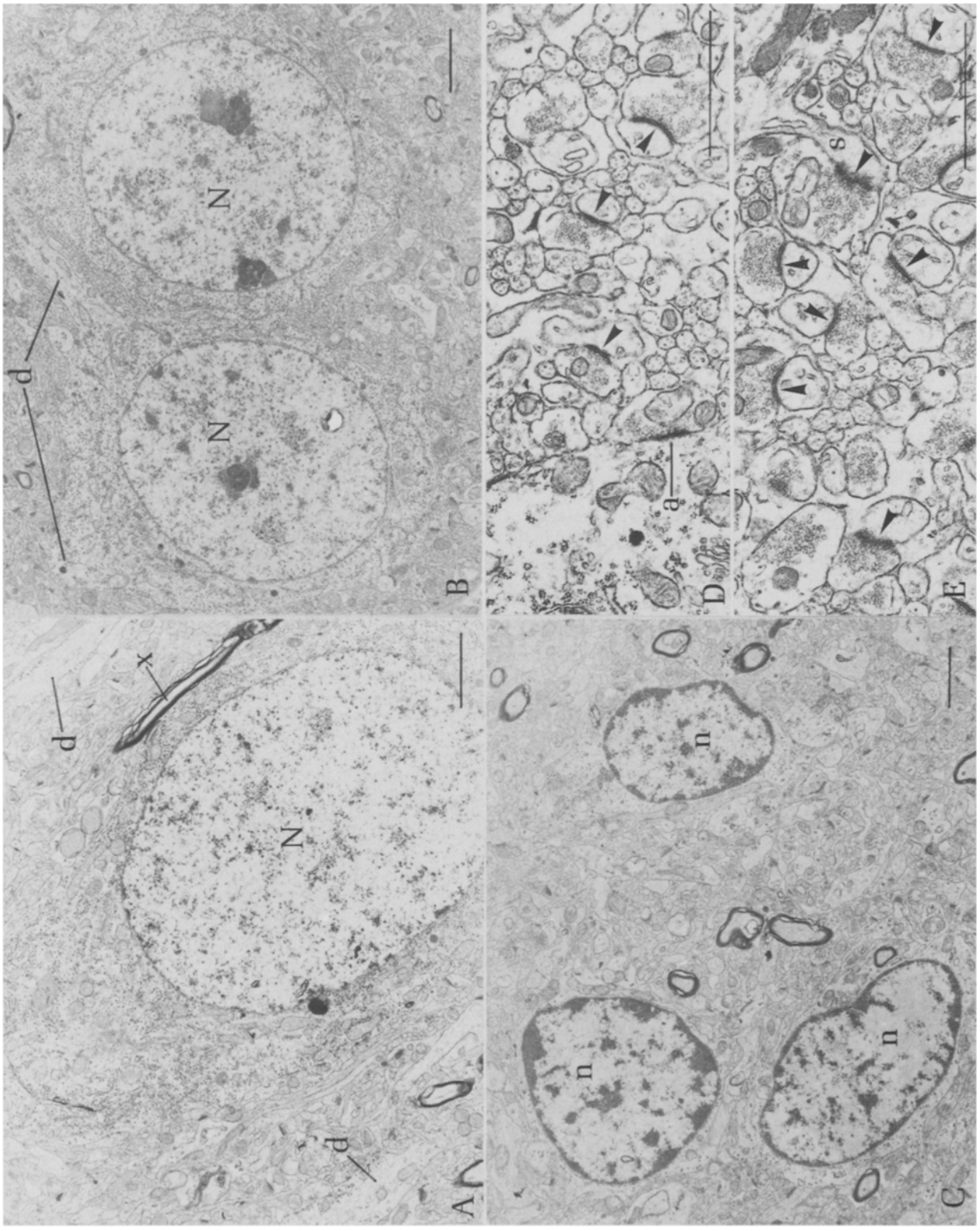

Figure 1. *A* Large pyramidal cell with its large pale nucleus (N). The orientation of the perikaryon towards the top left direction indicates the direction of its apical dendrite. Around this neuron, neuropil, dendritic processes (d) and myelinated axons (x) may be identified. *B* 2 stellate neurons (N). Their dendritic processes (d) emerging from the somata are indicated. *C* 3 oligodendroglia cells (n). Their nuclei are characterized by well-defined nuclear membrane and clumps of chromatin. Within the neuropil some thinly myelinated axons may be noted. *D* Axosomatic (a) and axodendritic (arrowheads) synapses. *E* A dendritic spine (s) receiving a synaptic terminal. The small size and shape of this spine suggests that other postsynaptic elements (arrowheads) too may be dentritic spines receiving synaptic terminals. Scale: 5 µm.

fied, but in the case of the apical dendrites of the large pyramidal cells, the cytoplasm appeared to extend into the dendritic trunk for a considerable distance. In addition to neurons, various neuroglial elements were observed in the transplants, and of them oligodendroglial cells were relatively more abundant than the astrocytes. The oligodendroglia cells were identified by their small-sized nuclei, that were somewhat electron dense and contained chromatin clustered within the nucleoplasm as well as along the inner aspects of the nuclear membrane (fig. 1C). They had a relatively small amount of cytoplasm, which was strikingly characterized by a large number of clusters of free ribosomes. The neuroglial elements were found all over the transplants within the neuropil, covering large surface areas of the somata as well as main dendrites of the neurons, and surrounding the capillaries.

The neuropil, within which the neurons and glia cells were embedded, was composed of dendrites and their processes, unmyelinated as well as thinly myelinated axons, synaptic profiles, glial processes, and small caliber capillaries. The axons with thin myelin were seen to course individually as well as in small bundles. They coursed in all planes in the transplants. These axons were organized in large bundles near the interface, but became separate inside the transplants. These observations indicated that they were the afferents from the host brain.

An analysis of synaptic profiles showed the presence of axosomatic (fig. 1D) as well as axodendritic synapses. In the latter case more synaptic endings were found on the spines of dendrites than on the dendrites themselves (figs. 1D, E). The synaptic profiles on the dendritic spines were densely distributed within the close vicinity of the dendritic fields of the neurons. In other regions, where glia cells were clustered together, relatively fewer synaptic profiles were observed.

Although all the interfaces were analyzed for this study, only two, namely neuropil and medullary interfaces, appeared to be of importance for a detailed ultrastructural characterization. The neuropil interfaces were formed between the neuropil of the transplant and molecular layer of the host cerebellum. These types of interfaces were characterized by a high density of synaptic profiles, and relatively large size of presynaptic terminals containing a large number of densely packed vesicles (fig. 2A). These synaptic profiles appeared different from those formed by the climbing fibers and parallel fibers in the molecular layer of the cerebellum. In comparison to this, the neuropil within the transplants contained relatively less densely packed synaptic profiles (fig. 2B). The presynaptic elements were small in size and contained a small number of synaptic vesicles. In some cases the synaptic vesicles were densely packed and in others they were loosely distributed. These features also were found at the regions of cellular interface. The medullary interfaces were characterized by the presence of myelinated axons of various sizes from the host cerebellum and neuropil of the transplants containing dendritic and glial processes. At these interfaces very few synaptic profiles were seen (fig. 2C). This finding was in contrast to that made on the neuropil interfaces. However, as one moved from the medullary interfaces to the center of the transplant, the density of synaptic endings increased, but it never reached as high as that seen at the neuropil interface.

Discussion. Neocortical transplants, as well as other neural transplants, that are parenchymally integrated with the host brain are found to be histologically well-differentiated and normal-looking. In support of these findings based upon light microscopic preparations, the observations presented in this report have shown that at ultrastructural level also these transplants look normal. These findings further confirm ultrastructual observations presented earlier[3]. In all these preparations of intraparenchymal neural transplants neither in light microscopic nor in electron microscopic preparations was any pathological reaction noticed. These observations, although valid for the intraparenchymal transplants, are not valid for the extraparenchymal transplants. A study of extraparenchymal transplants at light microscopic level revealed that they contain poorly differentiated, atrophying and shrunken neurons[12]. It is very likely that electron microscopic analysis of such extraparenchymal transplants would also reveal a variety of pathological features of the neurons, neuropil and synaptic profiles.

Studies on the connectivity of neocortical transplants in the cerebellum have shown that they receive afferents from the spinal cord, inferior olivary nuclei and pontine nuclei, the neural structures normally providing afferents to the cerebellum[11,13]. On the basis of these observations it may be suggested that very likely many, if not all, synaptic profiles found within the transplants may have been from the fibers providing afferents to the cerebellum. Thus, the afferents of the host cerebellum also became afferents of the neocortical transplants. Furthermore, since the afferents to the neocortical transplants, irrespective of their origin, arose from the fibers available in the immediate vicinity of the transplants, penetrated into them, and provided synaptic terminals on their neurons, it is safe to conclude that these afferents were non-specific in nature[9-11,13]. It is simply their physical proximity to the developing transplants, and not any specific chemical affinity, that determined the growth and penetration of the fibers or their collaterals into the transplants.

Neocortical transplants in the cerebellum of host animals receive afferents from those fibers that also provide afferents to the cerebellum in the form of climbing and mossy fibers. And yet, the ultrastructural characteristics of the synaptic endings within the transplants or at the neuropil interface did not show any characteristics of terminals of climbing fibers or glomerular formation of the mossy fibers. In a recent study on the cerebellar transplants that were transplanted in a cavity near hippocampus, Alvarado-Mallart and Sotelo[14] found that the axons arising from the nuclear mass, presumably the deep cerebellar nuclei, resulted in glomerular formation as they terminated upon the claw-shaped dendrites of the granule cells. These findings, when viewed comprehensively, indicate that the morphological characteristics of synaptic endings may be determined more by the postsynaptic elements upon which they terminate than the type of neurons from which they originate.

Earlier studies on connectivity of neocortical transplants have shown that medullary and neuropil interfaces have different characteristics of penetration of afferent fibers into the transplants[11]. In light of these observations it may be suggested that medullary interfaces, where very few synaptic profiles were seen, may be essential to provide afferent fibers that travel long distances and penetrate deep inside the transplants, and terminate within the neuropil or upon the somata and dendrites of the neurons. And, the neuropil interfaces, where a high density of synaptic profiles was observed, may be important for providing afferents to the neurons of transplants that are close to the host neural structure. Although from morphological viewpoint these differences appear interesting for further investigation, their bearing on physiological differences, if any, remains difficult to account for. Irrespective of these differences it is important to stress that presence of a viable interface between a transplant and the host brain is essential for establishment of anatomical connectivity between the two. In the absence of an interface, from anatomical viewpoint, a neural transplant is unintegrated with the host brain[9,10].

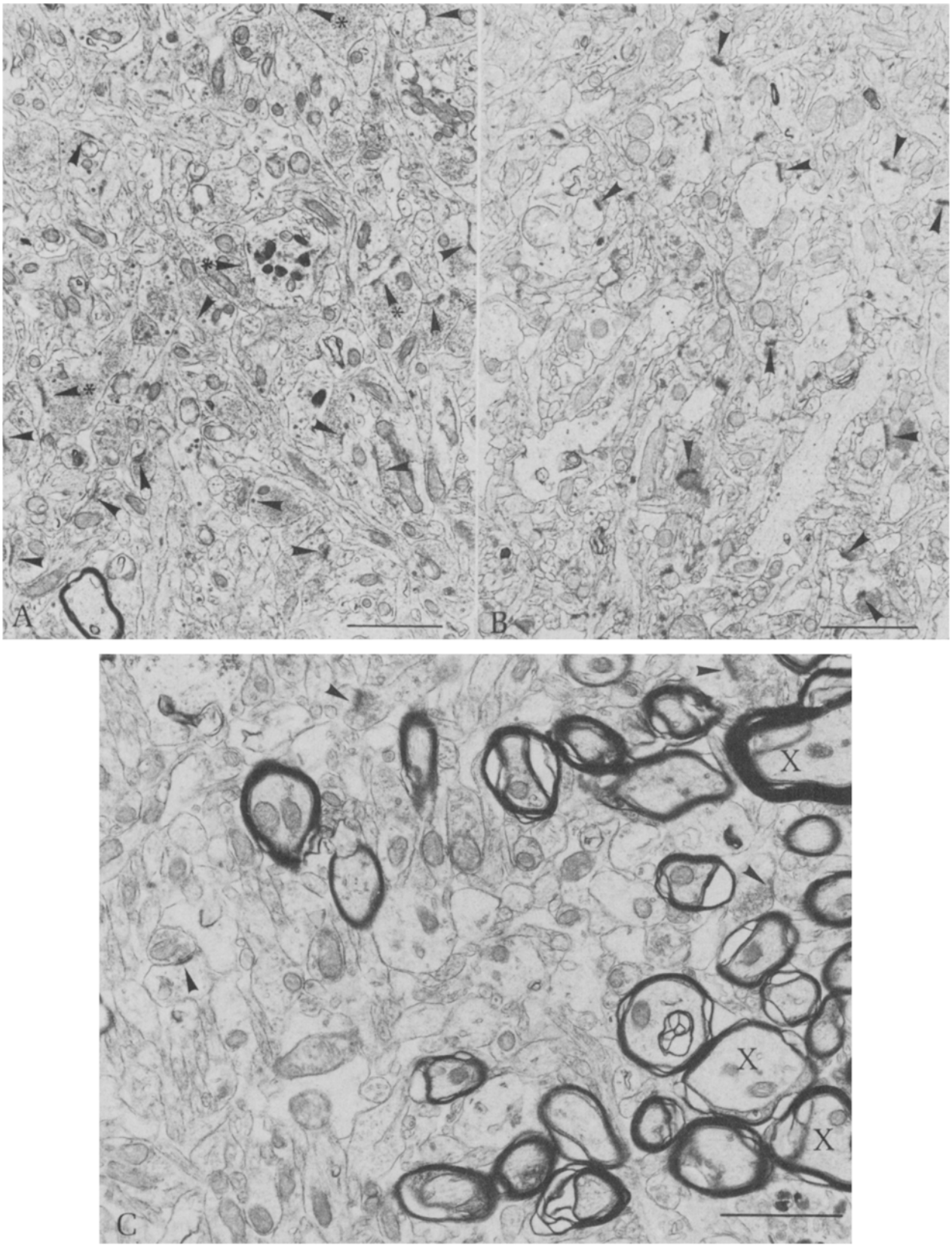

Figure 2. *A* Synaptic terminals (arrowheads) in the transplant very close to the neuropil interface. Note the high density of synaptic terminals, some of them very large in size (arrowheads with asterisks), and small size of the postsynaptic processes. *B* Synaptic terminals (arrowheads) in the neuropil of the transplant. They are relatively small in size and show a low density of distribution. The postsynaptic processes are relatively large in size. *C* The region of medullary interface. The afferent axons (x) of various sizes are seen to penetrate into the transplant (left). At such regions of integration very few synaptic terminals (arrowheads) are seen. Scale: 5 µm.

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Distribution of bilateral dorsal root potentials evoked by volleys in afferents entering lumbar and sacral segments of the spinal cord

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Summary. Ipsilateral dorsal root potentials evoked by volleys entering lumbar and sacral segments of the cord are largest at the level of entry of afferent volleys. Contralateral potentials resulting from stimulation of any of these nerves attain maximum amplitude in lower sacral and upper caudal segments.

The dorsal root potentials (DRPs) reflect depolarization of the central terminals of primary afferent fibers. It is considered that they indicate presynaptic inhibition, which controls the influx of sensory impulses into the cord[1-3]. The depolarization is produced in the immediate neighborhood of the place where the afferent volley enters the spinal cord and then it spreads to more distant segments. We have studied the DRPs produced by stimulation of L5 and Ca2 dorsal roots and recorded in 6 segments lying between them. In these experiments the DRPs evoked by volleys in each dorsal root were recorded after their spread in only one direction. It was found that ipsilateral potentials spreading caudally decrease to a smaller extent than the DRPs spreading cranially. On the contralateral side the DRPs transmitted cranially do not differ from ipsilateral potentials while depolarizations spreading caudally increase in segments close to the stimulated dorsal root[4]. These observations suggest that the effect of presynaptic inhibition on sensory information reaching more distant parts of the cord depends on the direction of spread and on the level of entry of afferent volleys. To obtain more insight into this question in the present experiments we investigated the longitudinal distribution of bilateral DRPs spreading in both directions. They were produced by volleys in peripheral nerves entering lumbar and sacral segments of the spinal cord.

Material and methods. The experiments were performed on 24 cats. Under short-acting thiamylal sodium anesthesia (25 mg/kg) the common carotids were tied and the animals were decerebrated at intercollicular level. After exposing the spinal cord the most caudal rootlets of equal thickness were separated bilaterally from 6 dorsal roots (L6 through Ca1) and prepared for recording the DRPs. 4 nerves; posterior tibial, superficial peroneal, caudal femoral cutaneous and pudendal were mounted on one side on the stimulating electrodes. The DRPs were produced by single pulses lasting 0.1 msec at 4 times the threshold strength.

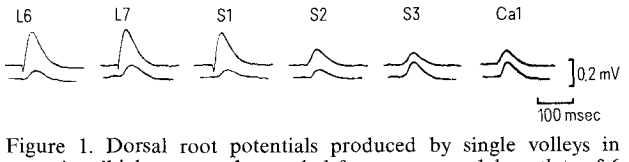

Figure 1. Dorsal root potentials produced by single volleys in posterior tibial nerve and recorded from most caudal rootlets of 6 consecutive dorsal roots. Upper trace of each record shows ipsilateral and lower trace contralateral potential. Symbols over records indicate the level of recording.

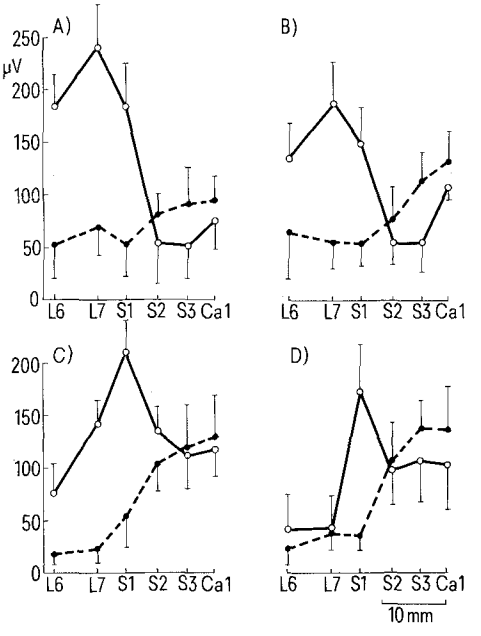

Figure 2. Distribution of the dorsal root potentials evoked by single volleys in posterior tibial (A), superficial peroneal (B), caudal femoral cutaneous (C) and pudendal (D) nerves and recorded from the most caudal rootlets of 6 consecutive dorsal roots. The distances between points of entry of dorsal rootlets into the cord are plotted as abscissae and the mean sizes of potentials as ordinates. Open circles show ipsilateral and closed circles contralateral potentials. Each point represents the mean ± SEM of 12–16 experiments.