penalties, 14; political channels and organizations, 14; establishment of involuntary fertility control, 14.

74. Davis was a strong advocate of family planning in India, and quite optimistic about its prospects even in the pre-IUD or pre-pill era. See K. Davis, in *The Interrelations of Demographic, Economic, and Social Problems in Selected Underdeveloped Areas* (Milbank Memorial Fund, New York, 1954). Davis concludes (pp. 87–88): "Although India is already well-launched in the rapid-growth phase of the demographic transition, there is no inherent reason why she should long continue in this phase. She need not necessarily wait patiently while the forces of urbanization, class mobility, and industrial development gradually build up to the point where parents are forced to limit their offspring on their own initiative and without help, perhaps even in the face of official opposition. . . . Realistically appraising her situation, India has a chance to be the first country to achieve a major revolution in human life—the planned diffusion of fertility control in a peasant population prior to, and for the benefit of, the urban-industrial transition."
75. K. Davis, in *The Interrelations of Demographic, Economic, and Social Problems in Selected Underdeveloped Areas* (Milbank Memorial Fund, New York, 1954), p. 86.
76. G. Myrdal, *Asian Drama: An Inquiry into the Poverty of Nations* (Pantheon, New York, 1968), p. 1503.
77. D. Kirk, "Population research in relation to population policy and national family planning programs," paper presented before the American Sociological Association, August 1968.
78. It begins to appear that the prospects for fertility control may be improving over the decades. Kirk, after reviewing several factors that "favor a much more rapid [demographic] transition than occurred in the West"—changed climate of opinion, religious doctrine, decline of infant mortality, modernization, fertility differentials, grass-roots concern, and improved contraceptive technology—shows, in a remarkable tabulation, that the later a country began the reduction of its birth rate from 35 to 20 births per thousand, the shorter the time it took to achieve this reduction: from 73 years (average) for the period 1831–60, for example, to 21 years after 1951; the trend has been consistently downward for over a century [D. Kirk, "Natality in the developing countries: recent trends and prospects," paper presented at the University of Michigan Sesquicentennial Celebration, 1967].
79. Nor, often, does such a conviction exist among the general public. For example, in midsummer of 1968 a national sample of adults was asked in a Gallup poll, "What do you think is the most important problem facing this country today?" Less than 1 percent mentioned population growth (Gallup release, 3 Aug. 1968, and personal communication).
80. For an old but enlightening review, see H. Dorn, *J. Amer. Statist. Ass.* **45**, 311 (1950).

Development of Specific Neuronal Connections

Marcus Jacobson

We generally conceive of the nervous system as an association of uniquely determined neurons, each possessing an essential nature of its own. One of the main expressions of the neuron's uniqueness is the formation of highly specific synaptic connections. Although this has been recognized for at least 50 years, almost nothing is known about the physicochemical basis of neuronal specificity or about the mechanisms of formation of specific synaptic connections. Therefore, the term *neuronal specificity* is not used here in any explanatory sense but is merely a convenient expression indicative of the unique properties of the neuron which result in the formation of specific connections.

There are many possible mechanisms that might be involved in the development of neuronal connections and in their maintenance and plasticity. We would like to discover the roles of genetic control mechanisms, metabolic control systems, and cellular interactions of various kinds, including intercellular transmission of molecules. Unfortunately, at present there is no direct evidence implicating even one of these mechanisms in the formation of neuronal connections.

Here I give evidence in support of a theory in which neuronal connectivity is regarded primarily from an ontogenetic point of view. According to this theory some neurons are highly specified and all their connections are fully determined, but there are also some incompletely specified neurons with relatively indeterminate connections. During ontogeny there is a tendency for neuronal specificity to increase and for connections to become more highly determined, but the developmental stage at which these changes occur, as well as their extent and duration, varies for different neurons. This theory takes into account the evidence of invariance and stability of the highly determined kinds of synapses, but it assigns the adaptive and plastic properties to the kinds of synapses that are not fully determined.

Variability of Neuronal Connections

Developing neurons sprout slender processes, their axons and dendrites, which in some cases grow to relatively great lengths to form connections with other neurons. The direction of growth of these processes and the targets on which they terminate appear to be constant in all individuals of the same species. Anatomical and physiological methods have shown the remarkable invariance of neuronal circuits and have given no evidence of random connectivity. A distinguished neuroanatomist has recently written (*1*), "The nervous system is not a random net. Its units are not redundant. Its organization is highly specific, not merely in terms of the connections between particular neurons, but also in terms of the number, style, and location of terminals upon different parts of the same cell and the precise distribution of terminals arising from that cell." This is a fair summary of what might be called the *deterministic,* as opposed to the *probabilistic,* theory of neuronal connectivity. According to the probabilistic theory, only statistical properties of the nervous system can be formulated, not the detailed properties or behavior of individual neurons or their connections.

It is easy to cite evidence of selective localization of synapses on neurons at constant positions in the nervous system, and difficult to find any evidence of nonselectivity. Perhaps this is because far more attention has been given to recording the invariant features of neuronal connections than to obtaining a measure of their variability.

There is ample evidence of specific synaptic patterns on neurons in invertebrates (*2*) as well as on vertebrate neurons—for example, Mauthner's neuron,

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the pyramidal neurons of the cerebral cortex, cerebellar Purkinje cells, spinal and cranial motor neurons, and retinal ganglion cells. These are all large neurons with long axons and with connections which are probably invariant in the adult, though they may be more variable at earlier stages of ontogeny. However, the total number of these large neurons is small compared with the number of interneurons, of many types, with short axons. The latter are generated much later in ontogeny than the large neurons with long axons, and continue to be formed after birth in mammals (*3*). The dendritic domains of the neurons with short axons in the cerebral cortex are highly variable in size and shape as compared with the domains of the neurons with long axons (*4*).

It seems very probable that the neurons with short axons may remain pluripotent in their capacity to form synaptic associations until fairly late in development, after the animal has become exposed to varied experience. Adaptive changes in their connectivity might then be brought about by functional activity. The modifiability of these connections may be one of the ways in which functional superiority of the human brain has been achieved without a comparable increase in the information content of the human genome.

It has been argued, in opposition to the deterministic theory of neuronal connectivity, that the information content of DNA is unlikely to be sufficient to determine all the connections of 10^{10} neurons in the human brain, or even of 10^{8} neurons in the brain of the rat. The conclusion drawn from this is that there must be a considerable degree of indeterminacy in the structure of the brain (*5*). However, the force of this argument is greatly reduced by our ignorance of the mechanisms of neuronal specificity and by the absence of any definite evidence of genetic control of the formation of neuronal connections. It would seem that a large proportion of the genome is not devoted to determining the structure of the brain, because obvious differences in the complexity of the brains of different orders of mammals are not reflected in corresponding differences in the DNA content of their cell nuclei (*6*). Each diploid cell nucleus contains 7.1×10^{-12} gram of DNA in man and 6.5×10^{-12} gram of DNA in the rat, in the form of a double strand containing about 10^{10} nucleotides per strand, which can code for a few million different proteins. This number is not sufficient for labeling every neuron with a different protein, but then it is more likely that neuronal specificity is achieved by a more economical use of genetic information. One way in which this might occur is for each neuron to be uniquely labeled by a small number of proteins or other macromolecules grouped in various combinations and permutations. Connections might then be made only between neurons with molecular labels which were complementary in some way, as Weiss and Sperry have suggested (*7*). However, it should also be kept in mind that different types of neurons might have different degrees of constraint put on their connectivity, so that the connections of some types of neurons might be much more variable than those of others.

Formation of Neuronal Connections with the Skin

In all vertebrates, cutaneous sensory localization depends on a topographical projection of the sensory nerves onto the sensory neurons of the central nervous system. It is not known how these point-to-point connections develop. Presumably the connections are formed as the result of selectivity between skin and cutaneous nerve terminals, or between cutaneous nerves and central neurons, or by selective connection in both the central and peripheral systems. There is some experimental evidence which shows that the order of the central connections is at least partly determined by local differences in the tissues with which the nerves connect peripherally (*8*).

Some neurons are able to adjust their connections so that there is congruence between the connections with skin or muscle and the central connections. This plasticity has been demonstrated in peripheral sensory and motor nerves of larval amphibians, and in the sensory nerves from muscle spindles in the newborn kitten (*9*). The modifiability of these connections is lost during metamorphosis in amphibians and shortly after birth in mammals (*10*). During the stage of modifiability, before the connections of these neurons are fixed, a peripheral switch, produced by skin or muscle transplantation or by crossing sensory or motor nerves, results in a compensatory switch in the central connections.

R. E. Baker and I recently performed some experiments on the formation of neuronal connections with skin grafts (*11*). If a large patch of skin extending from the back to the belly on one side of a frog tadpole is excised and replaced in a dorsoventrally inverted position, nerves regenerate into the skin graft and its sensitivity is restored. After metamorphosis, the cutaneous reflexes appear at the usual time and are at first normal: touching the graft results in a limb movement directed accurately at the point of stimulation. At this stage the original location of the grafted skin seems to have no effect in determining the reflex pathway from the skin to the muscles that move the limb to the point of stimulation on the graft. However, after a few days the normal reflexes are gradually replaced by misdirected reflex movements aimed at the original locus of the stimulated point on the grafted skin. Stimulating the belly skin grafted to the back results in a movement of the leg aimed at the belly, and vice versa. These misdirected reflexes are elicited at first only from a small region in the center of the graft, but during the following 2 weeks this region extends over almost the entire graft, and the misdirected reflexes then become permanent. The reflexes may revert to normal if the skin graft is excised and replaced in its normal position during larval or early adult stages, but in older adults the misdirected reflexes persist after the graft has been restored to its normal position. This shows that modifiability of the reflex mechanism is lost in the adult frog. We do not know whether the modifiability is lost as a result of changes in the skin or in the nervous system.

The misdirected reflexes indicate that the skin, after being grafted to a new position, is able to signal its original locus to the central nervous system. This might occur as the result of several alternative mechanisms. First, the cutaneous nerves might grow back to their appropriate places in the skin as the result of some sort of selective attraction of the nerves by the skin. We have ruled this out by electrophysiological recording from the cutaneous nerves and have shown that the nerves connect nonselectively with the nearest skin, regardless of the skin's original position. We have also found no changes in the pattern of receptive fields of cutaneous nerves during the period of change from normal to misdirected reflexes. Therefore the change of reflex behavior is

likely to be due to changes in the central nervous system rather than to changes in the connections of the nerves with the skin graft.

There is also no evidence of a specific nerve impulse code which might signal the peripheral origin of the sensation to the central nervous system. The most plausible explanation of our observations is that the nerves are instructed by the skin to form central connections that are congruent with their new peripheral positions in the skin graft. This might occur either by selective reconnection of the central terminals of the sensory nerves or by adjustment of the connections of spinal interneurons connecting the sensory nerves with motor neurons.

It is most likely that each region of the skin produces a specific biochemical change in the nerve as a result of which the central connections of the nerve become modified. The instructions for this modification may be encoded in molecules which may be transmitted from the skin to the nerve terminals, and which may travel in the axons, in a manner analogous to the transport of the neurotropic viruses, to the central nervous system (*12*). One cannot fail to notice similarities between this hypothetical mechanism for producing modifications of neuronal connections and some kinds of morphogenetic tissue interactions in which intercellular transfer of materials has been demonstrated (*13*).

Ontogeny of Neuronal Connections between Eye and Brain

At an early stage of their development the ganglion cells of the retina are pluripotent with respect to the connections that they can form in the brain, but at a later stage the central connections of each ganglion cell become uniquely specified. In order to discover the time at which this change occurs, I inverted the eye of the toad *Xenopus* at various stages of development before connections had formed between the retina and midbrain tectum, and then mapped the connections after they had formed (*14*). These experiments show that, if the left eye is inverted before the early tailbud larval stage 29 (*15*), its connections with the tectum are the same as the point-to-point connections of the normal right eye (Fig. 1). Before larval stage 29 the retinal ganglion cells are unspecified and will form connections in the tectum which are appropriate to the new position of the eye. The toad therefore has normal vision following eye inversion before larval stage 29. This capacity to regulate their connectivity is lost by the ganglion cells within a period of about 10 hours, for if the eye is inverted after larval stage 30 the retinotectal projection is inverted and the animal has permanently inverted vision (Fig. 2). Other experiments have shown that the retinotectal connections in *Xenopus* are completely determined immediately after the cessation of DNA synthesis and mitosis in the ganglion cells, and about a day before their axons start growing from the eye to the tectum (*16*).

At an early stage of their development all neurons may, like retinal ganglion cells, be pluripotent with respect to the connections which they can form. This means that if the neuron is moved to another place in the nervous system it will form connections that are appropriate to its new location, provided the neuron with which it connects is also pluripotent. At some later stage, which varies in different neurons, the connections may become uniquely specified.

One view of the development of neuronal connections is that homologous maps of neuronal specificities are laid down in parallel—for example, in the retina and the tectum—so that connections form as a result of affinities between neurons located at homologous positions in the retinal and tectal maps. The evidence for this parallel specification in the visual system comes from observations on the regeneration of optic nerve fibers in adult amphibians and fishes (*17*). For example, after the optic nerve of an adult goldfish is cut, the optic fibers grow back to their correct places in the tectum, and, if regeneration of optic fibers from a small region of retina is prevented, the corresponding region of tectum remains without optic connections (*18*). In the adult, or at some time after the connections form in the embryo, the position at which each optic nerve connects in the tectum is fixed. However, during the development of these connections in the embryo it seems that initially only the relative order of the connections is de-

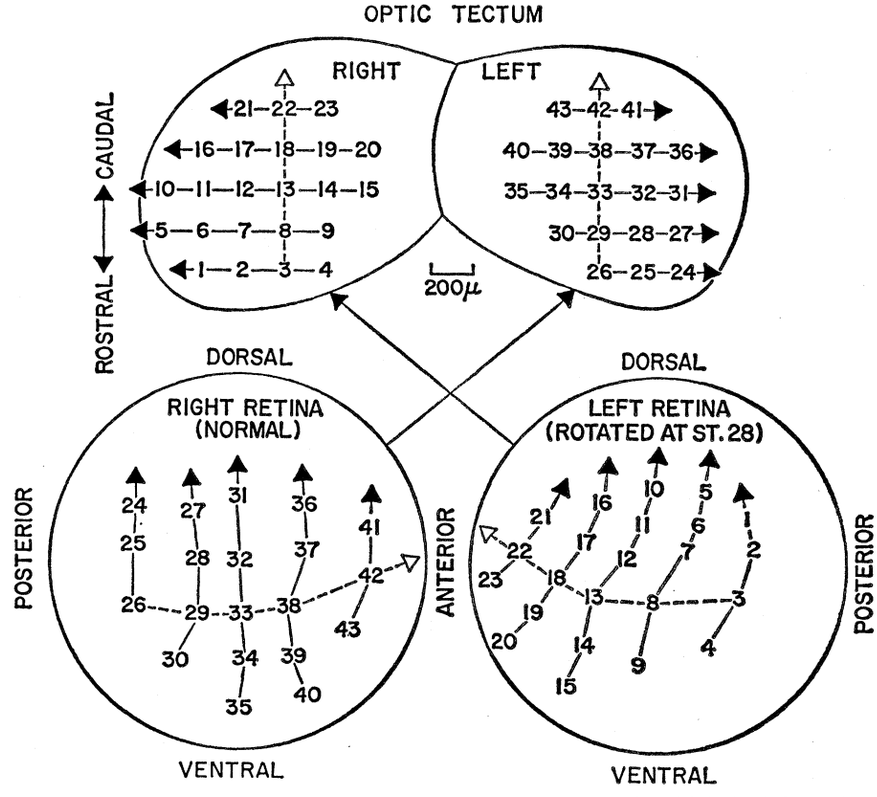

Fig. 1. Map of the retinotectal projection in an adult *Xenopus* in which the left eye had been inverted dorsoventrally and anteroposteriorly (rotated 180 degrees) at larval stage 28–29. The projection from the inverted eye is normal. Each number on the tectum represents the position at which a microelectrode recorded action potentials in response to a small spot of light at the position shown by the same number on the retina. [Adapted from Jacobson (*14*)]

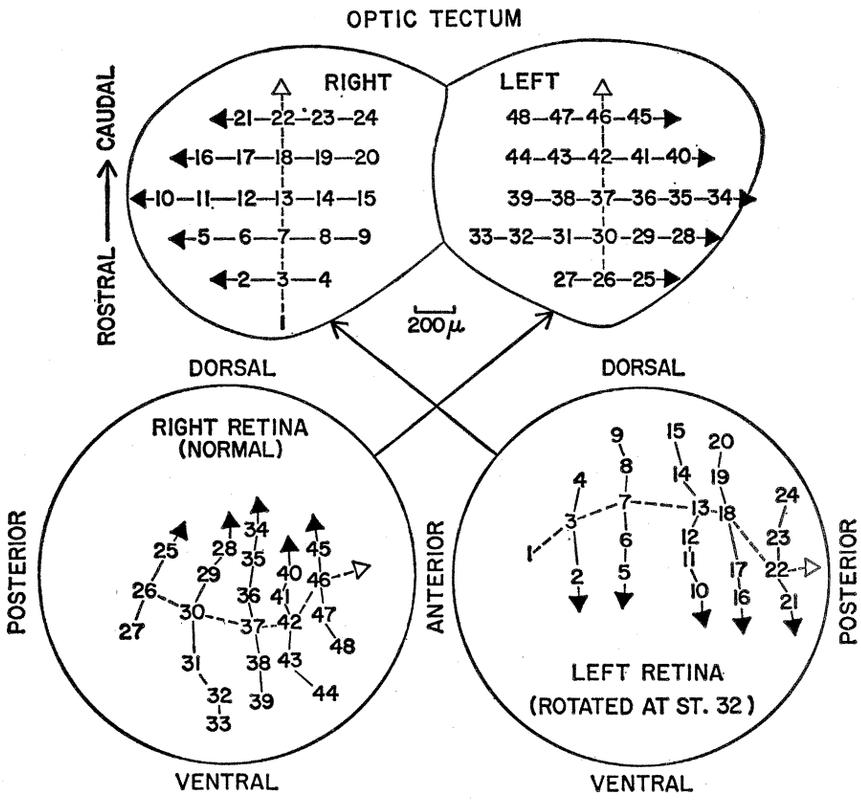

Fig. 2. Map of the retinotectal projection in an adult *Xenopus* to the left tectum from the normal right eye, and to the right tectum from the left retina which had been inverted at larval stage 32. The projection from the left retina is totally inverted. For the significance of the numbers on the tectum, see Fig. 1. [Adapted from Jacobson (*14*)]

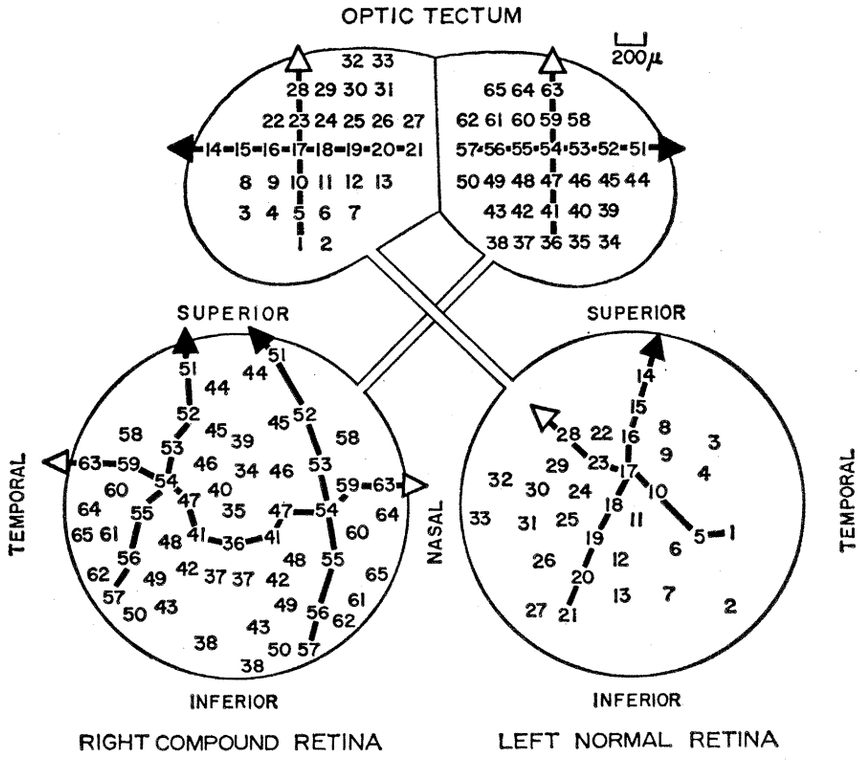

Fig. 3. Map of the retinotectal projection from the normal left retina to the right tectum and from the compound, double-nasal right retina to the left tectum in *Xenopus*. The projection from the left retina is normal. The projection from the double-nasal compound retina is reduplicated so that each half of the left retina projects to the whole right tectum. For the significance of the numbers on the tectum, see Fig. 1. [Adapted from Gaze *et al.* (*19*)]

termined, and the absolute position of each connection may become fixed at a later stage of development.

In the embryo there is evidence of elasticity in the retinotectal map. This has been shown in larval *Xenopus* in which two nasal or two temporal halves of the retina have been grafted together to form a double-nasal or a double-temporal compound eye (*19*). The compound eyes were made at larval stage 30, after the ganglion cells were specified but before they connected in the tectum, and the retinotectal connections were mapped after the connections had been formed. The ganglion cells in each half-retina were found to connect in point-to-point order with the whole tectum, not merely with the half of the tectum with which they normally would have connected (Fig. 3). It would seem that, during embryonic development, the mapping of the retina on the tectum is independent of the size or shape of either the retina or the tectum, and that the topographical order of the retinotectal map would develop regardless of the relative sizes of retina and tectum. The evidence of spreading out of connections in the tectum from a "compound eye" shows that a fixed system of place specificities cannot be laid down independently in the retina and tectum at a stage before the connections are first established in the embryo. Only the relative positions of the connections are determined, and only the topographical order of the retinotectal projection is invariant. The absolute position of each connection apparently becomes fixed at a later stage, as the experiments on adults show. We have, therefore, to discover the mechanisms by which the elastic properties of the retinotectal map in the embryo give way to a system of fixed place specificities that determines the position at which each optic axon terminates in the tectum.

Functional Modifiability of Connections

In the adult frog or fish, the regeneration of optic nerve fibers to the correct places in the tectum is not affected by function or experience, as Sperry's experiments have shown (*17*). His experiments on adults have led to the conclusion, which I think needs to be examined more critically as it applies to embryos, that the formation of retinotectal connections is determined entirely by genetic and developmental processes

which do not have any functional feedback. It is necessary to emphasize the fact that adequate experiments have not been performed to test the effects of visual deprivation or of alterations in visual function on the initial formation of retinotectal connections in amphibians and fishes.

In mammals there is good evidence that visual deprivation during development results in histological and physiological changes in the retina, lateral geniculate, and visual cortex (*20*). During the postnatal period there is a considerable decrease in the susceptibility of the visual system to sensory deprivation. For example, electrophysiological changes in the visual cortex occur in cats deprived of light from birth, but do not occur if the deprivation starts later than 6 weeks after birth (*21*). Visual stimulation is not necessary for the initial formation of connections from the retina to the visual cortex of the cat, for the connections are present in the newborn kitten before it opens its eyes (*22*). However, the maintenance of binocular connections in the visual cortex depends on patterned visual stimulation, not merely on stimulation by diffuse light. Monocular deprivation of pattern vision or a squint, which produces incongruence of the pattern of visual stimulation on corresponding points on the two retinas, has been shown to result in absence of cortical cells with binocular connections from the affected eye, in the kitten (*23*). This effect of function on the maturation of binocular connections in the kitten's visual cortex is important only for a short period between the 4th and 6th weeks after birth: temporary covering of one eye, ending before the 4th week or commencing after the 6th has little or no effect (*21*). Some kind of functional validation, during a relatively short critical period, is an essential stage in the maturation of these, and probably of other, types of synapses. What physicochemical changes occur in the neurons during this functional validation is not known.

It has often been postulated that permanent changes may occur in synapses as a result of functional activity, and may play some part in learning and memory (*24*). But little evidence of long-lasting synaptic facilitation, habituation, or functional adaptation of any kind has been found. To some extent this is because the neurons that are easiest to study, because they are large, have connections that are highly determined early in development and have lost their modifiability in mature animals. It is hardly surprising that no synaptic modifiability has been found in the large ganglion cells of the mollusk *Aplysia* (*25*), or in synapses of retinal ganglion cells in the lateral geniculate of the adult cat (*26*). When one is looking for modifiable synapses it seems important to consider their developmental history. Any evidence in support of theories of learning based on the modifiability of connections will have to come from studying the appropriate neurons while they are still at a modifiable stage of their ontogeny.

Summary

The mechanisms of formation of neuronal connections and the factors that are concerned with their stability and modifiability are largely unknown. I have given evidence for a theory that deals with neuronal connectivity from an ontogenetic point of view. During ontogeny, there is evidence of a progressive reduction of the capacity to form new neuronal connections and to modify existing ones. This reduction occurs at different times in different classes of neurons, so that those which are generated late in ontogeny and those which mature slowly have the greatest degree of modifiability in the mature animal. According to this theory, the modifiability of neuronal connections in the adult is regarded as a continuation of developmental processes that are much more pronounced in the embryo.

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