review article

Selective stabilisation of developing synapses as a mechanism for the specification of neuronal networks

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The specificity of synapses in the mammalian brain cannot possibly be accounted for by one-to-one biochemical matching. The alternative proposed in this article is that connections are genetically specified between classes of cells, but the final wiring pattern depends on the refinement of those collections by selective stabilisation during neuronal activity.

In his 1951 Ferrier lecture[1], J. Z. Young proposed that the mature synapse represents the end product of a continuous process of growth and degeneration of the terminal part of nerve fibres and further suggested that the "modification of such daily growth by functional activity would provide the basis of the plasticity of the nervous system". Since then, the portrait of the synapse has been drawn in more detail with the addition, in particular, of its fine organisation[2], the elementary mechanisms of its activity[3,4], the chemistry of some fine critical components[5] and the role of factors which selectively affect the growth of nerve terminals[6]. In parallel, and independently, the basic structural features of the genetic material and of its expression have emerged. Encouraged by the success of genetic analysis in the acquisition of this knowledge, molecular biologists interested in the nervous system have focused primarily on its genetic determination[7,8] and have thus raised the question of how such determination takes place. The DNA present in the nucleus of the fertilised egg may, at most, code for only a few million proteins and this quantity is practically the same in mouse, chimpanzee and man, moreover a significant fraction of it (more than 60%) does not correspond to genuine structural genes[9]. How is it then possible to engender the formidable complexity of the nervous system from such a limited number of genes?

The answer must lie in the mechanics of embryonic development. Models have been proposed which, from the combination of a small number of genes expressing themselves sequentially, and a few yes-no signals, produce a significant diversity of cell types[10]. They offer plausible, but still theoretical, explanations for the setting out, for example, of the neuronal somas. The establishment of adult connectivity, particularly of the vertebrate nervous system, is of a higher order of complexity. The call for "gene saving" mechanisms becomes pressing. The hypotheses which have been put forward to explain how nerve cells become interconnected during development differ chiefly on the question of whether the functional activity of the developing network plays a part. Schematically, the two main conceptual attitudes are the following.

(1) For Sperry[11] ("cytodifferentiation" or "chemoaffinity" hypothesis) the nerve fibres in the process of growth bear chemical labels complementary to those of their neuronal target; each label reflects the position of a given cell within a set. The pre- and postsynaptic partners recognise and assemble themselves with a selectivity which depends on the complementarity between cell surfaces. Gaze and Keating[12] and Prestidge and Willshaw[13] have amplified and extended this cytodifferentiation hypothesis introducing, in particular, a graded affinity between axons and postsynaptic targets and a competition between ingrowing nerve terminals and their postsynaptic partners, both being present in limited numbers. Gaze[14] and Jacobson[15] have also considered temporal factors in the establishment of connections. The differential growth of nerve fibres would be instrumental in bringing an order: reaching their target neurones at different times, the nerve terminals would "number" them in sequential order ("timing" hypothesis). The plausibility of these hypotheses has already been discussed[12–15,16].

(2) Since Ramon y Cajal[17] and others, three attitudes have been adopted; briefly they are the following (Fig. 1).

First, as in the "preformist" views just mentioned, the neuronal network is assumed to be specified before experience. Interaction with the environment merely triggers pre-established programmes and stabilises the genetically specified synaptic organisation ("functional verification" hypothesis[18]). Dysfunction at critical periods of development may, however, lead to the regression of preformed, but already specified, synaptic connections[18].

On the other hand, "empiricists" postulate that, to a large extent, the activity of the system specifies its connectivity, for instance by orienting the growth of the nerve terminals (see refs 17 and 19) or tracing pathways in more or less random networks[20,21]. Third, as a compromise between these two attitudes, we have postulated[22,23] ("selective stabilisation hypothesis"; see also refs 24 and 25) that the genetic program directs the proper interaction between main categories of neurones, for instance through the mechanisms presented below. However, during development within a given category, several contacts form at the same site; in other words, a significant but limited "redundancy" or fluctuation of the connectivity exists. The early activity of the circuits, spontaneous (in the embryo) and evoked (after birth) would increase the specificity or the order of the system, by reducing this transient redundancy. As a mechanism it was further postulated that the first synaptic contacts to form may exist under, at least, three states (synaptic plasticity): labile (L), stable (S) and regressed (D), the growth process being viewed as the emergence of labile states (Not→L). The labile and stable states would transmit nerve impulses but the regressed one (obviously) would not.

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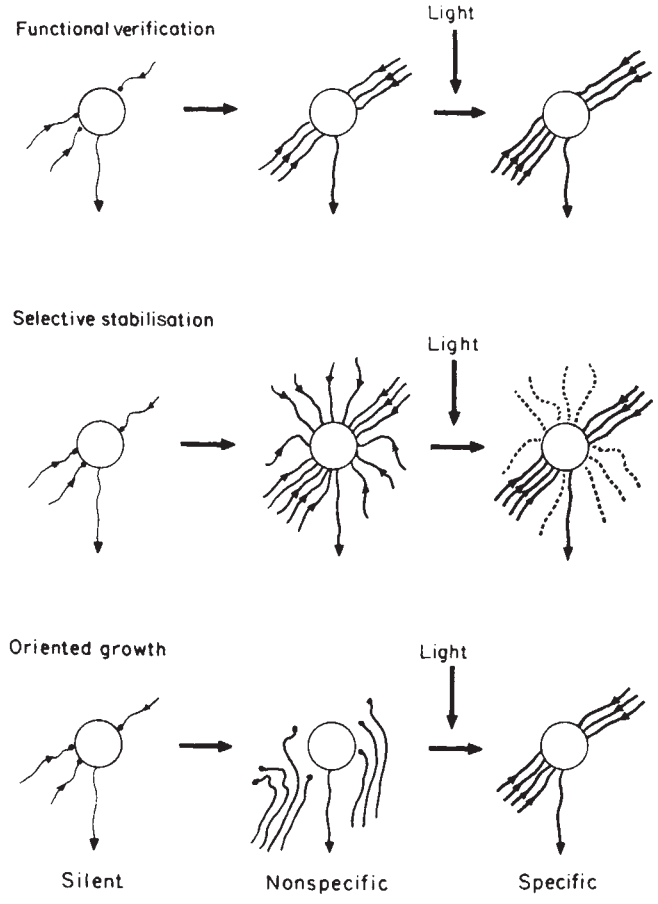


Fig. 1 Hypotheses regarding the effect of functional activity on the specification of a neuronal network: here a cell from the visual cortex which, in the adult, is specific for "orientation"[18]. According to Buisseret and Imbert[201], in the course of development the first active units to appear are "nonspecific" and respond to stimuli moving in any direction of the visual field. Subsequently, they give rise to "specific" units activated by light spots moving along only one precise orientation. The three possibilities considered deal with changes in the connectivity but alternative (or additional) mechanisms may take place such as changes of efficacy of excitatory and/or inhibitory synapses, growth of new sets of connections and so on.

The labile state may either become stabilised (L→S) or regress (L→D), regression being irreversible. (Regrowth (Not→L) may however take place.) An essential statement of the theory[22] is that the transitions of the labile state of the synapse (L→S and L→D) are regulated, in an "epigenetic" manner, by the total activity of the postsynaptic cell, including the activity of the considered synapse. Accordingly, the precocious activity of the developing network would "stabilise selectively" particular synapses among the many equivalent contacts which emerge during growth and thereby create diversity and specificity (in a concomitant manner the non-stabilised contacts will regress). As a consequence, a critical period exists in the development of a nerve terminal when it requires a given pattern of activity to become stable.

This selective stabilisation hypothesis has been formalised and applied to a few systems[22]. One of its advantages is that it may afford an economy of genes. Indeed some of the genes which dictate, for instance, the general rules of growth, the stability properties of the immature synapses (postulate 1), the regulation of their stability by the activity of the immature synapse (postulate 2), the integrative properties of the postsynaptic neurone, may be shared by different categories of neurones or even be common to all neurones. The set of genes involved (the genetic envelope) should therefore be smaller than if each synapse were determined individually. The amplitude of the limited redundancy subjected to functional epigenesis may vary from one area of the nervous system to another and with the "complexity" of the organism considered. Negligible in some invertebrates, it becomes most significant in high vertebrates.

In the following, we shall review experimental observations taken primarily from vertebrate neuromuscular junction and cerebellum which bring some support to the selective stabilisation hypothesis. Finally, we shall discuss biochemical mechanisms which may account for the stabilisation process and therefore make it more plausible.

Selectivity of surface recognition reconsidered

The exquisite specificity of synapse formation and its interpretation in terms of selective recognition between cell surfaces has often been emphasised[11–16] but functional synapses between non-homologous pairs of cells[12,14,26–28] may form. In mammalian cerebellum the afferent mossy fibres normally contact granular cell dendrites. By various means[29–38], the granular cells can be destroyed. In spite of the absence of their normal target, the mossy fibres make both anatomical[29,38] and functional[33] synapses on another category of neurones: the spines of the Purkinje cell dendrites. Functional heterologous synapses have also been obtained in the frog between the vagus nerve and the skeletal sartorius muscle transplanted into the thoracic region[39,40] and in the rabbit between the thoracic vagus and the diaphragm[41]; their occurrence has also been reported with the sympathetic ganglion after denervation[42–45] or *in vitro*, in tissue culture[46,47], even between cells which under normal conditions do not synthesise complementary transmitter and receptor[48].

These few examples illustrate that, in the absence of the normal target or after deviation of the axons from their usual route, functional synapses may form between non-complementary partners as long as the receptor for the neurotransmitter is present in the postsynaptic cell. In these instances, at least, the selectivity of surface recognition does not appear as stringent as one would expect from the strict "chemo-affinity" hypothesis. Compatibility between cell surfaces rather than complementarity might suffice for a synapse to be formed and additional mechanisms have to be invoked.

Variations in adult connectivity

If the paradigm of a highly selective and genetically determined development of synaptic connections were absolutely correct, one would expect the anatomy of the adult nervous system to be identical in genetically identical individuals. To obviate the genetic heterogeneity of a natural population, organisms have been studied that reproduce parthenogenetically: a small crustacean (*Daphnia magna*)[49] or even a viviparous tropical fish (*Poecilia formosa*)[50]. In a clone of such organisms, the different individuals have the same genetic equipment. The three-dimensional reconstruction of the fine structure of the optic system (eye and optic ganglia in *D. magna*) reveals that the gross features of the anatomy, in particular the number of cells, are invariant. A significant variability appears, however, in the branching and synaptic patterns of their processes at a resolution of a few tenth to a few micrometres. The variation seems to be as great when a given neurone is compared with the symmetrical cell on the opposite side of the same animal and with the same cell in other members of the clone. In spite of genetic homogeneity the "exact" form of identifiable neurones and of their processes shows a significant dispersion from one individual to another. The limits of genetic determination have been reached.

Activity of the growth cone and of developing nerve terminals

The primitive contacts present early in development transmit nerve impulses, long before the differentiated synapses of the adult are formed. In chick embryo, for example, spontaneous movements begin as early as 3 d of incubation[51] and are of neurogenic origin[51–54]. The rat foetus also moves spontaneously after 15 d of life[51]. At this precise date exploratory fibres enter the diaphragm and establish functional contacts (in 3 of 30

cells impaled) even before the presence of acetylcholinesterase can be detected by the Koelle method[55]. One day later, however, it is claimed that a single localised spot of acetylcholinesterase is present in 30% of the myotubes and distributed at random on the surface of the myotubes (although there is no convincing demonstration of this last point). In the majority of the cells impaled evoked postsynaptic potentials are recorded. In chick slow muscle anterior latissimus dorsi (ALD) a similar event takes place except that several spots of cholinesterase are observed at regular intervals along the length of the developing muscle fibre and persist in the adult fibre[55]. This post synaptic localisation process concerns both the esterase[56-59] and the cholinergic receptor protein which, dispersed evenly on the surface of the developing myotube, soon become concentrated under the ingrowing nerve terminal[60-62]. In tissue cultures the rudimentary synaptic contacts which form also transmit nerve impulses[63-66]. Even the growth cone or its filopodia show anatomical features indicating presynaptic or postsynaptic relationships with cell bodies or processes[67-70] with local accumulation of vesicles or postsynaptic thickenings and may give rise to typical postsynaptic potentials[71]. Chemical transmission therefore exists days before the pre- and postsynaptic differentiations of the mature synapse become apparent.

Cinematographic films of neurones in tissue culture show that the growing processes[72,73] are in perpetual motion. They advance, divide, retract, start again until a contact with a cell body becomes stable. It seems that through trial and error only a few of the transient contacts made by the growth cone or the developing neurites are selected. An interesting possibility is that such a behaviour accounts at least in part, for the fluctuation observed in the adult connectivity and that the activity of the growing processes play a role in this selection.

Spontaneous regressive phenomena

In the course of neurogenesis, regressive phenomena have been reported to occur at two distinct stages. (1) At the time of neuroblast proliferation or immediately after, a significant fraction of neuronal somas die[74-78]. For example, the ventral horns of the spinal cord of *Xenopus* tadpole may contain a maximum of 5,000 or 6,000 cells but 60 d later, at the time of metamorphosis, only 1,200, the adult number, persist. The number of cells which disappear during this period could even be larger (10,000) than the overall difference in total cell number[79]. Similar events take place in the spinal cord of chick embryo[78] and foetal mouse (between days 10–15 of intra uterine life[80,81]) and in several other parts of the nervous system (see ref. 77). At later stages of development a second regressive phase affects primarily axon collaterals and dendritic branches without significant changes in cell number. For example, in foetal rat diaphragm, at d 17, soon after the dense acetylcholinesterase (and acetylcholine receptor) deposit appears in the middle of the muscle fibre, motor axons develop multiple branches and make a complex network which increases in size until birth[55]. Observation of the endplate by electron microscopy shows several synaptic profiles in contact with the same synaptic folds. The "complexity" of the endplate potential (its stepwise increase of amplitude as a function of stimulus strength[82]) furthermore reveals that the several motor axons which converge on a given endplate are functional[55,82-84]. During the first postnatal week, the number of steps of the complex endplate potential progressively decreases: one of the endings finally dominates the others. At 3–5 weeks, all the synapses are innervated by a single nerve terminal and non-innervated muscle fibres are not seen. More than 60% of the functional contacts have, therefore, disappeared. The fine structure of the endplate during the regression phase does not reveal any gross degeneration of axonal branches and the number of axons in the ventral roots does not change significantly[83]. These observations have been repeated with other muscles[83], in particular with avian ALD[55] where each of the several endplates present per muscle fibre becomes innervated by several axon terminals.

Since the number of muscle fibres[85,86] and of motor axons[87,88,83] remains constant during this period, the number of muscle fibres innervated by a single motor neurone, that is the size of the "motor unit", should change[88,89,83]. Indeed, during postnatal development the average size of the motor units decreases. In the 3-d-old rat soleus, a single motor unit tension is about 1/4 of the total muscle tension[83]. In the adult, the fields of innervation of the motor neurones no longer overlap and a single motor fibre commands the contraction of a five times smaller set of fibres. This permits a progressive recruitment of a larger number of independent units and therefore a graded contraction of the muscle. The regressive process, which leads to the establishment of the "one motor terminal one muscle fibre" relationship and the corollary decrease in size of the motor units, corresponds to an improvement of the motor command of the muscle. One may say that the selective elimination of redundant synapses increases the "specificity" of muscle innervation.

In the adult cerebellum, the majority (more than 90%) of the Purkinje cells receive only one climbing fibre[17,90]. Unit recordings in 8–9-d-old rats show that, at this early stage, the response of the Purkinje cell to climbing fibre stimulation is "complex" as in the case of the immature neuromuscular junction: more than 50% of the Purkinje cells would be innervated by at least two distinct climbing fibres. A subsequent regressive phenomenon leads, as nearly as 15 d after birth, to the one-to-one relationship of the adult[91,92].

In these instances, the genetic program of the organism allows for an overproduction of labile and functional contacts but during maturation a significant fraction of them subsequently regresses.

Effects of target cell and its functional activity on synapse stabilisation

In the chick embryo[93-95] and in amphibians[96-98] early limb bud extirpation causes a massive depletion of both motor neurones and sensory ganglion cells accompanied by a decrease of the enzyme choline acetyltransferase in the spinal cord[99]. Removal of the peripheral target cells dramatically enhances the "normally occuring cell death". An interaction with the periphery is therefore required for the neurone to continue its development.

In the mutant mouse "staggerer"[31], similarly, a massive regression of the parallel fibres and granular cells occurs at late stages of development[100] and has been attributed to the failure of parallel fibres to establish normal synapses with the Purkinje cells (which show electrophysiological signs of abnormality[101] and lack a characteristic membrane protein[102]).

In none of these instances, however, has evidence been presented that the actual activity of the postsynaptic cell has any effect on these regressive phenomena. To test this possibility, several cholinergic agents or toxins known to block selectively neuromuscular transmission were injected into developing chick embryos[103-112]. These drugs stop the spontaneous movements of the embryo which may, nevertheless, survive until hatching. Such treatment causes a marked atrophy of skeletal muscles which show signs of delayed differentiation and of regression[103-112]. Pre- and postsynaptic toxins have similar effects, which suggests that the actual "functioning" of the muscle by way of the synapse is necessary for its growth, histogenesis and maintenance. Interestingly, these compounds affect the innervation[107-109]. For example, a typical postsynaptic blocking agent, the α-toxin from the venom of *Naja nigricollis*, causes the almost complete absence of typical motor endplates as revealed by the Koelle reaction (the embryos were injected at 3, 8 and 12 d of incubation with high doses of α-toxin and observed at 16 d). The same happens with another postsynaptic antagonist: *d*-tubocurarine[110-112]. In addition, and in a rather unexpected manner, despite the fact that α-toxin binds selectively to the nicotinic receptor site, marked regression takes place on the presynaptic side of the junction, as manifested by

a decrease in the specific and total activity of choline acetyltransferase in the muscle and sciatic nerve[107] and a reduction (more than 50%) in the total number (myelinated and non-myelinated) of axons in the ventral root of the spinal nerves (in the dorsal roots no significant change takes place)[109]. A selective loss of the motor neurones takes place. This regression seems to be less significant with the presynaptic botulinum toxin at the concentration tested possibly because of an intrinsic effect of the toxin on the nerve terminal (for example it is known that botulinum toxin poisoning causes axonal sprouting at the adult neuromuscular junction[113].) Although a direct action of the α-toxin on the neurones of the spinal cord[114–115] canno tbe ruled out in these experiments, no α-^{3}H-toxin binding was detected in spinal cord extracts; in addition, the number of fibres counted in the spinal roots clearly shows that the effect of the α-toxin is limited to the cholinergic motor neurones[109].

How can an essentially postsynaptic block influence, then, the motor nerve terminal? The possibility that the α-toxin interferes with the "recognition" step at the early stages of synapse formation is made unlikely by the observation that primitive junctions (as well as the content of choline acetyltransferase) in embryos injected at the 4th d of incubation do not significantly differ from those of the non-injected control until the 12th d of incubation. Similarly, *d*-tubocurarine or α-bungarotoxin do not prevent synapse formation *in vitro*[116–119] or re-innervation of adult rat diaphragm[120].

In agreement with this interpretation are recent findings on the effect of another snake α-toxin (from *Bungarus multicinctus*) on the development of retinotectal synapses in the toad: *Bufo marinus*[121]. The α-toxin was applied to a circumscribed region of tectal surface where it blocks the postsynaptic acetylcholine receptor in a stable manner for several weeks. In regeneration experiments, the optic nerve initially invades the toxin treated regions and subsequently retracts. In situations where the optic nerve was intact, chronic application of α-toxin results in a subsequent loss of optic nerve synaptic terminals. As in the case of the neuromuscular junction the stabilisation of developing nerve terminals requires the functional interaction of the neurotransmitter with its postsynaptic receptor.

Preliminary evidence suggests that the late phase of synapse maturation, that is the regression of multiple innervation, is also coupled with activity. Section of one of the tendons of sartorius muscle in infant rats indeed significantly delays this selective regression[84] but it is not yet known whether neonatal tenotomy modifies the chronic firing of the motor neurones. Also, in the cerebellum, as a consequence of the absence of the Purkinje cell major input (the lack of granular cells and their parallel fibres in X-irradiated rats[122–123] and "weaver"[124] mouse), the multiple innervation by climbing fibres observed in the infant rats persists in adulthood.

Biochemical models for selective stabilisation

These few biological examples briefly reviewed provide some support for the "selective stabilisation" hypothesis. In particular, it is clear that (1) at critical stages of development neuronal bodies as well as growing neurites may exist under a functional labile state susceptible to death or regression (postulate 1); (2) the number of neuroblasts and nerve connections produced at critical stages of development is significantly larger than that persisting in the adult (postulate 2); (3) the activity of the network may contribute in a direct or indirect manner, to this stabilisation process (postulate 3): in particular, retrograde signals emitted by the postsynaptic soma seem to have a significant role. Regarding this last point, the role of the "nerve growth factor" as a retrograde signal in the development of the sympathetic ganglion has already been extensively documented and reviewed[125–127].

Table 1 Degradation of the cholinergic receptor protein

Extrasynaptic:	
developing myotubes *in vitro:*	
—decay of bound α-toxin	16–28 h (ref. 148)
—chase of ^{35}S labelled receptor and decay of ..... bound α-toxin	16 h (ref. 158)
adult rat diagram:	
in vivo	19 h (ref. 153)
in vitro	8–11 h (ref. 154)
Subsynaptic:	
adult rat diaphragm:	
in vivo	7–5 d or more (ref. 153)
in vitro	6 d or more (ref. 154)

The following discussion deals with biochemical processes, relevant to the mechanism of synapse stabilisation. We shall distinguish events taking place on the postsynaptic side of the synapse (primarily the "localisation" of the receptor protein) from those concerning the nerve terminal and its selective "maintenance" or stabilisation. The examples and reasonings presented concern the developing neuromuscular junction in vertebrates but might be adapted to more complex neuronal networks.

Postsynaptic 'localisation' of the receptor protein

This discusion deals with the 'localisation' of the acetylcholine receptor, but it should be borne in mind that localisation of acetylcholinesterase takes place simultaneously. In the adult neuromuscular junction the density of acetylcholine receptor sites counted with radioactive snake α-toxins is 100–1000 times higher than in extrasynaptic areas[128–134]. It reaches an average of 8,000–9,000 sites per μm^2 (refs 128–132) in mouse junction with a higher density in the top (30,000±60,000 sites per μm^2) than in the bottom of the synaptic folds[133,134] and up to 50,000± 15,000 sites μm^2 in *Eloctrophorus* electroplaque (refs 135–137 and J. P. Bourgeois, J. L. Popot, A. Ryter and J. P. C., unpublished). The sub-synaptic membrane consists of a closely packed (lattice) assembly of the receptor molecules. Once formed the subsynaptic membrane appears remarkably stable: after denervation, the main features of the endplate region[138–140], the dense deposit of acetylcholinesterase revealed by the Koelle technique[2,141] and the high sensitivity to iontophoretically applied acetylcholine[142–144] persists for weeks. In *Electrophorus* electroplaque, the density of α-^{3}H-toxin sites in the subsynaptic membrane decreases by less that 50%, 52 days after denervation (refs 136, 137 and J. P. Bourgeois, J. L. Popot, A, Ryter and J.P.C., unpublished). No significant tendency for lateral diffusion of the receptor protein exists either in the adult "uncovered" neuromuscular endplate[145,146]. In the developing rat or chick myotubes, the density of acetylcholine receptor sites lies in between that of the extra and subsynaptic areas in the adult synapse (1,500/2,000 α-toxin sites per μm^2)[147–149]. Although the actual translation mobility or the receptor protein in embryonic cells has not been measured yet, it should be close to that of the bulk proteins in a highly fluid cytoplasmic membrane[150,151] that is much higher than in the subsynaptic membrane of the adult synapse.

The turnover rate of the cholinergic receptor protein in junctional and extra-junctional areas has been measured either with α-^{125}I-bungarotoxin (the particularly high stability of the toxin-receptor complex makes possible measurements of turnover rates faster than its own half life (9–15 d)[148,152–154]) or directly after pulse labelling of the receptor protein by ^{35}S-methionine[155,156] or heavy isotopes[157] followed by immunoprecipitation and/or purification. In developing calf myotubes *in vitro* the two methods give identical turnover rates[158]. Table 1 shows that the degradation of the receptor protein occurs at a rate considerably faster in extra than in subsynaptic areas both *in vivo* and *in vitro* and the rate in the extrasynaptic areas of the adult muscle after denervation is close to that observed on the surface of the developing myotube. The degradation process is blocked by inhibition of oxidative phosphorylations: DNP, cyanide (possibly because of an energy-dependent "internalisation" process) but insensitive to inhibitors of protein synthesis (cycloheximide, puromycin)[148]. The rate constant of

degradation of the extrasynaptic receptor appears independent of the culture conditions and in particular does not vary with the growth rate of the developing myotubes (ref. 149 and J. Merlie, unpublished). Under these conditions the regulation of the number of receptor molecules therefore depends directly on its rate of synthesis.

Pharmacological differences have been reported *in vivo* between the subsynaptic and the extrasynaptic receptors[60,61]; in addition, noise measurements reveal that the opening time of the cholinergic ionophore increases after denervation[4]; yet, they may not reveal differences in the primary structure of the two classes of receptor molecules but, for instance, differences in packing or environment of the receptor protein in the membrane. In agreement with this interpretation are the following observations. (1) *In vitro* the binding constants for agonists and antagonists of the membrane-bound receptor do not change in chick embryo leg muscles from 8 d (extrasynaptic) before hatching until 70 d after hatching (subsynaptic) (G. Giacobini, unpublished); the receptor proteins purified by affinity chromatography from extra and subsynaptic regions of rat diaphragm have been reported to show identical binding properties in one laboratory[161] but different in another one[161]. (2) The equivalence point by immunoprecipitation seems to be identical for the two receptors (ref. 162, and H. Sugiyama, unpublished). (3) These two molecules have the same hydrodynamic properties[162]. Only isoelectric focusing unambiguously separates the two receptors, revealing a charge difference[162] but interconversion between the two structural forms has been obtained *in vitro*[163]. The most likely interpretation of the data is that the extrasynaptic and subsynaptic receptors derive from the same protein molecule, the observed difference would result, for instance, from a covalent modification[164] but a different subunit composition of the two forms is not excluded.

Little is known yet about the factors which regulate the distribution and concentration of acetylcholine receptor in developing neuromuscular junction; however, the increase of sensitivity in extra-junctional areas after denervation is accounted for entirely by a neo-synthesis of receptor molecules[155,165,166]. Direct electrical stimulation of adult denervated muscle through chronically implanted electrodes (refs 167–169 and T. Lømo, unpublished) or of embryonic non-innervated myotubes[170] maintained *in vitro* abolishes the hypersensitivity to ACh in the extra-junctional areas. The result depends critically on the amount and pattern of the stimuli. Maximal rate of decline of ACh sensitivity is approximately exponential with a half time in the range of that reported for the degradation in extrasynaptic areas[168,169]. It is likely that optimal stimulation blocks receptor synthesis, the acetylcholine sensitivity falling at a rate determined by the degradation of the extra-junctional receptor[169] (recent experiments[170] actually show that activity slows down this rate by about a factor of two).

To account for the localisation of the receptor protein and for eventual intercorrelations between developing synapses mediated by the postsynaptic cell we propose, that during development (Fig. 2):

(1) The receptor may exist under two interconvertible forms: *l*, labile and diffusible and *s*, stable, resistant to degradation and immobilised.

(2) The *s* state derives from the *l* state by way of a stabilisation reaction $l \rightarrow s$ which is not reversible. This reaction takes place when both an anterograde factor liberated by the nerve terminal during activity and an "internal coupling factor" are present above a critical concentration and within a given lapse of time, on the two faces of the subsynaptic membrane.

(3) On the membrane area underlying the developing nerve terminals, the neurotransmitter, in addition to its electrogenic effects, triggers the emission of a signal whose amplitude is given by the concentration of internal coupling factor which propagates inside the cell. (The propagation of the internal signal may result from a regenerative process. The initiation of the signal may take place with a significant delay in such a manner that its amplitude would reach the threshold for stabilisation only at a certain distance from the synapse. This would prevent, or delay, the self-stabilisation of the emitting synapse.)

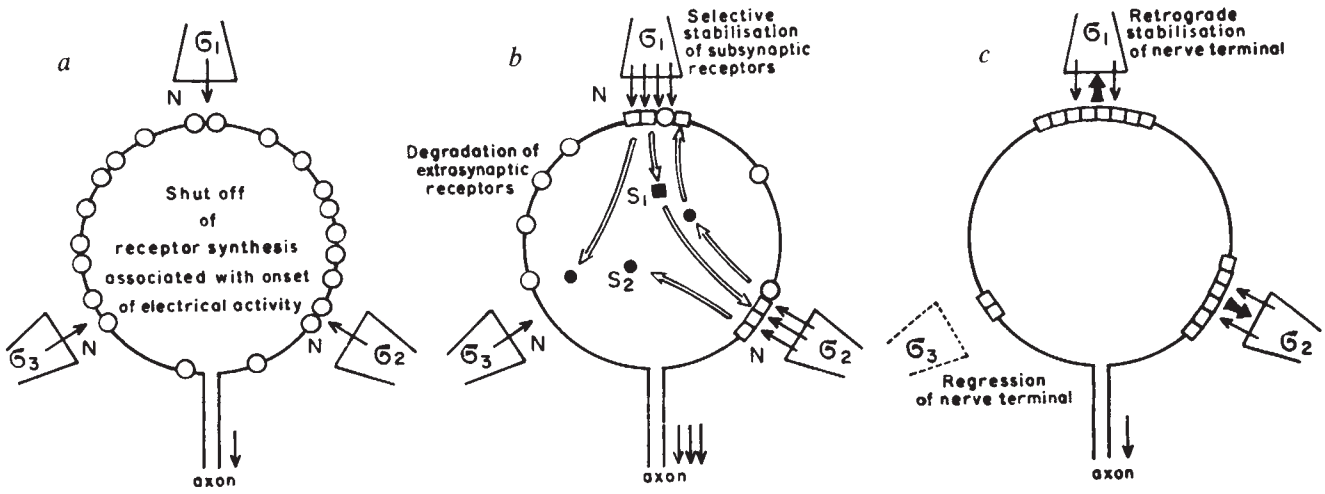
(4) The synthesis of the labile form of the receptor protein stops in the postsynaptic cell when activity starts in the myotube. An internal shut-off factor would act selectively on the protein synthesis machinery.

Accordingly, the selective localisation process would correspond to a management of a fixed and limited stock of *l* via lateral diffusion and stabilisation reactions.

This model is being formalised[172] and may account for the redistribution of the receptor protein during localisation. The still hypothetical stabilisation reactions of the receptor molecule might for instance be a covalent modification of the receptor molecule such as a phosphorylation[173–175], an adenylation[175,176], a glycosylation[177,178] or any other mechanism providing protection against proteolysis[179,180]. The modification might for instance change the tendency of the receptor molecule to make aggregates under which it would be altogether resistant to degradation and immobile. It might also facilitate the anchoring of the receptor molecule inside the postsynaptic cell to fibrillar proteins[181]: actin, tubulin, or collagen, or, on its external surface to the basement membrane or any "cleft substance".

The most likely candidate for the "anterograde factor" is the neurotransmitter[104,107,111,164]. This role in localisation has been

Fig. 2 The selective localisation of the receptor on the surface of a neurone receiving several nerve terminals. The labile and mobile state of the receptor for the neurotransmitter is represented by a circle, the stable and immobilised one by a square. The different signals postulated are represented by arrows: N, the anterograde factor, for instance the neurotransmitter; S, the internal coupling factor; a thick arrow indicates the retrograde factor. At the onset of activity a "shut off" factor stops the synthesis of labile receptor molecules. The model allows for the segregation of different receptor molecules if the nerve terminals secrete different anterograde factors.



challenged[118] despite the fact that it provides a simple explanation for the segregation of different receptor molecules in a complex situation such as that of a neuronal soma receiving nerve terminals with different neurotransmitters. Other factors liberated by the nerve terminals such as, for instance, ATP[182,183] or some of its degradation product enzymes or polypeptide hormones might have to be considered. The liberated ATP might offer a convenient energy supply for reactions taking place on the external surface of the membrane. Alternative (or additional) processes which do not require the emission of a diffusible signal might also have to be envisioned such as a "patching"[61,181] of the receptor molecules in the postsynaptic membrane; spontanenus or resulting from a direct physical contact with the nerve terminal.

The postulation of one (or several) internal "coupling factor(s)", in addition to the membrane potential[184] is not necessary to account for the localisation of the receptor protein in the focally innervated muscle fibre. It may, however, offer plausible explanations for the development of complex patterns of synapses on neuronal somas (Fig. 2) or dendrites or, in the case of the avian ALD, of a regular distance between synapses. In any case it allows for interactions between synapses on a given soma in a more discriminative manner than the membrane potential. The postulated mechanism for the propagation of the signal is even more hypothetical than its very existence but plausible reactions may be written down which lead to the propagation of a "chemical" wave. (They might for instance be those of the nucleotide cyclase protein kinase and phosphatase system if, in one manner or another, the protein kinase target of the cyclic nucleotide exerts a positive feedback on the nucleotide cyclase.)

The "shut-off" of protein synthesis may be triggered by the internal coupling factor or by a different one. Possible candidates are the often evoked Ca^{2+} ions[185,186], cyclic nucleotides[173,187] or both. Ca^{2+} would be a rather simple "shut-off" factor since it might enter through the cholinergic ionophore[188-191] or the tetrodoxin sensitive ionic channels[192-194] when they are activated. Signals directly coupled with the mechanical contraction of the muscle may also have a role.

In any case, despite its non-uniqueness the proposed model gives rise to several predictions which may offer experimental tests for the validity of its critical assumptions.

(1) If localisation corresponds to the management of a limited pool of receptor protein, changing the size of this pool by selectively blocking receptor synthesis before the arrival of the nerve terminals should interfere with localisation and/or reduce the number of endplates formed in a muscle like ALD. The "immunity" to innervation of the extrasynaptic areas in an adult muscle fibre would then simply result from the absence of receptor molecules available in the extrasynaptic membrane to form a novel endplate.

(2) The activity of the postsynaptic cell is expected to regulate the synthesis of receptor through the shut off factor but also the management of the membrane pool of receptor through the stabilisation reaction. Once the synthesis of receptor has stopped, changes in the program of activity of the developing nerve terminals by chronic stimulation (or blocking) may lead to the formation of supernumerary endplates (or prevent localisation) or, thanks to the internal coupling factor, for instance modify the distance between endplates. In avian ALD this may occur[110-112]. In agreement with these views is the recent[195] observation that the spontaneous activity markedly differs in the slow ALD with multiple endplates and the focally innervated fast PLD from chick embryo (16–18-d-old): ALD fires continuously at a rate of 0.2 to 5 Hz, while in PLD only occasional activity is seen at a rate of 4 to 8 Hz. Chronic blocking of synaptic transmission may also modify the pattern of endplates and their relative distance in these muscles[110-112].

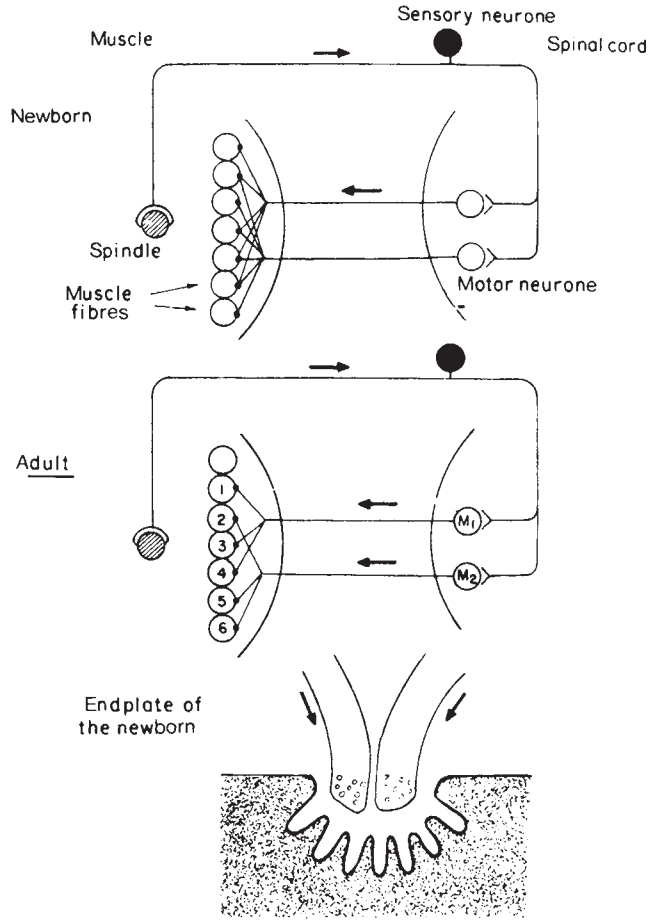
(3) According to the theory, the development of a characteristic geometric pattern of synapses may be determined by the exact temporal pattern of activity integrated by the postsynaptic cell. To illustrate this point, if Ca^{2+} is the shut off factor its internal concentration might be regulated by two competing kinetic processes: the opening of the ionophores in the postsynaptic cell and the pumping rate of calcium by the mitochondria. A critical firing rate shall then be required to let the internal Ca^{2+} concentration reach a critical value to turn off the synthesis of receptor.

Presynaptic selection of a single nerve terminal at multiply-innervated endplates

Less information is available as yet on this second step of synapse maturation (Fig. 3), we may nevertheless propose that: (1) all the motor nerve terminals converging on a given endplate receive the same average number of impulses but in a randomly non-synchronous manner, (2) at the peak of the multi-innervation stage, all terminals share almost equivalent areas of the stabilised subsynaptic membrane surface and/or dispose of a limited stock of a postsynaptic "retrograde factor" x, (3) the arrival of impulses cause an increase of surface occupancy and/or of utilisation of x, (4) when the surface occupied by the nerve terminal (and/or the amount of x utilised) becomes lower than a critical value this nerve terminal regresses.

This set of minimal assumptions may explain both the stabilisation of a single nerve terminal and the constant size of the motor units. Accordingly, the size of the motor unit should be determined by the ratio of the number of muscle fibres to that of motor neurones. Indeed, it is known from human patients that a partial neurogenic atrophy (which reduces the number of motor neurones) causes a decrease in the number

Fig. 3 The differentiation of motor units in a skeletal fast muscle by retrograde selective stabilisation of nerve terminals. In the neonate one motor neurone innervates many more muscle fibres than in the adult and each muscle fibre receives several nerve terminals. A few weeks after birth only one nerve terminal persists per muscle, the others regress.



of motor units with a marked increase in their size[196–198]. An increase of surface occupancy by nerve terminals as a consequence of activity has been described[199, 200], but the biochemical mechanisms by which a nerve terminal becomes stabilised once a critical surface of the postsynaptic membrane is occupied remains obscure. The postulated retrograde factor (substance *x*) has not been identified chemically; in the case of the neuromuscular junction: the concentration of calcium in the cleft[108] or of any nerve growth factor are possible candidates. An interesting possibility would be the direct covalent modification of the fibrillar or tubular proteins engaged in the maintenance of the shape of the nerve terminal and thereby regulating its internal "cytoplasmic flow".

Conclusions

From a biochemical point of view, the selective stabilisation hypothesis appears plausible. Four different signalling mechanisms have been postulated to govern the evolution of a developing synapse: (1) for the postsynaptic localisation of the receptor protein: an anterograde factor liberated by the nerve terminals (possibly the transmitter) which governs the stabilisation reaction of the receptor, an internal shut off signal which informs the protein biosynthesis machinery (transcription and/or translation) of the activity of the postsynaptic cell, an internal coupling factor which permits exchange of information between synapses and 2) for the selective presynaptic stabilisation of the nerve terminals, a retrograde factor emitted by the postsynaptic cell. Additional mechanisms and regulatory signals may have to be postulated in the future to account for the complete description of the stabilisation and maturation of the synpase which is certainly a complex process lasting for days or even weeks. In any case, at this level of understanding the word "trophic factor" is of no help and should be abandoned.

The basic assumption of the theory that an increase of "specificity" accompanies the active stabilisation of particular synapses (and the regression of others) remains to be tested with neuronal networks (cerebellum, visual cortex) more complex than the motor innervation of the striated muscle. For instance, an intriguing possibility would be that in the much studied visual cortex (Fig. 1) the acquisition of a given specificity such as the specificity of orientation[18], results from such a selective stabilisation (Fig. 1). It would be of primary interest to investigate through intracellular recordings if the transition from "nonspecific units"[201] to "specific" ones would correspond to a decrease of synapse redundancy via the regression of transiently multi-innervated neuronal somas and to what extent the activity of the system, spontaneous and/or evoked, governs these transitions.

In a more general manner, these speculations place emphasis on critical features of developing neuronal networks which are directly accessible to the experiment: (1) their early activity (spontaneous or evoked) preceding synapse maturation: (2) the existence and limits of a transient redundancy (in the case of motor innervation the limits of the fluctuation are the boundaries of the muscle[202]); (3) an eventual modification of this redundancy by chronic changes of the early activity.

Even if the limits of the redundancy are narrow, they may be sufficient to provide an opportunity to save genes during the differentiation of neuronal networks and to provide a plausible biochemical mechanism for "learning" without postulating any synthesis of new molecular species.

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articles

Ophiolites in south-western Newfoundland

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Ophiolite and ophiolite-related rocks have long been recognised in northern and western Newfoundland[1,2]. The presence of these rocks has, to a great extent, controlled the plate tectonic models developed for the tectonic evolution of the Newfoundland Appalachians. Such models have been extrapolated through south and south central Newfoundland with little geologic control. I describe here the ophiolites in south-western Newfoundland, discuss their relationship to a cryptic suture, the Cape Ray Fault, and evolve an independent model, which I compare with the model developed for northern and western Newfoundland.

SOUTH-WESTERN Newfoundland consists essentially of two basement complexes separated by a zone of intense mylonitisation, the Cape Ray Fault. The Long Range Gneiss, to the North of the fault, forms part of the Western Crystalline Belt[3] and, as such, defines the western margin of the Proto-Atlantic Ocean. The Port aux Basques Gneiss, to the South of the fault, forms part of the Eastern Crystalline Belt[3] and defines the eastern margin of the Proto-Atlantic Ocean. The Cape Ray Fault has been interpretated as a cryptic suture, along which complete closure of the Proto-Atlantic Ocean took place[4].

Lower sheet

Recent work (P. A. Brown, unpublished) in south-western Newfoundland has shown that the Long Range Mafic-Ultramafic Complex[5] occurs as thrust sheets overlying a