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Nimodipine and Neural Plasticity

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Abstract

Mental deterioration associated with aging is often described as a decrease in the adaptive capacity, or plasticity, of the nervous system. The peripheral nervous system has the ability to respond to traumatic injury by regeneration and re-establishment of functionally appropriate connections. In experimental animals, this repair capacity diminishes with age. It is likely that the reduced ability to respond to damage and the overall deterioration in nervous system function are related phenomena. The point at which nerve growth occurs, the nerve growth cone, is acutely sensitive to changes in calcium concentrations, thus alterations in calcium levels might be expected to modulate both injuryinduced regeneration and agerelated deficits. The known disturbances in calcium homeostasis in the aged brain may thus be causally related to decreased plasticity. In young adult rats oral administration of the calcium channel blocking agent, nimodipine, has been shown to enhance recovery of peripheral nerves following crush lesion. Rats treated with nimodipine respond earlier to noxious stimuli applied to the hind paw and more rapidly re-establish a normal walking pattern, as measured by footprint analysis. A similar acceleration of recovery of normal locomotion subsequent to peripheral nerve crush results from intraperitoneal dosing with nimodipine. Aged rats show deficits in peripheral nerve conduction velocity, peripheral nerve fibre density and locomotion in the absence of experimental trauma to the nerves. Oral treatment with nimodipine reduces these abnormalities in aged rats. The results suggest that the neurotrophic actions of the calcium channel blocker measured in peripheral nerve regeneration also occur in the aged nervous system and that this is a valuable approach for the treatment of mental decline in senescence.

Introduction

Neural plasticity is the capacity of the nervous system to adapt continuously to a changing internal or external environment. It can be studied at the molecular,

morphological, neurophysiological and the behavioural level. Neural plasticity is an essential feature of neural function, and forms the basis of development, learning and memory formation. Neuromuscular junctions of mammalian skeletal muscle fibres are modified (synaptic remodelling) throughout the animal's life as noted by Barker and Ip (5). Later studies showed that in the normal adult frog, the nerve endings sprout continuously (73). Recent studies of the plasticity of cortical sensory maps indicate that map organization can also be modified during adult life (70, 71). Thus the nervous system is dynamic rather than static, and adaptive changes occur at all levels of complexity.

Neural plasticity is of great importance in relation to a number of serious human health problems such as injury to the peripheral nerve, spinal cord or brain, developmental disorders, and dementia (22). The mental decline associated with brain aging is commonly considered to result in part from reduced neural plasticity. For these reasons further insight into the mechanisms underlying neural plasticity mechanisms is of vital importance. It is evident that neural Ca^{2+} homeostasis is a key factor in the control of plasticity. Evidence is accumulating to suggest that intracellular Ca^{2+} is a major factor in the regulation of growth cone motility. Different growth cone behaviours such as protrusion, refraction and elongation seem to have different Ca^{+2} dependencies (34). Furthermore, the synaptic plasticity that can be observed during the establishment of long-term potentiation in rat hippocampal synapses involve Ca^{2+} entry (40) and activation of Ca^{2+}-sensitive processes including phosphatidylinositol 4,5-biphosphate breakdown (4), protein kinase C-mediated phosphorylation of specific substrates, such as the protein B-50/F1 (1, 15) and selective proteolysis (39). Severe disturbance of Ca^{2+} homeostasis in the aged brain, in which neural plasticity is diminished has been reported (24, 33, 36).

Evidence is rapidly accumulating that Ca^{2+} antagonists can modulate many aspects of nervous system function. Recent studies have suggested that nimodipine, a Ca^{2+}-entry blocker of the dihydropyridine type, may reduce functional deficits in spinal cord injuries (6), and improve both peripheral and central nervous system function in aged and lesioned laboratory animals (54 and LeVere this volume).

These findings strongly suggest a therapeutic utility of nimodipine in both CNS and PNS dysfunction associated with trauma or aging. In this paper we highlight some of the earlier data and report for the first time that intraperitoneal administration of nimodipine accelerates functional recovery following peripheral nerve damage in the rat.

Calcium and Neural Plasticity

Calcium ions play an important role in the regulation of many neuronal functions. It is evident that they have a key role in the control of neural plasticity. Neurons have a free Ca^{2+} concentration of about 10^{-7} M. This concentration can rise as a result of release of Ca^{2+} from intracellular stores of Ca^{2+} bound to membrane surfaces and cytosolic components and segregated within membrane-bound organelles. Several chemical stimuli (hormones and neurotransmitters) act upon cell surface receptors and stimulate the breakdown of the phospholipid phosphatidylinositol biphosphate (5), which in turn generates diacylglycerol (DAG) and inositol triphosphate (IP_3). Activation of intracellular receptors for IP_3 leads to release of Ca^{2+} into the cytoplasm (21). The other major cause of increased free cellular Ca^{2+} is the opening of voltage-sensitive calcium channels (VSCC) on the plasma membrane through which Ca^{2+} can pass into the cytoplasm. Recent electrophysiological studies have provided evidence that there are at least three types of VSCC, the L-, N- and T-type (62). Thus the free calcium within synapses is modulated by electrical and chemical stimuli, thereby altering synaptic function via a series of calcium-sensitive modulating steps.

In addition to its role in the function of pre-existing synapses, Ca^{2+} is important in the remodelling of neuronal connections. Formation of new synapses involves growth and is dependent on a specialised region, known as the growth cone, at the tip of the growing nerve process. Intracellular Ca^{2+} is of major importance in the regulation of growth cone motility. Changes in the membrane potential caused by a variety of stimuli are integrated at the level of the growth cone membrane. Voltage-gated Ca^{2+} channels transduce the net effect on the membrane potential into an intracellular Ca^{2+} pulse. The free Ca^{2+} level within the growth cone acts as a final integrator of the environmental stimuli by influencing the elements (cytoskeleton and vesicular apparatus) that underlie the motility of the growth cone. The effects of Ca^{2+} on the cytoskeleton elements may be direct or may require intervening Ca^{2+} binding proteins such as Ca^{2+}-dependent kinases and proteases. Intracellular levels of free Ca^{2+} have been shown to be lower in spontaneously inactive growth cones than in those that were active. Apparently the balance between influx, efflux and Ca^{2+}-buffering systems such as pumps, Ca^{2+} binding proteins and organelle sequestering systems play a crucial part in the regulation of growth cone behaviour. Both excessive increases and excessive decreases in cellular Ca^{2+} impair growth cone function and thus the ability of the axon to reach its proper target (34).

There are indications that nerve terminals at the end plates of skeletal muscle fibres are being remodelled throughout life (5, 63, 72), and these changes seem

to be to some extent related to the activity of the synapse (28). It has been reported (12, 61) that the activity induced elimination of nerve-muscle contacts is dependent on Ca^{2+} and regulated by calcium activated neutral proteases (CANP). By increasing calcium, nerve-muscle contacts are disrupted and this effect of calcium was found to be prevented by concurrent inhibition of the CANP. Conversely, reducing Ca^{2+} or inhibiting the CANP reduces the rate of the naturally occurring elimination of polyneural innervation. Adult mammalian nerve endings are known to continue extending and retracting small branches (28) and it may be that the retracting branches are normally removed by CANP activity, which itself is activated by calcium entry (61).

Nimodipine as a Potential Neurotrophic Agent

Many of the molecular processes underlying neuronal repair are similar, if not identical, to those that govern neurite outgrowth and synaptogenesis during development. It is therefore logical to consider the factors that influence the development, differentiation, and maturation of neurons as potential promotors of repair processes in damaged neurons in adult life (3, 64). Indeed, nerve growth factor, gangliosides and melanocortins not only were shown to stimulate fetal neuronal development in culture, but also stimulate recovery of function following peripheral nerve injury in vivo (17). In view of the important role that calcium plays during neuronal development and neurite outgrowth (see above) it is not surprising that research is focussing on drugs that modulate intracellular calcium levels. The demonstration by Azmitia (this volume) that nimodipine is able to influence the maturation of cultured serotonergic cells obtained from fetal rat raphe nuclei suggests that modulation of calcium channels can influence developmental processes. In view of the parallels between development and repair discussed above, nimodipine may also affect neuronal plasticity in mature neurons in vivo.

Nimodipine and Neural Plasticity in Aged Animals

Old rats show a diversity of behavioural symptoms such as: diminished learning and memory capacities, reduced social and sexual behaviour, decreased locomotor and exploratory behaviour in novel environments, reduced body care, disturbed circadian rhythms of sleep, food intake abnormalities, and impaired balance (21). This makes the old rat an attractive model for the study of behavioural aspects of senile dementia. Schuurman et al. (this volume) examined the behavioural effects of orally administered nimodipine on

cognitive functions in old Wistar rats, and reported that the total number of errors in a water maze was significantly smaller in the nimodipine treated rats. Also treated rats showed a higher level of exploration, and duration of inactivity was significantly lower. In aging rabbits, intravenous administration of nimodipine has been shown to accelerate acquisition of associative learning (Disterhoft, this volume). At the molecular level, nimodipine has been shown to influence the Ca^{2+}-dependent afterhyperpolarization in isolated CA1 hippocampal neurons from aged rats (36). Age related deficits in motor function in rats have been noted by many researchers and are discussed in detail by Coper et al. (13). It is suggested that the decline of motor performance with age is primarily due to a loss of precision and a slowing down of central mechanisms. Schuurman et al. (54) were able to show for the first time that oral administration of the Ca^{2+}-entry blocker nimodipine improves motor coordination (locomotion and balance, suspended hanging and climbing in a pool) and walking of aged rats. In separate experiments reported by Gerritsen van der Hoop et al. (23) in addition to walking pattern analysis both sensory and motor conduction velocities in the sciatic nerve were measured using the technique described by De Koning and Gispen (19). At 29 weeks the motor and sensory conduction velocities in the untreated rats were much slower than in the young adult animals, whereas in rats given oral nimodipine, they were close to the young adult rats. Histological analyis showed that fibre density in the aged, control rats was much lower than seen in young animals. In contrast, in nimodipine treated aged animals fibre densities were similar to those observed in untreated young control rats (67). Collectively, these data warrant further and careful evaluation of nimodipine as a potential drug in the treatment of age related loss of neural and behavioral plasticity (see Schuurman this volume).

Nimodipine and Postlesion Plasticity in the CNS

During the last few years, evidence has been accumulating that some calcium antagonists are useful in the treatment of several CNS disorders. The neuronal effect of nimodipine appears to be mediated through slowly inactivating Ca^{2+} channels (L-type) in a voltage-dependent manner; the blockade of these channels leads to reduction of Ca^{2+} entry into neurons (56). Le Vere et al. (this volume) investigated the effect in rats of repeated administration of nimodipine on the recovery of preoperatively learned behaviour following a brain lesion known to disrupt that behaviour. Nimodipine significantly reduced the number of errors made by rats. The facilitatory effect of nimodipine on recovery has been reported by these authors to be probably due to an effect on the memory

process. Isaacson (personal communications) has also observed a nimodipine-induced enhancement of recovery following a septal lesion in rats. These observations suggest that nimodipine might be of use in the treatment of brain injuries in addition to its protective effect in ischaemia.

Nimodipine and Postlesion Plasticity in the PNS

The neuron is an extremely specialised and differentiated cell and has proved to be the most vulnerable cell in the mammalian central and peripheral nervous system. In general, it is assumed that damage to cell bodies of neurons results in irreversible degeneration and cell death. If damage is restricted to the neuronal processes (dendrites and axons) regeneration with resulting reinnervation of the target is possible. For reasons still not completely understood, the neurons in the PNS regenerate better than neurons in the CNS. The above described behavioural effects of nimodipine in CNS-damaged and old animals strongly suggest therapeutic utility of nimodipine in PNS dysfunction associated with trauma.

The effects of nimodipine on plasticity of the peripheral nerve have been studied in rats using a crush model. Return of both sensory and motor function can be monitored easily by applying techniques described by De Koning et al. (18). The speed of recovery can be assessed by using foot flick withdrawal, and the quality of recovery can be monitored with an analysis of the free walking pattern. Van der Zee et al. reported that orally administered nimodipine enhanced recovery of both sensory and motor function in this model, reducing the days needed for recovery by 2–3 days, and improving the walking pattern (65).

In a separate experiment, a crush lesion was produced unilaterally in the sciatic nerve of four groups of rats. Animals received 5 mg/kg, 10 mg/kg or 20

Table 1. Sciatic Functional Index (SFI) (mean ± SEM). The figures were analyzed with ANOVA on repeated measures.

Ways after crush	−1	12	14	16	18	20	22	24
Control	+2.3±1.5	−77.0±2.9	−66.4±5.1	−42.2±5.6	−23.1±5.1	−11.3±3.3	−12.7±2.2	−2.4±2.7
5 mg/kg*	−1.8±3.2	−77.2±2.7	−57.7±5.3	−32.9±7.3	−17.6±4.7	−16.4±2.6	−3.7±3.3	–
10 mg/kg**	−0.5±2.3	−78.8±3.0	−57.7±5.2	−26.5±3.7	−7.6±1.8	0.2±4.5	−0.8±2.6	–
20 mg/kg**	−4.0±2.3	−73.2±3.0	−45.7±4.3	−17.4±4.9	−9.7±3.1	−7.8±3.1	−2.5±3.1	–

Statistical significance levels for the values given in the table are:
* not significant v. control and ** $p < 0.001$ v. control.

mg/kg intraperitoneal injections of nimodipine every 48 h, starting on the day of the crush. The control group was injected with vehicle (polyethyleneglycol/saline 2:1). The quality of recovery was assessed by analysing the free walking pattern (Fig. 1). In all three nimodipine treated groups the sciatic functional index (SFI) returned to normal earlier than the control group. The 10 mg/kg and 20 mg/kg administered rats showed an overall better recovery than the controls. Improvement of walking pattern was significantly faster only in the high-dose groups (Table 1).

The Growth-Associated Protein B-50

In the search for the mechanism of action of nimodipine in postlesion neuronal repair, attention should be given to the newly identified neuron-specific class of so-called growth-associated proteins. Comparison of anterogradely transported proteins in intact versus regenerating nerves revealed a small family of proteins synthesised at levels up to 100-fold higher during neurite outgrowth (58, 59, 60). Based on these initial metabolic labeling studies, the GAP hypothesis was postulated: Induction of a small subset of growth-associated proteins (GAPs) may be a prerequisite for axonal growth during development and regeneration (38). The best characterised member of this family is GAP43 (=B-50).

An acidic neuron-specific phosphoprotein has been studied independently for many years by a number of different laboratories and given a different name by each research team. Recently, cross-laboratory studies and sequencing data confirmed that all the originally named proteins B-50, F1, GAP43, GAP48, pp46 and γ5 are equivalent (7). This list has now been extended to include the neuron-specific, atypical calmodulin-binding protein P-57 (11). The protein will further be referred to as B-50.

Increased Levels of B-50

During the development of the CNS, B-50 levels are highest in the perinatal period, when axon outgrowth and synaptic organization occur in rabbit (58, 59), rat (31, 77), hamster (44) and human (47, 48). A sharp decline in synthesis (mRNA level) is seen thereafter, followed by a slower decrease of B-50 levels (31). In human brain, B-50 expression declines with age, but remains relatively high in some associative brain areas (47, 48). Induction of the protein accompanies successful regeneration of peripheral nerves, but does not occur in damaged central nerves, which fail to restore their projections (7).

Whether the amount of B-50 molecules determines the axonal growth rate is not clear. In tissue culture, growth cones of young neuronal origin grow faster and also display stronger B-50 immunoreactivity than morphologically matched ones from older animals (32). Interestingly, when regeneration from a crush lesion in the rat sciatic nerve is accelerated by a preceding, conditioning lesion, B-50 levels raise earlier and higher than after a single crush lesion (66). Furthermore, B-50 levels decline to normal several weeks after the lesion (38, 66) and this normalization may be independent of whether successful target connection has taken place (75). All these in vivo and in vitro studies of the last five years confirm that its expression is highly correlated with axon growth. Nonetheless, a causal relationship between expression of the protein and axonal outgrowth and synaptic organization has still not been established. Some indication of the possible role of B-50 may be obtained by determining its exact localization in growing neurites.

B-50 Localization

Immunostaining for B-50 of explanted dorsal root ganglia grown in culture shows a halo of strong immunoreactivity in the distal growth cone region, with low intensity staining in neurites (43, 45). Monitoring the developing pyramidical tract at the third cervical spinal segment revealed a transient wave of high B-50 immunoreactivity which coincides with the passage of growth cones of outgrowing corticospinal axons (27). In the regenerating sciatic nerve, B-50 is associated with newly formed sprouts (68). In humans, nearly all fetal endplates in the skeletal muscles were shown to be immunoreactive for B-50. The percentage of B-50 positive endplates drops significantly during the peri- and postnatal period, but in children and adults a low percentage of B-50 positive endplates remains present (30). In adult rats B-50 is virtually absent in intact neuromuscular junctions, but appears during reinnervation in association with the presynaptic membrane and with synaptic vesicle-like structures (69).

B-50 immunoreactivity is highest in growth cones and much lower in neurites of cultured dorsal root ganglion cells. This distribution is abolished when axonal transport is inhibited by colchicine so that B-50 immunoreactivity becomes evenly distributed between neurites and growth cones (53). Thus the proximo-distal gradient of B-50 appears to be built up by fast axonal transport. Preliminary studies of Meiri and Gordon-Weeks (43) indicate, that in growth cones isolated from fetal and neonatal rat brain. B-50 is detectable in cytoskeleton-associated membranes, but not in a pure cytoskeleton preparation of the growth cone. From these correlative and immunolocalization

studies, B-50 appears to be transported along the cytoskeleton towards the growth cone, where it becomes somehow associated with the plasma membrane.

B-50 and Protein Kinase C (PKC)

Protein B-50 is a substrate for the Ca^{2+} – and phospholipid-dependent kinase PKC (2). PKC is concentrated in differentiating, neuropil-rich regions and nerve fibres of the developing rat brain (45, 25), whereas in adult rat brain the kinase is closely associated with presynaptic terminals (74, 25). A very similar localisation has been described for B-50 (50, 51, 26, 8). Like B-50 (77), the kinase C system develops during prenatal (10), or perinatal (29), development of rat brain. This co-localisation and co-purification of B-50 with its kinase through several steps (77, 2) suggest that PKC phosphorylation of the protein is very important for its function.

Nimodipine, Neurotrophic Effects and B-50 Function?

An attractive hypothesis is that in postlesion plasticity nimodipine is modulating intracellular calcium levels crucial to growth cone motitlity and neurite outgrowth. As B-50 is identical to the atypical calmodulin binding protein P-57 (or neuromodulin) the intracellular Ca-CaM concentration may be codetermined by the degree of phosphorylation of B-50, as CaM only binds to the unphosphorylated protein. Furthermore, B-50 phosphorylation is strongly correlated with presynaptic neurotransmitter release (17) and has been shown to be a pre-requisite for the vesicular release of noradrenalin from isolated rat brain synaptosomes (16). One might speculate that neurite outgrowth involves similar membrane fusion processes as observed during presynaptic transmitter release. Hence, the degree of phosphorylation of B-50 in growth cones may also be crucial to neurite growth. Work is now in progress to study the effect of nimodipine on growth cone calcium and the PKC-B-50 system.

Nimodipine and Cisplatin Induced Neurotoxicity

Mechanical injury is but one of a broad spectrum of conditions that present a potentially life-threatening hazard to the vulnerable neuron. A substantial number of compounds can induce neurotoxicity that is then manifested by a peripheral polyneuropathy. Neurotoxic chemicals include not only environ-

mental and occupational hazards such as heavy metals and organic solvents, but also drugs such as vincristine and cisplatin (57). The latter is effectively used in the treatment of ovarian and testicular cancer. At present, cisplatin-induced neuropathy is a major and dose-limiting side-effect. After cessation of treatment the neuropathy is only partially reversible (52).

In order to study the effects of cisplatin on the PNS, and to investigate compounds that might be of benefit in the prevention and treatment of this neuropathy, an animal model has been established (20). Rats are given cisplatin in a dose of 1 mg/kg body wt, twice a week, by intraperitoneal injection for a period of 12 weeks or more. Using this method the animals lose weight but morbidity is low. The motor (MNCV) and sensory (SNCV) nerve conduction velocities can be measured by stimulating the sciatic nerve consecutively at two points with percutaneous needle electrodes and recording the muscle action potential from the plantar muscles of the foot in an anaesthetised rat. This can be repeated a number of times, so that every animal serves as its own control. After a cumulative dose of 13 mg/kg the SNCV is significantly lower than that in control untreated rats. The SNCV further decreases in a dose-dependent manner. When the administration of cisplatin is stopped, a slow but complete recovery is seen.

This model was used to study the possible beneficial effect of nimodipine on cisplatin induced neurotoxicity. One group of rats received nimodipine orally, dispersed into food pellets in a concentration of 1000 ppm. Another group was fed with control food pellets. After a cumulative dose of 13 mg of cisplatin the SNCV of the rats given control food was 20 % lower than that of nimodipine fed rats. This difference became more pronounced with an increasing cumulative dose of cisplatin. No difference was seen between nimodipine treated rats and normal age controls. No rats died during the experiment and the weight loss was comparable in both groups.

The experiment suggests that nimodipine can prevent cisplatin induced neurotoxicity in rats. Unfortunately, the mechanism of toxicity of cisplatin, and indeed most other neurotoxins, is unknown. It has been proposed, however, that increased intracellular calcium is a common feature of several otherwise unrelated neurotoxins (35). The preliminary data on the protective effect of nimodipine in cisplatin neuropathy are consistent with this view and further studies on the effect of nimodipine in this and other toxic neuropathies are urgently needed.

Concluding Remarks

In this paper we discussed aspects of neural plasticity and the role of Ca^{2+} homeostasis in the PNS and the CNS in the context of both normal function and

a number of health-associated problems. The calcium entry-blocker nimodipine has been shown to exert beneficial effects in cases of CNS damage, aging and PNS damage. These observations, combined with the data pointing to possible anti-ischaemic effects of the drug make it a potential therapeutic agent in certain conditions. The precise mechanism of action of nimodipine is not known, but is tempting to suggest that the drug directly affects parameters involved in neurotrophic processes, perhaps influencing the function of the growth-associated protein B-50/GAP43. These neuronal responses are most evident following trauma but are also required for maintenance of normal function and become inadequate during aging. The key role of Ca^{2+} in growth and plasticity suggest that the effects of nimodipine on these processes are related to modulation of neuronal calcium channels. However, indirect effects via altered release of hormonal and growth factors (14) cannot at present be excluded. More detailed information is required to allow proper speculation on the precise mechanism of action of nimodipine. Nonetheless, the present data further support the potential significance of nimodipine in the pharmacotherapy of neural repair and age-related deficits in nervous system function.

References

(1) Akers RF, Lovinger DM, Colley PA, Linden DJ, Routtenberg A. Translocation of protein kinase C activity may mediate hippocampal long-term potentiation. Science 1986; 231: 587–9.

(2) Aloyo VJ, Zwiers H, Gispen WH. Phosphorylation of B-50 protein by calcium-activated phospholipid-dependent protein kinase and B-50 protein kinase. J Neurochem 1983; 41: 649–53.

(3) Appel H, Ojika K, Tomozawa Y, Bostwick R. Trophic factors in brain aging and disease. In: Senile Dementia of the Alzheimer Type. Traber J, Gispen WH (Eds). Berlin, Heidelberg, New York: Springer 1985, 218–31.

(4) Bär PR, Wiegant VM, Lopes da Silva FH, Gispen WH. Tetanic stimulation affects the metabolism of phosphoinositides in hippocampal slices. Brain Res 1984; 321: 381–5.

(5) Barker D, Ip MC. Sprouting and degeneration of mammalian motor axons in normal and deafferented skeletal muscle. Proc R Soc (London) Series B 1966; 163: 538–54.

(6) Beatt M, Stokes B, Bresauhan J, Somerson S. Anatomical, behavioural and physiological studies using a new computer controlled spinal injury device. Abstract 2nd Int Clare-Luxemburg Neurosci Conf 1986, p6.

(7) Benowitz LI, Routtenberg A. A membrane phosphoprotein associated with neural development, axonal regeneration, phospholipid metabolism and synaptic plasticity. Trends Neurosci 1987; 12: 527–32.

(8) Benowitz LI, Apostolides PJ, Perrone-Bizzozero N, Finkelstein SP, Zwiers H. Anatomical distribution of the growth-associated protein GAP43/B-50 in the adult rat brain. J Neurosci 1988; 8: 339–52.

(9) Betz E, Deck K, Hoffmeister F (Eds). Nimodipine: Pharmacological and Clinical Properties. Stuttgart: Schattauer 1985.

(10) Burgess SK, Sahyoun N, Blanchard SF, LeVine III H, Chang K-J, Cuatrecasas P. Phorbol ester receptors and protein kinase C in primary neuronal cultures: development and stimulation of endogenous phosphorylation. J Cell Biol 1986; 102: 312–9.

(11) Cimler BM, Giebelhaus DH, Wakim BT, Storm DR, Moon RT. Characterization of murine cDNAs encoding P-57, a neural-specific calmodulin-binding protein. J Biol Chem 1987; 262: 12158–63.

(12) Connold AL, Evers JV, Vrbova B. Effect of low Ca^{++} and protease inhibitors on synapse elimination during postnatal development in the rat soleus muscle. Dev Brain Res 1986; 28: 99–107.

(13) Coper H, Janicke B, Schulze G. Biophysiological research on adaptivity across the life-span of animals. In: Life-span Development and Behaviour. PD Baltes, Featherman DL, Lerner RM (Eds). Hillsdale: Erlbaum 1988, 207–32.

(14) Costa G, Saija A, Padovano I, Trimarchi GR, De Pasquale R, Caputi AP. The calcium antagonist nimodipine increases B-endorphin release from rat hypophysis through an action on adrenal glands. An "in vivo" and "in vitro" study. Pharmacol Res Comm 1984; 16: 959–68.

(15) De Graan PNE, Oestreicher AB, Schrama LH, Gispen WH. Phosphoprotein B-50: localization and function. Prog Brain Res 1986; 69: 37–50.

(16) De Graan PNE, Oestreicher AB, Dekker LV, van der Voorn L, Gispen WH. Determination of changes in the phosphorylation state of the neuron-specific protein kinase C substrate B-50 (GAP43) by quantative immunoprecipitation. J Neurochem 1989; 52: 17–23.

(17) Dekker A, Gispen WH, De Wied D. Axonal regeneration, growth factors and neuropeptides. Minireview: axonal regeneration, growth factors and neuropeptides. Life Sci 1987; 41: 1667–87.

(18) De Koning P, Brakkee JH, Gispen WH. Methods for producing a reproducible crush in the sciatic and tibial nerve of the rat and rapid and precise testing of return of sensory function. J Neurol Sci 1986; 74: 237–41.

(19) De Koning P, Gispen W.H. A rationale for the use of melanocortins in the treatment of

nervous tissue damage. In: Pharmacological Approaches to the Treatment of Brain and Spinal Cord Injuries. DG Stein, Babal B. (Eds). New York: Plenum 1988, 233–58.
(20) De Koning P, Neijt JP, Jennekens FGI, Gispen WH. Evaluation of cis-Diamminedichloroplatinum(II)(Cisplatin) neurotoxicity in rats. Toxicol Appl Pharmacol 1987; 89: 81–7.
(21) Gage FH, Dunnett SB, Björklund A. Spatial learning and motor deficits in aged rats. Neurobiol Aging 1984; 5: 43–8.
(22) Gelijns AC, Graaff PJ, Lopes da Silva FH, Gispen WH. Future health care applications resulting from progress in the neurosciences: the significance of neural plasticity research. Health Policy 1987; 8: 265–76.
(23) Gerritsen van der Hoop R, Van der Zee CEEM, Gispen WH. Nimodipine and neural plasticity. In: Diagnosis and Treatment of Senile Dementia. Bergener M, Reisberg B (Eds). Berlin, Heidelberg, New York: Springer 1989, 288–94.
(24) Gibson GE, Perrino P, Daniel G. Alterations of in vivo brain homeostasis with aging. J Am Assoc 1984; 14: 62.
(25) Girard PR, Mazzei GJ, Wood JG, Kuo JF. Polyclonal antibodies to phospholipid/calcium-dependent protein kinase and immunocytochemical localization of the enzyme in rat brain. Proc Natl Acad Sci USA 1985; 82: 3030–4.
(26) Gispen WH, Leunissen JLM, Oestreicher AB,Verkleij AJ, Zwiers H. Presynaptic localization of B-50 phosphoprotein: the ACTH-sensitive protein kinase substrate involved in rat brain polyphosphoinositide metabolism. Brain Res 1985; 328: 381–5.
(27) Gorgels TGMF, Oestreicher AB, De Kort EJM, Gispen WH. Immunocytochemical distribution of the protein kinase C substrate B-50 (GAP43) in developing rat pyramidal tract. Neurosci Lett 1987; 83: 59–64.
(28) Grinnel AD, Herrera AA. Specificity and plasticity of neuromuscular connections: long-term regulation of motor neuron function. Prog Neurobiol 1981; 17: 203–82.
(29) Hashimoto T, Ase K, Sawamura S, Kikkawa U, Saito N, Tanaka C, Nishizuka Y. Postnatal development of a brain-specific subspecies of protein kinase C in rat. J Neurosci 1988; 8: 1678–83.
(30) Hesselmans LFGM, Jennekens FGI, van den Oord CJM, Oestreicher AB, Veldman H, Gispen WH. A light and electron microscopical study of B-50 (GAP43) in human intramuscular nerve and neuromuscular junctions during development. J Neurol Sci 1989; 89: 301–11.
(31) Jacobson RD,Virag I, Skene JHP. A protein associated with axon growth, GAP43, is widely distributed and developmentally regulated in rat CNS. J Neurosci 1986; 6: 1843–55.
(32) Johnson MI, Meiri K, Willard M. Comparison of the growthassociated protein, GAP43, in perinatal and postnatal sympathetic neurons regenerating in culture. Ann Neurol 1986; 20: 420–1.
(33) Katchaturian Z. Towards theories of brain aging. In: Handbook of Studies in Psychiatry and Old Age. Kay DW, Burrows GD (Eds). New York: Elsevier 1984, 7–30.
(34) Kater SB, Mattson MP, Cohan C, Connor J. Calcium regulation of the neuronal growth cone. TINS 1988; 11: 315–20.
(35) Komulainen H, Bondy SC. Increased free intracellular Ca^{2+} by toxic agents: an index of potential neurotoxicity? Tips 1988; 9: 154–6.
(36) Landfield PW. Increased calcium currents in rat hippocampal neurons during aging. In: The Calcium Channel: Structure, Function and Implications. Morad M, Nayler W, Kazda S, Schramm M (Eds). Berlin, Heidelberg, New York: Springer 1988, 465–77.
(37) Landfield PW, Pitler TA. Prolonged Ca-dependent afterhyperpolerisation in hippocampal neurons in aged rats. Science 1984; 226: 1089–91.
(38) Levine J, Skene P, Willard M. GAPs and fodrin novel axonally transported proteins. Trends Neurosci 1981; 4: 273–7.
(39) Lynch G, Baudry M. The biochemistry of memory: a new and specific hypothesis. Sience 1984; 224: 1057–63.
(40) Lynch G, Larson J, Kelso S, Barrionuevo B, Schottler F. Intracellular injections of EGTA block induction of hippocampal long-term potentiation. Nature 1983; 305: 719–21.

(41) Martinez A, Vitorica J, Satrustegui J. Cytosolic free calcium levels increase with age in rat brain synaptosomes. Neurosci Lett 1988; 88: 336–42.
(42) Meiri KF, Pfenninger KH, Willard MB. Growth-associated protein, GAP43, a polypeptide that is induced when neurons extend axons, is a component of growth cones and corresponds to pp46, a major polypeptide of a subcellular fraction enriched in growth cones. Proc Natl Acad Sci USA 1986; 83: 3537–41.
(43) Meiri KF, Gordon-Weeks PR. Distribution of GAP43 within isolated neuronal growth cones. Soc Neurosci, 1987, abstract 13, 1481.
(44) Moya KL, Benowitz LI, Jhaveri S, Schneider GE. Enhanced visualization of axonally transported proteins in the immature CNS by suppression of systemic labeling. Dev Brain Res 1987; 31: 183–91.
(45) Murphy KMM, Gould RJ, Oster-Granite ML, Gearhart JD, Snyder SH. Phorbol ester receptors: autoradiographic identification in the developing rat. Science 1983; 222: 1036–8.
(46) Neve RL, Perrone-Bizzozero NI, Finkelstein SP, Zwiers H, Bird E, Kurnit DM, Benowitz LI. The neuronal growth-associated protein GAP43 (B-50, F1): neuronal specificity, developmental regulation and regional distribution of the human and rat mRNAs. Mol Brain Res 1987; 2: 177–83.
(47) Ng S-C, De la Monte SM, Conboy GL, Karns LR, Fishman MC. Cloning of human GAP43: growth association and ischemic resurgence. Neuron 1988; 1: 133–9.
(48) Nishizuka Y. Turnover of inositol phospholipids and signal transduction. Science 1984; 225: 1365–70.
(49) Nixon RA. Fodrin degradation by calcium-activated neutral proteinase (CANP) in retinal ganglion cell neurons and in optic glia: preferential localization of CANP activities in neurons. J Neurosci 1986; 6: 1264–71.
(50) Oestreicher AB, Zwiers H, Schotman P, Gispen WH. Immunohistochemical localization of a phosphoprotein (B-50) isolated from brain synaptosomal plasma membranes. Brain Res Bull 1981; 6: 145–53.
(51) Oestreicher, AB, Gispen WH. Comparison of the immunocytochemical distribution of the phosphoprotein B-50 in the cerebellum and hippocampus of immature and adult rat brain. Brain Res 1986; 375: 267–79.
(52) Roelofs RI, Hrushesky W, Rogin J, Rosenberg L. Peripheral sensory neuropathy and cisplatin chemotherapy. Neurology 1984; 34: 934–8.
(53) Schmidt-Michels M, Edwards PM, Oestreicher AB, Gispen WH. Colchicine effect on B-50 localization in rat dorsal root ganglion in culture. 1989 (submitted).
(54) Schuurman T, Klein H, Beneke M, Traber J. Nimodipine and motor deficits in the aged rat. Neurosci Res Commun 1987; 1: 9–15.
(55) Scott B, Leu J, Cinader B. Effects of aging on neuronal electrical membrane properties. Mechanisms of ageing and development 1988; 44:203–14.
(56) Scriabine A, Schuurman T, Traber J. Pharmacological basis for the use of nimodipine in central nervous system disorders. FASEB J 1989; 3: 1799–1806.
(57) Spencer PS, Schaumburg HH. An expanded classification of neurotoxic responses based on cellular targets of chemical agents. Acta Neurol Scand 1984; 70: 9–19.
(58) Skene JHP, Willard M. Changes in axonally transported proteins during axon regeneration in toad retinal ganglion cells. J Cell Biol 1981; 89: 86–95.
(59) Skene JHP, Willard M. Axonally transported proteins associated with axon growth in rabbit central and peripheral nervous system. J Cell Biol 1981; 89: 96–103.
(60) Skene JHP, Willard M. Characteristics of growth-associated polypeptides in regenerating toad retinal ganglion cell axons. J Neurosci 1981; 1: 419–26.
(61) Swanson GT, Vrbova G. Effects of low Ca^{++} and inhibition of calcium-activated neutral protease (CANP) on mature nerve terminal structure in the rat sternocostalis muscle. Dev Brain Res 1987; 33: 199–203.
(62) Tsien RW, Lipscombe D, Madison DV, Blay KR, Fox AP. Multiple types of neuronal calcium channels and their selective modulation. TINS 1988; 11: 431–7.

(63) Tuffery AR. Growth and degeneration of MEPs in normal cat hindlimb muscles. J Anat 1971; 110: 221–47.
(64) Varon S. Factors promoting the growth of the nervous system. In: Discussion in Neurosciences, FESN, Geneva, 1985; 2, no 3.
(65) Van der Zee CEEM, Schuurman T, Traber J, Gispen WH. Oral administration of nimodipine accelerates functional recovery following peripheral nerve damage in the rat. Neurosci Lett 1987; 83: 143–8.
(66) Van der Zee CEEM, Vos JP, Nielander HB, Lopes da Silva SD, Verhaagen J, Oestreicher AB, Schrama LH, Schotman P, Gispen WH. Expression of growthassociated protein B-50/GAP43 in dorsal root ganglion and sciatic nerve during regenerative sprouting. Effects of ORG2766 and conditioning lesion. 1989, (submitted).
(67) Van der Zee CEEM, Schuurman T, Gerritsen van der Hoop R, Traber J, Gispen WH. Beneficial effect of nimodipine on peripheral nerve function in aged rats. Neurobiology of Aging (in press).
(68) Verhaagen J, van Hooff COM, Edwards PM, de Graan, PNE, Oestreicher AB, Jennekens FGI, Gispen WH. The kinase C substrate protein B-50 and axonal regeneration. Brain Res Bull 1986; 17: 737–41.
(69) Verhaagen J, Oestreicher AB, Edwards PM, Veldman H, Jennekens FGI, Gispen WH. Light and electron microscopical study of phosphoprotein B-50 following denervation and reinnervation of the rat soleus muscle. J Neurosci 1988; 8: 1759–66.
(70) Wall JT. Variable organization in cortical maps of the skinas an indication of the lifelong adaptive capacities of circuits in the mammalian brain. TINS 1988; 11: 549–57.
(71) Wall JT, Kaas JH. Cortical reorganization and sensory recovery following nerve damage and regeneration. In: Synaptic Plasticity. Cotman CW (Ed). New York: Guildford Press, 1985, 231–60.
(72) Werning A, Cormody JJ, Anzil AP, Hansert E, Marciniak M, Zucker H. Persistance of nerve sprouting with features of synapse remodelling in soleus muscles of adult mice. Neurosci 1984; 11: 241–53.
(73) Werning A, Pécot-Dechavassine M, Stöver H. Sprouting and regression of the nerve at the frog nmj in normal conditions and after prolonged paralysis with curare. J Neurocytol 1980; 9: 277–303.
(74) Wood JG, Girard PR, Mazzei GJ, Kuo JF. Immunocytochemical localization of protein kinase C in identified neuronal compartments of rat brain. J Neurosci 1986; 6: 2571–7.
(75) Yoon MG, Benowitz LI, Baker FA. The optic tectum regulates the transport of specific proteins in regenerating optic fibers of goldfish. Brain Res 1986; 382: 339–51.
(76) Zwiers H, Schotman P, Gispen WH. Purification and some characteristics of an ACTH-sensitive protein kinase and its substrate protein in rat brain membranes. J Neurochem 1980; 34: 1689–99.
(77) Zwiers H, Oestreicher AB, Bisby MA, de Graan PNE, Gispen WH. Protein kinase C substrate B-50 in adult and developing rat brain is identical to axonally transported GAP43 in regenerating peripheral rat nerve. In: Axonal Transport. Smith R, Bisby M (Eds). New York: Alan R. Liss, 1987, 421–33.