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SUMMER COLLEGE IN BIOPHYSICS

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DEVELOPMENT I and II

DENERVATION AND REINNERVATION I and II

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C.T.P. COLLEGE ON BIOPHYSICS OF THE NERVOUS SYSTEM

DEVELOPMENT I: EARLY EVENTS IN THE DEVELOPMENT OF THE NERVOUS SYSTEM

Inductive interactions in the early embryo - primary neural induction

Hilde Mangold and Hans Spemann first showed in 1924 that the determination of the nervous system was the result of the interaction of mesoderm, invaginating during gastrulation, with the overlying ectoderm of the dorsal surface of the early embryo. The process of induction - the initiation of developmental processes in a particular region of the embryo by interaction with adjacent structures - became a key concept in embryology. The induction of the neural plate, the anlage of the nervous system, is known as primary embryonic induction. If the close relationship between ectoderm and mesoderm is not established, as for example when gastrulation fails, then the nervous system does not develop. Very little is known about the mechanism of inductive interactions in general and in particular the way in which the inducing signal is transferred from mesoderm to ectoderm, during primary embryonic induction.

Consequences of primary neural induction - the fate of different areas of the neural plate is settled by mid neural fold stages. Carl-Oloff Jacobson's grafting experiments on neural plate stage embryos: rotation of small areas of the neural plate at different times after gastrulation. Rotation before the mid-neural fold stage results in a normal adult nervous system. Rotation of parts of the neural plate after mid-neural fold stages results in a changed nervous system - the grafted cells develop according to their original position. Jacobson concluded that by the mid-neural fold stage the future anatomical structure of the CNS has been laid down and the fate of different areas of the neural plate is settled.

> Jacobson, C.O. 1964. Motor nuclei, cranial nerve roots and fibre pattern in the medulla oblongata after reversal experiments on the neural plate of axolotl larvae. Zool. Bidr. Upps. 36, 73-160.

Are there detectable changes in the membrane properties of neural plate ectoderm cells as a consequence of the transfer of the inducing signal? In the developing nervous system the consequence of induction of ectoderm cells by mesoderm is reflected in clear changes in the membrane properties of those ectoderm cells destined to become the nervous system. During the neural plate stages the resting membrane potential of neuroectoderm cells rises above that of the [illegible] ectoderm cells and has a different developmental fate. The rise in resting membrane potential of neuroectoderm cells is abolished by cardiac glycosides and is thought to be due to an increase in the activity of the sodium pump.

> Blackshaw, S.E. & Warner, A.E. 1976. Alterations in resting membrane properties during neural plate stages of development of the nervous system. J. Physiol. 255, 231-247.

Are these changes in membrane properties of neuroectoderm cells important for further development of the nervous system? Will inhibition of the sodium pump at neural plate stages affect the subsequent differentiation of nerve cells? Neural tube ectoderm dissected out of the embryo at late neural fold stages, disaggreagated and plated, will differentiate in monolayer cultures. The proportion of neurones differentiating can be measured quantitatively by cell counting. These experiments show that the treatment of amphibian embryos with cardiac glycosides during neurulation substantially reduces the number of morphogenetically identifiable nerve cells which differentiate in culture.

> Messenger, Angela E. & Warner, Ann E. 1979. The function of the sodium pump during differentiation of amphibian embryonic neurones. J. Physiol. 292, 85-105.

A relatively high resting membrane potential could be a necessary prerequisite for the differentiation of the voltage dependent conductance channels responsible for the action potential. Differentiation of the action potential mechanism occurs in cells which grow out from the spinal cord shortly after the neural tube closes.

> Spitzer, N. 1979. Ion channels in development. Ann. Rev. Neuroscience 363-340.

CELL PROLIFERATION & MIGRATION

How are neurones and glia generated in proliferative zones? His showed in 1887 that cell proliferation in the developing neuroepithelium is limited to the population of epithelial cells that lines the lumen of the neural tube.

[illegible] and neurones, the major components of the nervous system, [illegible] His microscope study suggested to him that the germinal epithelium was stratified, and that there were 2 distinct embryonic kinds of cells, one that gave rise to neurones and a second [illegible] gave rise to glial cells. More recent

^{3}H-thymidine studies have shown that in fact the epithelium is not stratified but consists of a single type of columnar cell which spans the epithelium between its ventricular (lumen of the tube) and external (pial) surface and whose nucleus migrates up and down within the cell during its mitotic cycle. The nucleus synthesizes DNA near the pial surface then migrates down to the ventricular surface for cell division.

Sauer, F.C. 1935. Mitosis in the neural tube. J. comp. Neurol. 62,377-405.
Sidman, R.L. 1970. Autoradiographic methods and principles for study of the nervous system with ^{3}H thymidine. In 'Contemporary research methods in neuroanatomy'. Ed. Nauta & Ebbesson. Springer Verlag.

Thus all types of neurones and glia are presumed to arise from a single type of stem cell. (This does not imply however that the germinal cell is totipotent - Jacobson's work shows that within the neural plate the germinal cells already form a mosaic of areas with different prospective fates).

Distinct sets of neurones originate in a fairly invariant timetable

Information about the time of origin of neurones in the developing nervous system comes from experiments using the technique of thymidine autoradiography. This method makes use of the fact that when a cell stops dividing it migrates away from the ventricular zone and is forever postmitotic. Pregnant mammals are injected with tritiated thymidine, the foetuses killed at varying times after injection and their brains examined autoradiographically. The heavily labelled cells seen in the autoradiographs are those neurones which were undergoing their final round of DNA synthesis at the time of injection - the 'birthdate' of the neurone. What has emerged from these autoradiographic studies is that each subpopulation of neurones in a given region tends to finish dividing during a limited and well-defined period. So for example, Rakic has shown that neurones in a given cortical layer of the rhesus monkey have similar birthdays. The first neurones of the cortical plate to be born are generated around embryonic day 45 (in a gestation period of 165 days). These are the neurones of layer 6, the deepest cortical layer. Layer 5 neurones are born next, about 15 days later, and subsequently neurones of layer 4, 3 etc. Thus the deepest cortical layers are generated first, the most superficial last.

Rakic, P. 1974. Neurones in rhesus monkey visual cortex: Systematic relation between time of origin and eventual disposition. Science 183, 425-427.

What is not known is how the germinal cells are programmed to produce the different types of neurones and glia, nor how their proliferation is controlled to produce the correct numbers of cells in each part of the nervous system.

Neuronal migration: Cells that ultimately form the neurones of the brain and spinal cord migrate away from the ventricular zone when they have finished dividing. How do migrating cells know where to migrate to and when to stop? Some classes of neurones, such as those in the developing mammalian cortex, appear to move along the surface of a particular non-neuronal cell type, the radial glia. Golgi studies show that the processes of the radial glia span the entire thickness of the wall of the neural tube from ventricular to pial surfaces, and might therefore form a scaffolding along which migrating neurones can climb. Rakic' EM studies show that there is a close relationship between migrating neurones and the processes of the radial glial cells.

Rakic, P. 1972. Mode of cell migration to the superficial layers of foetal monkey neocortex. J. comp. Neurol. 145, 61-84.

Experimental evidence for a relationship between radial glia and the appropriate migration of cortical neurones comes from observations on a mutant mouse - Weaver - a genetic mutant with cerebellar defects - in which disordered radial glial fibres are associated with a failure of granule cells to migrate to their correct locations.

Neuronal lineage and differentiation

Cell lineage in vertebrates - the ontogeny of the neural crest.
Neural crest cells that give rise to the primary sensory neurones of the dorsal root ganglia, the peripheral ganglia of the autonomic nervous system and the Schwann cells of the peripheral nervous system make extensive migrations through embryonic mesenchyme to their final destinations.

Le Douarin, N.M. 1980. The ontogeny of the neural crest in avian embryo chimaeras. Nature 286, 663-669.

The tissue environment during early development is important for differentiation of neurones: neurotransmitter phenotype of neural crest cells is selected by the tissue environment. The addition of certain types of non-neuronal cells to postmitotic sympathetic neurones in cell

culture markedly affects the proportion of cholinergic to noradrenergic neurones.

Patterson, P.H. & Chun, L.L.Y. 1977. The induction of acetyl choline synthesis in primary cultures of dissociated rat sympathetic neurones. 1. Effects of conditioned medium. Dev. Biol. 56, 263-280.

Single cell cultures. Electrophysiological recording from developing autonomic neurones in culture show that individual cells have the capacity to change their transmitter metabolism in response to changes in their fluid environment.

Furshpan, E.J., McLeish, P.R., O'Lague, P.H. & Potter, D.D. 1976. Chemical transmission between rat sympathetic neurones and cardiac myocytes developing in microcultures: Evidence for cholinergic, adrenergic and dual-function neurones. P.N.A.S. 73, 4225-4229.

DEVELOPMENT II

Neuronal death in normal development. During normal development of the nervous system, large numbers of the cells initially generated in a particular nucleus or ganglion, degenerate and die. Early evidence for a competitive interaction between neurones came from the work of Victor Hamburger and Rita Levi-Montalcini who showed in 1949 that as many as 50% of the nerve cells originally generated in the spinal ganglia of the chick embryo die during later development, and that the amount of this cell death is modulated by the target.

Hamburger, V. and Levi-Montalcini, R. 1949. Proliferation, differentiation and degeneration in the spinal ganglia of the chick embryo under normal and experimental conditions. J. exp. Zool. 111, 457-501.

Hamburger, V. 1975. Cell death in the development of the lateral motor column of the chick embryo. J. comp. Neurol. 160, 535-546

The importance of the target in regulating cell death was also confirmed in a recent study of the dependence of ciliary ganglion cells on the iris. The normal amount of cell death is diminished if the amount of available target is increased.

Pilar, G., Landmesser, L. & Burstein, L. 1980. Competition for survival among developing ciliary ganglion cells. J. Neurophysiol. 43, 233-254

Together these findings suggest that death of some neurones is not a predestined affair but depends on the ability of individual neurones to respond to some property of the target, and gave rise to the idea that there is competition for the target among innervating axons. Not all results however are consistent with competition as the sole cause of neuronal death. Lamb has direct evidence against the idea. He amputated one hind limb bud of a Xenopus tadpole at an early stage of development and diverted the nerves that should have innervated it to the remaining hindlimb, which was therefore supplied from both sides of the spinal cord. The proportion of motoneurones that die in this case is practically the same as normal - so the total number of survivors innervating the single remaining limb is twice as great as normal. He concluded that the

limb does not limit the number of surviving neurones.

Lamb, A.H. 1980. Motoneuron counts in Xenopus frogs reared with one bilaterally innervated hindlimb. Nature 284, 347-350

Does motoneurone death act to eliminate errors in the pattern of connections? Landmesser has shown by tracing axon projections with horseradish peroxidase in the chick, that particular muscles are innervated by particular pools of motoneurones lying in distinctive and reproducible positions in the spinal cord. This pattern is accurately established before the period of motoneurone death and doesn't change i.e. motor neurones innervate only appropriate regions of the limb from the start and extensive errors in projection which might be corrected during the period of cell death are not made.

Landmesser, L. 1978. The distribution of motoneurones supplying chick hind limb muscles. J. Physiol. 284, 371-390

Landmesser, L. 1978. The development of motor projection patterns in the chick hind limb. J. Physiol. 284, 391-414

Synapse elimination: Rearrangement of synaptic connections in Early Life
In several parts of the nervous system there is a gradual tuning of synaptic connections and it seems likely that competition regulates the number and arrangement of synaptic connections.

Elimination of synapses in the periphery: both muscle fibres and autonomic ganglion cells are innervated by more axons at birth that they are a few weeks later, indicating an extensive rearrangement of connections in early postnatal life:

Bennett, M.R. & Pettigrew, A.G. 1974. The formation of synapses in striated muscle during development. J. Physiol 241, 515-545

Brown, M.C., Jansen, J.K.S. & Van Essen, D. 1976. Polyneuronal innervation of skeletal muscle in new born rats and its elimination during maturation J. Physiol. 261, 387-422

Lichtman, J.W. 1977. The reorganisation of synaptic connections in the rat submandibular ganglion during postnatal development. J. Physiol. 273, 155-177.

Elimination of synapses in the CNS: postnatal segregation of thalamic projections to visual cortex of monkeys and cats. In adult cats and some species of monkey, cortical neurones in layer IV of the primary visual cortex are segregated into columns dominated alternately by the right eye and left eye. At birth however, axon terminals from either right or left geniculate nucleus (the relay from the retina) are distributed throughout layer IV of the primary visual cortex. During the subsequent weeks the projections from the 2 eyes become separated so that cortical neurones in this layer are ultimately driven by either the right or the left eye. Both anatomical and electrophysiological evidence suggests that the progressive definition of ocular dominance columns involves the elimination of some initial synaptic connections.

Hubel, D.H., Wiesel, T.N. & LeVay, S. 1977. Plasticity of ocular dominance columns in monkey striate cortex. Phil. Trans. R. Soc. 278, 377-409

What is the relevant property of the target that regulates both cell death and synaptic connections? It seems likely that a trophic factor provided by the target is an essential ingredient.

Nerve growth factor. There is now considerable evidence that the well characterised protein called Nerve Growth Factor is such a trophic agent in the autonomic nervous system. In the early 1950's Levi-Montalcini, Hamburger and their colleagues extracted from a mouse tumour an agent that stimulates the growth of 2 types of mammalian neurones (sympathetic and dorsal root ganglion cells)

Levi-Montalcini, R. & Hamburger, V. 1951. Selective growth stimulating effects of mouse sarcoma on the sensory and sympathetic nervous system of the chick embryo. J. exp. Zool. 116, 321-362

Neonatal animals immunologically deprived of NGF by injection of an antiserum grow to maturity with dramatically stunted sympathetic and sensory ganglia.

Gorin, P.D. & Johnson, E.M. 1979. Experimental autoimmune model of nerve growth factor deprivation: Effects on developing peripheral and sympathetic sensory neurones PNAS 76, 5382-5386

C.T.P. COLLEGE ON BIOPHYSICS OF THE NERVOUS SYSTEM

DENERVATION & REINNERVATION I

Unlike most other types of cell in the body, nerve cells are unable to reproduce themselves by dividing once the embryonic nervous system is complete. If a nerve cell dies, and cells are apparently dying all the time even in a healthy adult nervous system, it is not replaced. However the nervous system is still flexible: nerve cells have mechanisms for replacing lost connections. Some nerve cells can survive damage and regenerate their processes to restore the functions that were lost when the damage occurred. So for example, primary motoneurones whose cell bodies lie in the spinal cord and whose axons run in peripheral nerves, not only survive peripheral nerve lesions but regrow their axons along previous pathways to remake synapses with the correct target muscle enabling them to work again. Similarly the axons of sensory neurones will regenerate to reinnervate the skin and restore sensation to an area paralysed by damage to a peripheral nerve.

This is not the case however for the majority of cells in the CNS of higher vertebrates. Neurones whose processes ramify within the CNS degenerate and die within weeks if their axons are cut or crushed. Thus injury to the spinal cord or brain can be irreversible resulting in permanent muscle paralysis, permanent sensory deficits or deficits in higher cortical functions because CNS neurones, for reasons which are not understood, fail to regenerate properly after damage. In spite of their failure to regenerate there are remarkable examples of plasticity of the CNS after injury. Recent findings show that neuronal circuitry is highly adaptable. In response to lesions or in response to changes in the environment, neuronal circuitry often actively reorganises by forming new synapses, or by strengthening or weakening existing connections. These lectures are concerned with the types of changes that occur as a result of damage to the nervous system, their significance, and the experimental approaches to the underlying mechanisms.

REACTIONS OF NEURONES TO INJURY

Responses of axotomized neurones: chromalytic reactions of the cell body. Different neurones respond to axotomy in different ways. Within hours after an axon has been injured, visible changes may occur in the nerve cell body. This was first described in 1892 by Franz Nissl for motoneurones innervating the face of the rabbit. He cut the motor axons in the facial nerve and described the changes in the motoneuron cell bodies

that were visible in the light microscope with the staining techniques then available. 24 hours after cutting the axons, the characteristic granular appearance of the cytoplasm after staining with basic dyes (the substance now referred to as Nissl bodies) began to disintegrate. The anatomical changes have since been described at the EM level. E.g., the ultrastructural changes following axotomy of rat sympathetic neurones have been described in detail by Matthews and Raisman.

Matthews M.R. 1973. An ultrastructural study of axonal changes following constriction of postganglionic branches of the superior cervical ganglion of the rat. Phil. Trans. R. Soc. 264, 479-508

Matthews M.R. & Raisman, G. 1972. A light and electron microscope study of the cellular response to axonal injury in the superior cervical ganglion of the rat. Proc. R. Soc. 181, 43-79

At the EM level the dispersal of Nissl substance is seen to be due to a change in the arrangement and concentration of RNA carrying organelles in the cell. There is a considerable increase in free ribosomes, as well as in ribosomes connected to cisternae of the granular endoplasmic reticulum. Together these changes are generally considered to be the characteristic response of a neurone to axotomy, although they are not invariably seen - Purkinje cells for example do not undergo chromatolysis.

So from the ultrastructural observations it can be deduced that following axotomy the synthesis and turnover of ribosomal RNA is increased. This is supported by other kinds of data - in parallel with the morphological changes are alterations in the neurones' metabolism, e.g. increased incorporation of RNA precursors.

It seems reasonable to assume that a neurone about to regenerate its processes might be expected to increase synthesis of proteins. This is borne out by numerous observations of increased incorporation of radioactive amino acids into proteins during regeneration. Interestingly, a regenerating neurone produces a quantitatively different spectrum of proteins from the normal nerve cell, e.g. increase in synthesis of polypeptides known as 'growth related proteins' or GAP's.

Transynaptic or transneuronal changes: responses in connected uninjured neurones

Axotomy leads to changes in cells to which the damaged neurone connects. Transneuronal degeneration first seen in the visual system. Section of ganglion cell axons in the optic nerve leads to degeneration of the post-

-synaptic neurones in the lateral geniculate nucleus of the thalamus.

Legros Clark & Penman, 1934. The projection of the retina in the LGN. Proc. R. Soc. 114, 291-313.

Transneuronal degeneration may cross more than one synapse - atrophy of LGN neurones after cutting the optic nerve induces degeneration of cortical cells - illustrates that widespread changes can be brought about by damage to a small part of the brain.

Transneuronal degeneration is one facet of a broad class of trophic interactions known to occur between neurones that are in synaptic contact as well as between neurones and their peripheral target organs. The clearest example of this type of interaction is found at the neuromuscular junction:

The denervated muscle membrane: neurones can influence the chemosensitivity of the muscle membrane. ACh receptors of vertebrate skeletal muscle normally are restricted to the region of the endplate where the motor nerve synapses on the muscle. Here the density exceeds 20,000 receptor molecules /um^2. A few micrometres away the density of receptors falls to extremely low levels, less than 50/ um^2. Denervation supersensitivity: Julius Axelsson and Stephen Thesleff in Sweden found that if the nerve is cut, within one week of denervation ACh receptors appear in extrajunctional regions - receptors are no longer restricted to the endplate but rather the whole surface of the muscle is uniformly sensitive to ACh -

Axelsson, J. & Thesleff, S. 1957. A study of supersensitivity in denervated mammalian skeletal muscle. J. Physiol. 149, 178-193.

Physiological evidence for appearance of new receptors after denervation has since been found between neurones:

Kuffler, S.W., Dennis, M.J. and Harris, A.J. 1971. Development of extrasynaptic receptors in nerve cells. Proc. R. Soc. 177, 555-563.

Roper, S. 1976. The acetyl-choline sensitivity of the surface membrane of multiply innervated parasympathetic ganglion cells in the mudpuppy before and after partial denervation. J. Physiol 254, 455-473.

This diffuse distribution of new receptors does not simply represent the unmasking of receptors that are already present in the membrane in ocult form, but the synthesis of new receptors and their insertion into the membrane. Fragments of muscle separated from the endplate will develop new receptors:

Katz, B. & Miledi, R. 1974. The development of acetyl-choline sensitivity in nerve free segments of vertebrate skeletal muscle. J. Physiol 170, 389-396.

What restricts the distribution of ACh receptors with innervation and leads to their appearance with denervation? One idea was that the transmitter, or some trophic substance, flows from nerve to muscle and is responsible for restricting the ACh receptors. Lomo and Rosenthal however showed that inactivity without denervation can mimic the changes of denervation, and that direct activation of denervated muscles can restore extrajunctional properties towards normal

Lomo, T. & Rosenthal, J. 1972. Control of ACh sensitivity by muscle activity in the rat. J. Physiol. 222, 493-513.

Sprouting as a direct effect of denervation of a target After partial denervation of a muscle, the undamaged motoneurones sprouts either from the nodes of Ranvier or from the axon terminals, which reinnervate the denervated fibres. Individual motor units can increase by as much as 4 to 5 X their normal size

e.g. Brown, M.C. & Ironton, R. 1978. Sprouting and regression of neuromuscular synapses in partially denervated mammalian muscles. J. Physiol 278, 325-348

Partial denervation is not the only effective stimulus. Muscle paralysis i.e. almost any treatment that blocks neuromuscular junctions or muscle activity and brings about denervation like changes in mammalian muscle, can by itself elicit sprouting

Holland, R.L. & Brown, M.C. 1980. Postsynaptic transmission block can cause motor nerve terminal sprouting. Science 207, 649-651

It has been shown recently that sprouting and synapse formation are not restricted to denervated tissues. After crushing the motor nerve to one cutaneous pectoris muscle in the frog, the motor nerve to the opposite muscle sprouts and forms additional synapses on intact and already innervated muscle fibres.

Rotshenker, S. 1979. Synapse formation in intact innervated cutaneous pectoris muscles of the frog following denervation of the opposite muscle. J. Physiol. 292, 535-547

Changes in synaptic transmission to neurones whose axons have been interrupted:

Damage to a bundle of axons in the CNS results in degenerative changes not only to the damaged neurones and to the postsynaptic cells that receive synapses from the damaged neurones, but the presynaptic neurones also may be affected. Depression of synaptic transmission after axotomy first observed by Acheson et al. 1942. J. Neurophysiol 5,269-273, in recordings of the discharge of respiratory neurones whose axons had been cut. Since these initial experiments impairment of transmission to injured neurones has emerged as a general feature of the reaction to axotomy e.g. spinal motoneurones:

> Kuno, M. & Llinas, R. 1970. Alterations of synaptic action in chromatolysed motoneurones of the cat. J. Physiol. 210, 823-838.

sympathetic neurones:

> Matthews, Margaret R. & Nelson, Victoria H. 1975. Detachment of structurally intact nerve endings from chromatolytic neurones or rat superior cervical ganglion during the depression of synaptic transmission induced by postganglionic axotomy. J. Physiol 245, 91-135.
>
> Purves, D. 1975. Functional and structural changes in mammalian sympathetic neurones following interruption of their axons. J. Physiol. 252, 429-463.

Stripping of synapses: Synaptic depression could be due to a defect in any of the steps of chemical transmission but several studies are general agreement that axotomy is followed by a loss of synapses from the surfaces of the affected cells. This has been studied quantitatively in sympathetic ganglia, see Matthews and Nelson 1975. The number of vesicle filled profiles per unit area of EM section declines by nearly the same amount as the number of synapses; therefore in addition to disjunction of most endings, presynaptic terminals also involute.

Causes of synapse loss after axotomy not fully known. Matthews and Nelson saw that presynaptic terminals were sometimes seen to be separated from the postsynaptic element by a finger of satellite cell cytoplasm which lead to the suggestion that glial cells play an active role in the process of synaptic detachment

Proliferation of glial cells around chromatoltyic neurones: proliferation of microglial cells leads to loss of approximately 80% of synapses on soma and dendrites.

> Blitzinger, K. & Kreutzberg, G. 1968. Displacement of synaptic terminals from regenerating motoneurones by microglial cells. Z Zellforsch. 85, 145-157.

Suggested that axotomy interrupts the flow of some trophic substance moving back along the axon from terminal to cell body. Most of the functional and morphological effects of axotomy in autonomic ganglia can be elicited in the absence of nerve injury by agents which block the fast component of axoplasmic transport. First demonstrated by:

> Pilar, G. & Landmesser, L. 1972. Axotomy mimicked by localised colchiane application. Science 177, 1116-1118.

Within a few days of localised application to postganglionic nerves, chromatolysis and depression of transmission occurred through avian ciliary ganglion. Also in mammalian superior cervical ganglion, local colchicine treatment leads to most of the electrophysiological and ultrastructural changes associated with axotomy.

> Purves, D. 1976. Functional and structural changes in mammalian sympathetic neurones following colchicine application to post-ganglionic nerves. J. Physiol. 259, 159-175.

The apparent dependence of the integrity of the axoplasmic transport system suggests that maintenance of synaptic contacts depends on either removal of some material from the neurone by anterograde transport, or the supply of something brought to the cell by retrograde transport. In the peripheral sympathetic system recent evidence suggests that retrograde transport of NGF plays a part in the development and maintenance of synaptic contacts on ganglion cells.

> Nja, A. & Purves, D. 1978. The effects of nerve growth factor and its antiserum on synapses in the superior cervical ganglion of the guinea-pig. J. Physiol. 277, 53

DENERVATION & REINNERVATION II

"Once the development was ended the founts of growth and regeneration of axons and dendrites dried up irrevocably. In adult centres the nerve paths are something fixed, ended, immutable. Everything may die, nothing may be regenerated."

Cajal, S.R. in 'Degeneration and regeneration of the nervous system'. London: Oxford University Press. 1928

Is failure of regeneration in the CNS due to the inability of neurones to form synapses? CNS neurones were long thought to be incapable of growth and regeneration. That central neurones can in fact sprout processes was first demonstrated by Liu and Chambers who demonstrated collateral sprouting from intact axons after partial denervation of the spinal cord in the cat.

Liu, C.N. & Chambers, W.W. 1958. Intraspinal sprouting of dorsal root axons Arch. Neurol. Chicago 79, 46-61

Subsequently collateral sprouting and formation of new synapses in response to lesions in the CNS was shown to be a widespread phenomenon occurring at all levels of the neuraxis. eg. synaptic plasticity in the red nucleus. Tsukahara and his colleagues looked at the physiological properties of newly formed synapses on neurones of the red nucleus by corticorubral fibres after destroying the input to these cells from the cerebellum.

Tsukahara, N. 1978. Synaptic plasticity in the red nucleus. In 'Neuronal Plasticity' ed. Cotman. Raven Press, New York, 113-130.

Synaptic plasticity in the hippocampus

Cotman, C.W. & Nadler, J.V. 1978. Reactive synaptogenesis in the hippocampus in 'Neuronal Plasticity' ed Cotman. Raven Press, New York, 227-272

Formation of new connections in CNS transplants Peripheral target tissues such as smooth muscle of the iris implantated into the brain reinnervated by cholinergic and noradrenergic neurones:

Bjorklund, A. & Stenevi, U. 1979. Regeneration of monoaminergic and cholinergic neurones in the mammalian central nervous system. Phys. Rev. 59, 62-100

CNS neurones transplanted to novel sites within the brain, either in chunks or as dissociated cells can also regenerate axons and form new synapses:

Schmidt, R.H., Bjorklund, A. & Stenevi, U. 1981. Intracerebral grafting of dissociated CNS tissue suspensions: a new approach for neuronal transplantation to deep brain sites. Brain Research 218, 347-356

Is failure of regeneration in the CNS due to the inability of adult neurones to grow long enough distances? A possible explanation for the failure of regeneration is that adult regenerating axons cannot grow over great enough distances to re-establish their normal connections. Aguayo and his colleagues have looked at the role of CNS and PNS environments in promoting the growth of central and peripheral neurones. Peripheral axons such as those of the sciatic nerve which normally regenerate well, will only grow a little way into a CNS graft such as optic nerve.

In the converse experiment segments of sciatic nerve were used as bridges to connect the hindbrain to the spinal cord. Both spinal and medullary neurones penetrated the CNS graft and grew long distances in this altered glial environment i.e. CNS neurones are capable of extended axonal elongation.

David, S. & Aguayo, A.J. 1981. Regenerative axonal elongation in the adult mammalian CNS. Neuroscience Abstracts VII, 221.

Conclusion: CNS neurones do retain the ability to grow after embryonic differentiation is over. It has been unequivocally demonstrated that axons can regenerate long distances under favourable conditions. Furthermore axon sprouting and synaptic remodelling are now generally recognized as a basic property of many CNS neurones.

THE RESTORATION OF SPECIFIC NEURONAL CONNECTIONS: SELECTIVE SYNAPSE FORMATION IN THE PERIPHERY

All major classes of peripheral nerves regenerate and form new synapses after axotomy. It is also possible to transpose nerves to novel targets or to novel sites. Mature neurones and skeletal muscle fibres can be induced through denervation to accept innervation from foreign sources.

Katz, b. & Miledi, R. 1964. The development of acetycholine sensitivity in nerve free segments of skeletal muscle. J. Physiol 170, 389-396.

Does foreign innervatio remain functional when the correct nerve reinnervates its target? Dennis and Yip showed in lower vertebrates that native axons compete with foreign ones that have become established following denervation,

and the incorrect synapses are eliminated in favour of the correct ones.

Dennis, M.J. & Yip, J.W. 1978. Formation and elimination of foreign synapses on adult salamander muscle. J. Physiol. 274, 299-310.

Selective synapse formation also occurs in the mammalian sympathetic chain of the PNS.

Purves, D., Thompson, W. & Yip, J.W. 1981. Reinnervation of ganglia transplanted to the neck from different levels of the guinea-pig sympathetic chain J. Physiol. 313, 49-63

Factors accounting for precise reinnervation at the neuromuscular junction
Axons spontaneously reinnervating muscle after nerve damage form neuromuscular junctions at the original endplate regions of the muscle fibre:

Letinsky, M.S., Fischbeck, K.H. & McMahan, U.J. 1976. Precision of reinnervation of original postsynaptic sites in frog muscle after nerve crush. J. Neurocytol. 5, 691-718.

The muscle fibre is not required for this precise reinnervation to occur, and extracellular components associated with the basal lamina direct the growth of axons to the original synaptic sites.

Sanes, J.R., Marshall, L.M. & McMahnan, U.J. 1978. Reinnervation of muscle fibre basal lamina after removal of myofibres. J. Cell. Biol. 78, 176-198.

REINNERVATION OF NERVE CELLS IN THE CNS

Regenerating neurones have long been thought to recognize their targets by means of specific chemical labels.
Regeneration of specific connections in fish and amphibia: severed axons of retinal ganglion cells reinnervate the tectum and restore visual function in lower vertebrates.

Sperry, R.W. 1963. Chemoaffinity in the orderly growth of nerve fibres. PNAS 50, 703-710.

Specificity at the level of single cells: Regenerations between neurones in the CNS of invertebrates occurs at the level of single identified cells.

Muller, K.M. 1979. Synapses between neurones in the central nervous system of the leech. Biol. Rev. 54, 99-134.

Individual identified neurones isolated from the CNS of the leech and maintained in culture form selective connections.

Fuchs, P.A., Nicholls, J.G. & Ready, D.F. 1981. Membrane properties and selective connections of identified leech neurones in culture. J. Physiol. 316. 203-223.