D. F. Swaab, E. Fliers, M. Mirmiran, W. A. Van Gool and F. Van Haaren (Eds.)
Progress in Brain Research, Vol. 70.
© 1986 Elsevier Science Publishers B.V. (Biomedical Division)

CHAPTER 15

Neuronal cell membranes and brain aging

A. B. Oestreicher, P. N. E. De Graan and W. H. Gispen

Division of Molecular Neurobiology, Rudolf Magnus Institute for Pharmacology and Institute of Molecular Biology, University of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands

Introduction

Intercellular communication is one of the characteristic features of neuronal networks. Arrival of a nerve impulse at the presynaptic terminal induces a complex train of events which consists of calcium influx, vesicle membrane and presynaptic membrane fusion, exocytosis of the chemical transmitter, its diffusion across the synaptic cleft and postsynaptic receptor activation followed by receptor-mediated effects on ion channels in the postsynaptic membrane (Krnjevic, 1974). The physico-chemical properties of the participating membranes are of importance for the proper functioning of chemical neurotransmission. In this review we shall discuss some of the molecular events in synaptic membranes that are involved in synaptic functions and that are subject to age-related changes.

Membrane lipid fluidity

Effect of age

The plasma membrane which encloses every cell is more than a passive barrier between cell content and environment. The cell membrane maintains a concentration gradient of the various ions on either side and it serves as a highly selective barrier for chemicals that are of use for cellular metabolism or that are waste products. It is generally accepted that eukaryotic cell membranes consist of a lipid bilayer in which protein molecules float (Alberts et al., 1983). While lipids are providing the basic structure and subserving functions of the membrane, the membrane proteins, that act as the major transport molecules, catalyze membrane-associated chemical reactions and form a cell-specific recognizable outer surface. Membrane proteins are responsible for receiving and transducing chemical messengers from neighboring cells or the intercellular fluid.

The activity and functioning of these proteins in or associated with the membrane are largely determined by the fluidity of their lipid microenvironment (Shinitzky and Henkart, 1979). In general, changes in lipid fluidity affect the lateral migration and the interaction of proteins embedded in the lipid bilayer. Protein displacement across the membrane lipid bilayer may result in changes in their exposure and hence their accessibility to chemicals in and outside the cell (Shinitzky and Henkart, 1979). The fluidity of the lipid bilayer depends on its lipid composition. Notable determinants are the degree of saturation of the fatty acid side chains and the content of cholesterol. During aging, there is a marked increase in the ratio of cholesterol to phospholipids in the membranes of various tissues and, in particular, in the brain (Rouser et al., 1972).

In collaboration with Dr. Hershkowitz, we repeated some of his studies (Hershkowitz, 1983; Hershkowitz et al., 1982) using synaptic plasma membranes (SPM) obtained from brains of 24-

months-old male Wistar rats (TNO, Zeist, The Netherlands). Measurements of the mobility of the lipid probe 1,2-diphenyl-1,3,5-hexatriene (DPH) by steady-state fluorescence polarization established that SPM obtained from the brains of old rats were more micro-viscous than those of adult controls (6 months). Regional differences between cerebral cortex and hippocampus were not detected. Since the measurements were performed over a wide temperature range (4–37°C), the data indicated not only the absence of lipid phase transitions in the SPM, but also that in the SPM of the aged rat the decreased lipid fluidity was apparent over the total temperature range tested (Fig. 1; Van Dongen et al., 1983). Therefore, these data are in good agreement with the reports on the age-related increases in micro-viscosity of a crude SPM fraction obtained from mouse forebrain (Hershkowitz, 1983) and rat cerebral cortex and neostriatum (Cimino et al., 1984). The latter study established the diminished lipid fluidity both by means of fluorescence polarization and electron spin resonance.

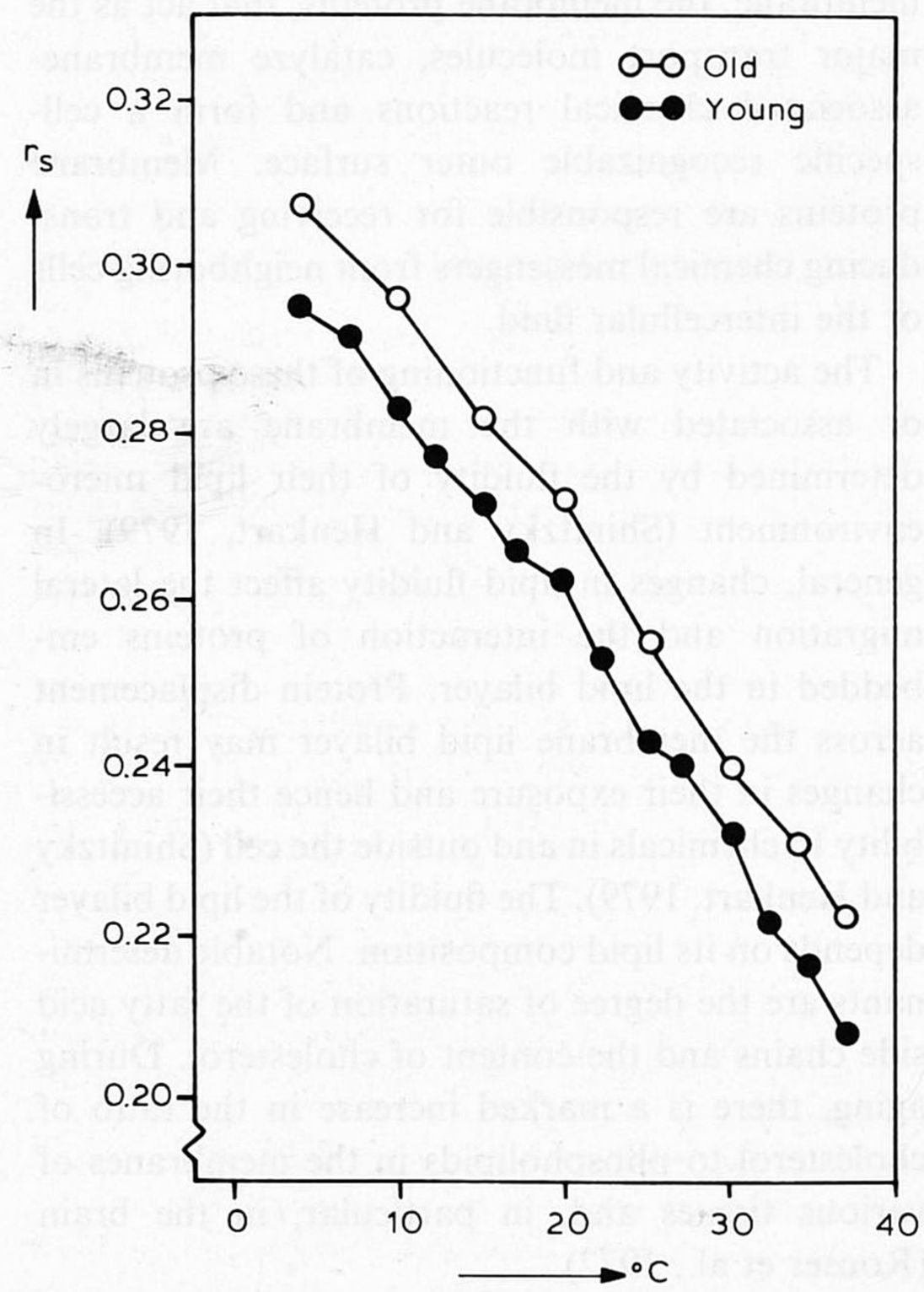

Fig. 1. Steady-state fluorescence anisotropy values of hippocampal SPM as a function of temperature. SPM was prepared from hippocampus from young and old rats. (From Van Dongen et al., 1983)

Receptors

As reports on the consequences of age-related increases in micro-viscosity of brain membranes are scarce, it is difficult to assess the significance of such membrane changes for synaptic function. Since some receptors may become more exposed with increasing micro-viscosity, while others may be less accessible, one can expect to find diverse rather than uniform effects of aging on neurotransmission, depending on the protein and the functional aspect studied. Likewise, some embedded proteins that are involved in signal transduction may collide more frequently, whereas the interaction of others may be impaired.

Based on their experience with membrane lipid fluidity and the serotonin receptor (Heron et al., 1980), Hershkowitz et al. (1982) selected this receptor for study in relation to aging of brain membranes. They reported that in the SPM obtained from the forebrains of 26-months-old mice there is a 50% reduction in basal serotonin binding, with concomitantly decreased lipid fluidity, in comparison with that observed in membranes obtained from brains of 3-months-old controls. Interestingly, scatchard analysis revealed that the loss of binding was primarily due to a loss of high-affinity binding sites, as the young mouse brain contained both high- and low-affinity sites and the old mouse brain only the low-affinity sites (Hershkowitz et al., 1982). These data are in good agreement with previous studies of these authors in which they show that *in vitro* rigidification of adult mouse brain SPM with cholesterol results in a loss of serotonin binding (Heron et al., 1980).

Cimino et al. (1984) reported that in aging rat

brain cerebral cortex, neostriatum and pineal gland, there is a marked drop in β-adrenergic binding sites, while in the striata of these animals a lower number of dopamine binding sites was observed. Such a loss of striatal dopamine receptors with age has been reported for various animal species as well as man (see Roth et al., 1986).

With respect to the serotonin receptor, it was shown that by *in vivo* treatment of old mice with a known membrane fluidizer AL721 (see below) the number of binding sites became in the order of that seen in the young control mice (Hershkowitz et al., 1982). Chronic treatment of old rats with *S*-adenosyl-L-methionine, a cofactor in phospholipid methylation — a process that increases lipid fluidity (Hirata and Axelrod, 1978) — resulted in a decreased membrane viscosity and an increased β-receptor binding. However, the reduction of putative dopamine receptor sites was not affected by the fluidizing treatment of the old rats (Cimino et al., 1984). This suggests that a decrease in receptor synthesis rather than membrane sequestration, plays a role in the diminishing dopamine receptor availability in old age (Roth et al., 1986), whereas the availability of the other receptors studied seems to be determined by the lipid fluidity of the membrane.

Membrane protein phosphorylation

Post-translational, covalent modification, e.g. phosphorylation, is a common phenomenon in the regulation of the structure and function of membrane proteins. The synaptic membrane is extremely rich in protein kinases and phosphoprotein phosphatases, substrate proteins and receptor-coupled signal transducing systems that mobilize calcium or elevate cAMP, both known modulators of protein kinases (Weller, 1979; Gispen and Routtenberg, 1982). In a series of studies, Hershkowitz et al. (1982) investigated whether membrane lipid fluidity influenced intrinsic membrane protein phosphorylation. If the lipid fluidity of crude mouse brain SPM was decreased (cholesterol) or increased (lecithin) *in vitro*, the phosphorylation of several proteins in the 40–60 kDa molecular weight range was influenced. One of these, a 47 kDa protein, is of special interest because this protein is most likely identical to protein B-50, which seems to play an important role in transmembrane signal transduction (see below). An *in vitro*-induced small decrease in SPM lipid fluidity of about 15% was accompanied by a marked increase in the endogenous phosphorylation of this protein (Hershkowitz, 1983).

When membranes were isolated from brains of old mice, the basal phosphorylation pattern differed from that of young mice only with respect to this 47 kDa/B-50 protein (Hershkowitz, 1983; Hershkowitz et al., 1982). Thus, this protein showed a higher degree of phosphorylation in the more viscous membranes of the aged mice. Why there were no concomitant changes in the phosphorylation of other proteins is unclear. It may suggest that the phosphorylation of this protein is especially sensitive to changes in membrane lipid fluidity that occur under *in vivo* conditions of aging. If, however, cAMP-sensitive phosphorylation was studied, membranes obtained from aged mouse brains responded less to the addition of cAMP in the endogenous phosphorylation assay as compared to that seen in membranes obtained from young mice (Hershkowitz, 1983). Again, the differences in protein phosphorylation could be counteracted by *in vivo* treatment of the old mice with the active lipid extract AL721. The treatment resulted in an increase of the lipid fluidity of the isolated brain membranes, a decrease in the basal phosphorylation level of the 47 kDa protein and an enhancement of the responsiveness of other proteins to cAMP (Hershkowitz, 1983).

Studies with the membrane fluidizer AL721

Based on the assumption that abnormal membrane lipid viscosity is implicated in the etiology of disorders which are relevant to aging (Rouser et al., 1972; Shinitzky, 1984), Shinitzky and coworkers developed a lipid mixture (AL721) from egg yolk that fluidizes membranes. The active ingredi-

ents are phospholipids, mainly phosphatidylcholine (Hershkowitz et al., 1982). It was found that old mice and man, both suffering from suppressed immune reactivity, could be effectively treated (per os) with AL721, substantially restoring their immuno-responsiveness (Shinitzky, 1984).

As discussed in the previous paragraph, the *in vivo* treatment of mice with AL721 was also effective in counteracting membrane changes in aging brain. Currently, this group of researchers is testing AL721 in clinical trials that aim to assess the efficacy in improving the condition of geriatric patients (Hershkowitz, 1985).

Signal transduction

Cells function in a coordinated manner by a continuous exchange of signals that are transferred through the plasma membrane from the exterior to the interior and vice versa. External information carried by neurotransmitters, hormones, growth factors and other first messengers, is translated by the constituents of the cell membrane into internal signals, the second messengers. The second messengers, cAMP, cGMP and calcium, govern intracellularly a variety of physiological responses, such as changes in metabolism, neurotransmission and growth, resulting in adaptations to alterations in external environment (Weller, 1979; Nestler and Greengard, 1983, 1984).

In the previous section, marked changes in brain cell membrane lipid fluidity observed during aging have been shown to influence plasma membrane properties. Some of these are directly involved in the homeostatic and communicative functions of the neuronal plasma membrane: i.e. in the processes of signal transduction. Here, we will summarize the features of these processes in general terms, followed by presentation of a model for one particular mechanism, the phosphoinositol-mediated signal transduction and we indicate age-related changes in participating components (Van Dongen et al., 1983).

Recent studies at the level of the plasma membrane in various cell types have resolved in part the train of events occurring in transmembrane signal transduction. Extracellular messengers bind to and activate specific outer membrane receptors. In its activated form, the receptor can interact with one of the types of guanine nucleotide regulatory proteins (N protein) and this complex may activate either a nucleotide cyclase producing cAMP or cGMP as intracellular second messenger (Nestler and Greengard, 1984), or a phosphodiesterase that specifically hydrolyzes polyphosphoinositides associated with intracellular calcium mobilization (Berridge and Irvine, 1984; Nishizuka, 1983).

We will limit our discussion to the latter signalling pathway that mobilizes intracellular calcium using inositol lipids in the transduction mechanism (Michell, 1975; Michell and Kirk, 1981; Downes and Michell, 1982).

Receptor-stimulated inositide metabolism was discovered by Hokin and Hokin (1954), who showed that the incorporation of [^{32}P]phosphate into phospholipids in pancreas was stimulated by acetylcholine. Since then the concurrence between calcium mobilization and stimulated inositide turnover has been observed in many types of cells and tissues in response to a wide range of external stimuli (Berridge and Irvine, 1984; Nishizuka, 1984a).

The current view on the signal transduction involving polyphosphoinositides (PPI) is depicted in Fig. 2. In this Fig., the presumed role of the protein B-50 is also illustrated. Agonist-receptor

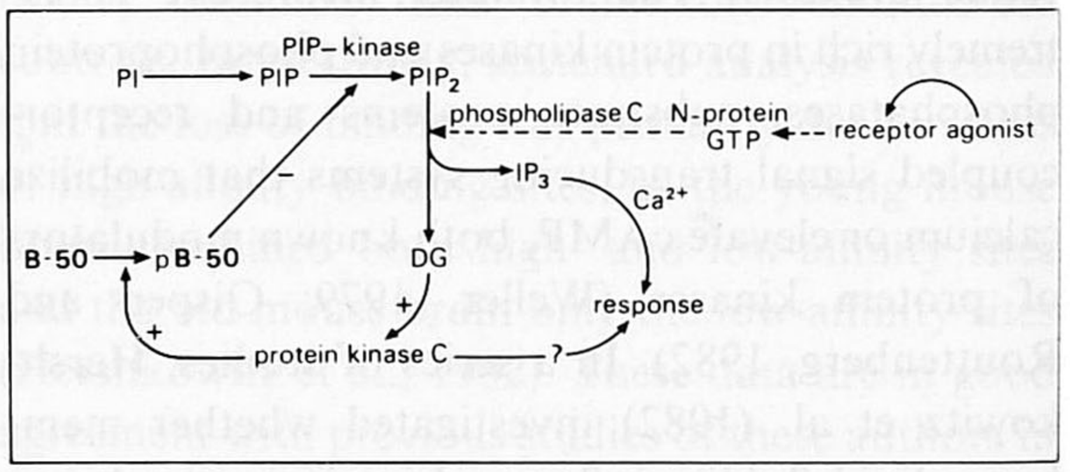

Fig. 2. Working model for the PPI-mediated signal transduction across SPM and for the role of the phosphoprotein B-50 in the feedback response of the PIP_2 turnover. See the text for explanation.

interaction induces phosphodiesteratic cleavage of phosphatidylinositol 4,5-bisphosphate (PIP_2) into inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DG). Possibly, a GTP nucleotide-binding protein conveys the information from the occupied receptor to phospholipase C (Cockcroft and Gomperts, 1985). IP_3 has recently been characterized as a second messenger (Berridge, 1983). It leaves the membrane and releases calcium from intracellular stores, most likely calcium that is sequestered in the endoplasmatic reticulum and not that present in mitochondria (Prentki et al., 1984; Berridge and Irvine, 1984). The increased intracellular level of calcium activates various enzyme systems that trigger physiological responses. Nishizuka and colleagues (Nishizuka, 1980, 1983; Takai et al., 1979a; Kishimoto et al., 1980) found that DG greatly increases the affinity of protein kinase C for calcium, thereby activating this enzyme. Therefore, Nishizuka (1984a) has suggested that during inositol lipid-dependent signal transduction, activation by enhanced calcium levels and enhanced protein phosphorylation catalyzed by protein kinase C probably act separately as well as synergistically in the control of a wide range of cellular events. Studies in our laboratory indicated that the degree of phosphorylation of the membrane protein B-50, a substrate of protein kinase C, may act as a negative feedback control in the receptor-mediated PPI response: enhancement of the phosphorylation of B-50 by protein kinase C inhibits PIP kinase. Consequently, less PIP_2 will be available for the receptor-induced breakdown of IP_3 and DG (Gispen et al., 1985a).

The role of the phosphoprotein B-50

Phosphorylation and properties

B-50 (apparent molecular weight 48 kDa, isoelectric point 4.5) is a nervous tissue-specific (Kristjansson et al., 1982), membrane-bound protein with several sites for phosphorylation (Zwiers et al., 1985). The protein was discovered in the course of studies on the neurochemical mechanism of action of behaviorally active peptides. Zwiers et al. (1976) observed, while screening membrane protein phosphorylation, that in SPM isolated from rat brain the endogenous phosphorylation of several proteins including B-50 was dose-dependently inhibited by ACTH peptides and not affected by cAMP or cGMP (Wiegant et al., 1978). It turned out that ACTH did not influence the activity of protein phosphatases and ATP-ases of SPM, but inhibited a particular membrane protein kinase. B-50 and its protein kinase were purified by extraction from rat brain membranes, DEAE-cellulose column chromatography, ammonium-sulfate precipitation (ASP) and two-dimensional electrophoresis (Zwiers et al., 1980). Aloyo et al. (1982, 1983) demonstrated that the B-50 protein kinase, being an ACTH-sensitive, nucleotide-independent protein kinase, is indistinguishable from calcium- and lipid-activated protein kinase C, isolated by Inoue et al. (1977; Nishizuka, 1984b; Kuo et al., 1980; Takai et al., 1979b). Recent studies on brain membrane protein phosphorylation in several laboratories have indicated that the B-50 protein may be identical to protein γ-5 (Gower and Rodnight, 1982), protein 47 kDa (Hershkowitz et al., 1982), protein P54p (Mahler et al., 1982) and protein F_1 (Routtenberg, 1982; Routtenberg et al., 1985).

In the nervous system there is a rapid PPI turnover, particularly in non-myelin membranes (Gonzalez-Sastre et al., 1971). Parallel to the studies on the effects of peptides on protein phosphorylation, Jolles et al. (1979) began to investigate whether interaction of ACTH with the neuronal membranes influenced the metabolism of PPI. Examining a partially purified preparation of B-50 and B-50 kinase (the ASP fraction of Zwiers et al., 1980), Jolles et al. (1980) discovered that when the lipid PIP and [γ-^{32}P]ATP were added as substrates, $ACTH_{1-24}$ in concentrations of 10^{-7} to 10^{-4}M stimulated the conversion of PIP to PIP_2, while simultaneously inhibiting the [^{32}P]-incorporation in B-50. Thus, this ASP fraction contained a protein kinase as well as a PIP kinase. The ASP fraction was checked for related enzyme

activities and was found to be devoid of phosphodiesteratic and protein phosphatase activity. Interestingly, a link between the B-50 and PIP kinase appeared to exist. When, in this ASP fraction, B-50 was prephosphorylated for increasing reaction times, and thereafter PIP was added, the amount of PIP_2 formed was inversely correlated with the extent of B-50 phosphorylation (Jolles et al., 1980). Next, Jolles et al. (1981a) demonstrated that also in rat brain membranes $ACTH_{1-24}$ affected simultaneously both protein and PPI metabolism. The structure-activity profile for the effect of the neuropeptide on B-50 phosphorylation and PIP_2/phosphatidic acid formation was similar (Jolles et al., 1981b). These and other findings led to the hypothesis that PPI metabolism in SPM is regulated by the degree of phosphorylation of B-50.

By several approaches we have tried to test the proposed feedback regulation as summarized in Fig. 2. The following evidence was recently collected:

(1) Affinity-purified anti-B-50 immunoglobulins (IgGs) or $ACTH_{1-24}$ added to SPM markedly and specifically inhibited the B-50 phosphorylation and concomitantly enhanced the [^{32}P]-labelling of PIP_2 several-fold. Control IgGs added in equal amounts were without effect (Oestreicher et al., 1983a).

(2) An inverse relationship between B-50 phosphorylation and [^{32}P]-labelling of PIP_2 was observed in a study on the effect of incubation with $5 \cdot 10^{-4}$M dopamine of rat hippocampal slices. The phosphorylation assays were carried out by 'post-hoc' phosphorylation of the SPM prepared from dopamine-treated and control slices. The effects were reversed when the antagonist haloperidol was added during the incubation of the hippocampal slices, indicating that a receptor-mediated process was involved (Jork et al., 1984).

(3) The proposed regulation of PIP kinase was tested in a system of reconstituted components. When B-50 preparations enriched in either the highly phosphorylated components or the dephosphorylated forms (Zwiers et al., 1985) were examined for their effect on the activity of the purified PIP kinase (45 kDa, isoelectric point 5.8; Van Dongen et al., 1984), it was found that using equal amounts of both B-50 preparations, only the phosphorylated forms decreased significantly the conversion of PIP to PIP_2 (Van Dongen et al., 1985).

These findings support the proposal that B-50 phosphorylation regulates the activity of PIP kinase. Since these proteins and B-50 kinase can be extracted together from the neuronal membrane, they may occur in a complex in the presynaptic plasma membrane (see below).

Currently, we are interested in the interaction of B-50 and PIP kinase in the signal transduction cycle in relation to development, maturation and aging of neural tissue. Previously, it has been demonstrated that after birth inositol lipid kinases reach an activity peak around the time of myelination (Salway et al., 1968; Eichberg and Hauser, 1969; Shaik and Palmer, 1976; Uma and Ramakrishnan, 1983).

We found that the absolute levels of phosphatidylinositol (PI), PIP and PIP_2 in SPM from forebrain of 24-months-old male rats did not differ from those of 6 months old (Van Dongen et al., 1983). However, it should be kept in mind that the total level of PPI in the membrane itself may be a rather meaningless figure. The amount associated with receptor-mediated signal transduction is of much more relevance. When the influence of age was studied on the turnover of the PPI, it was revealed that the activity of the PIP kinase was specifically decreased in membranes prepared from old rat hippocampus (Van Dongen et al., 1983). This observation and that of Hershkowitz (1983), who demonstrated that the endogenous phosphorylation of specifically the 47 kDa/B-50 protein is high in membranes from old mouse brains, again supports the notion that the B-50 phosphorylation may regulate the activity of PIP kinase (Gispen et al., 1985a).

Does the localization of B-50 in rat brain change during maturation and aging?

Earlier studies on the distribution of B-50 were carried out with adult rat nervous tissue. Kristjans-

son et al. (1982) demonstrated that B-50 is localized exclusively in rat brain particulate fractions and is not detectable by phosphorylation, two-dimensional gel electrophoresis and immunochemical analysis in non-nervous tissues. The immunohistochemical localization of B-50 was studied in paraffin-embedded sections of cerebellum and hippocampus of the adult rat (inbred Wistar strain, TNO, Zeist, The Netherlands), by means of a rabbit antiserum raised against purified B-50 using the peroxidase-antiperoxidase (PAP) method (Oestreicher et al., 1981). The findings were that B-50 immunoreactivity (BIR) appeared to be concentrated in neuropil-rich brain areas (Oestreicher et al., 1981). In contrast, the white matter and the cell bodies were virtually without immunostaining.

Sörensen et al. (1981) analyzed various subcellular fractions of adult rat brain by endogenous phosphorylation and concluded that B-50 is mainly associated with the presynaptic membrane. Recently, we used immuno electron microscopy for detection of the binding of affinity-purified anti-B-50 IgGs by means of protein-A-coated gold particles, and demonstrated that in the adult hippocampus *in situ*, B-50 is predominantly localized at presynaptic sites of nerve terminals and associated with the inner face of the plasma membrane (Oestreicher et al., 1983b; Gispen et al., 1985b). In order to support this ultrastructural localization, we examined the subcellular sites of B-50 in isolated intact and post-sectioned synaptosomes. When ultra-thin cryosections were first cut from aldehyde-fixed synaptosomes so that the interior of the synaptosomes was exposed to the immuno reagents and then processed for immuno electron microscopy, BIR was detected close to the SPM, at the cytoplasmic face. In contrast, pre-embedding immunostaining of the intact synaptosomes followed by fixation and processing for electron microscopic analysis resulted in micrographs devoid of any immunolabelling (Gispen et al., 1985b). This led us to conclude that B-50 is located at the inner face of the cell membrane, being not accessible to the antibodies when the synaptosomal membrane is not ruptured. In the experiments on the *in situ* labelling of the hippocampus some of the nerve terminals were found to be devoid of BIR. The observation of the absence of immunostaining from some synapses raises many questions, for example whether B-50 is associated selectively with specific populations of nerve terminals involved in certain transmitter and signalling systems.

In view of the restricted localization in hippocampal neurons of adult rat, the question arose whether the localization of B-50 differed as a function of age. Previously, we had demonstrated that in 8-days-old rats, B-50 was one of the prominent proteins in neural membranes that had become labelled after intracranial injection of radiolabelled orthophosphate (Oestreicher et al., 1982). Employing affinity-purified anti-B-50 antibodies (Oestreicher et al., 1983a), we showed in a comparative immunocytochemical study (Oestreicher et al., 1983b) of brain regions in the 8-days-old and adult rat that BIR was distributed throughout the rat brain in regions rich in synaptic contacts. In addition, in the developing brain BIR was also observed in outgrowing axons, probably present in their growth cones (De Graan et al., 1985a).

After finding that the site of localization of B-50 is dependent on the stage of differentiation and growth of the neurons in various brain regions (Oestreicher and Gispen, 1986), we started to explore immunocytochemically changes in the location of B-50 in the hippocampus of young adult and senescent rats (BN strain, obtained from REP Institutes, GO-TNO, Rijswijk, The Netherlands) in more detail. The distribution of BIR in the neuropil areas of the stratum oriens and the stratum moleculare lacunosum of CA1 was rather similar (Fig. 3). However, a conspicuous difference was revealed in the hippocampus of the 27-months-old rat, in which BIR appeared to be present in 'aggregates' at the periphery of the cell body or as 'particulate' material unevenly distributed in the cytosol of the pyramidal neurons of CA3. The same BIR deposits were seen to a

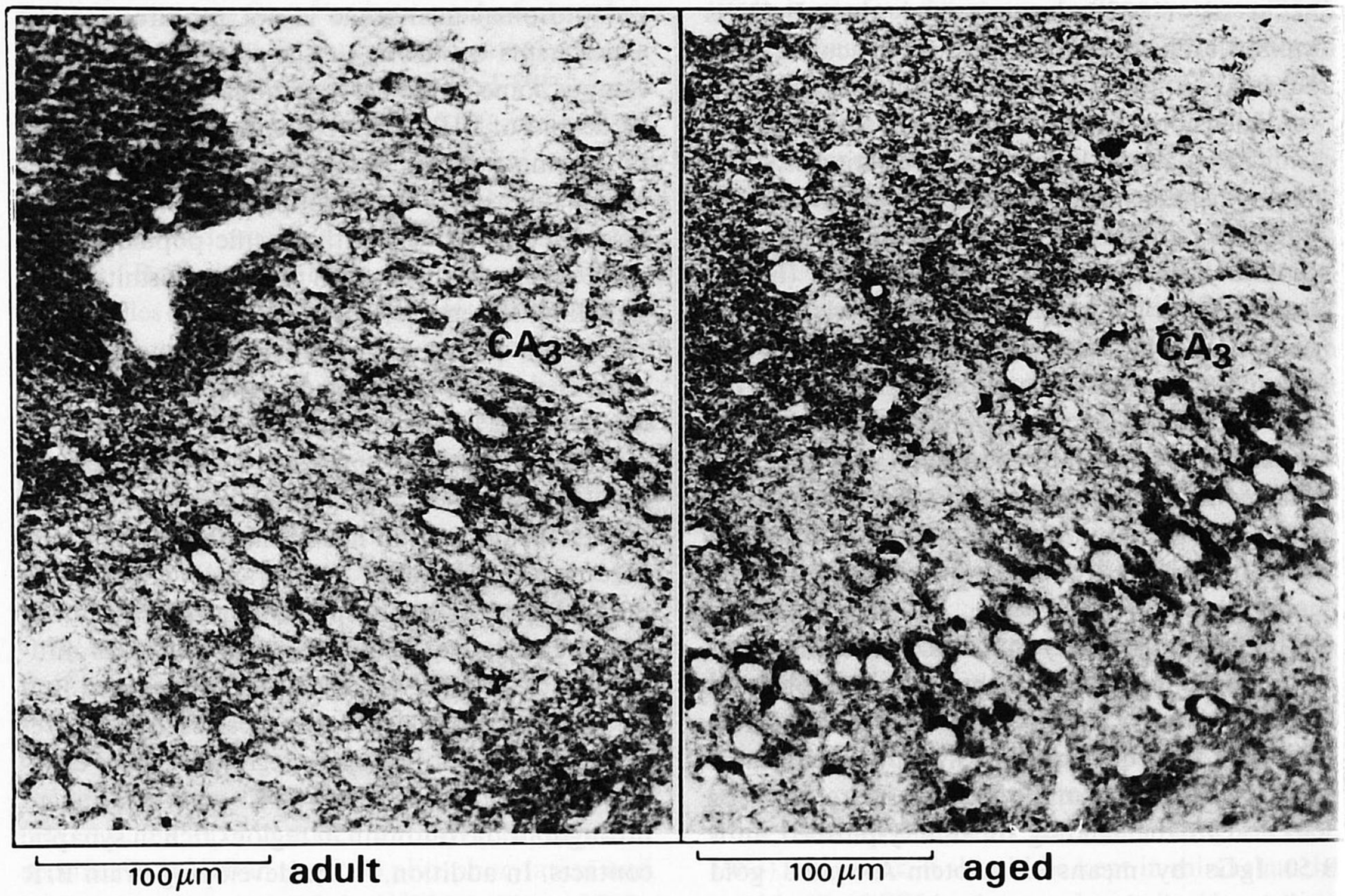

Fig. 3. Distribution of B-50 immunoreactivity (BIR) in the CA3 field of the hippocampus of young adult (left) and senescent (right) rats. The procedures used to perfuse and fix the brains, to obtain the tissue sections and to perform the immunocytochemistry are described in detail in a recent study by Oestreicher and Gispen (1986). Sections were cut from the rat brains perfused with fixative, containing 2% paraformaldehyde, 0.1% dimethylsulfoxide, 0.5% glutaraldehyde in phosphate-buffered saline at pH 7.4, post-fixed and impregnated with paraffin. 5-μm thick parasagittal sections were incubated with anti-B-50 IgGs and the immunoreaction was detected by the PAP method. The micrographs show that small dot-like deposits of BIR are present in the stratum radiatum of the young and senescent hippocampus. The density of the BIR deposits diminishes at the proximal side of the innervation of the apical dendrites of the pyramidal neurones (left and right). Mainly, in the CA3 of the young (left), but also in the old (right) rats, unstained thin dendrites and unstained pyramidal neurons appear to be outlined by small punctuate BIR, probably present in presynaptic terminals of afferents terminating on their membrane (Gispen et al., 1985b). In addition, in the senescent rat (right), there are conspicuous, relatively large deposits of BIR close to the neuronal membrane of the pyramidal cells. This BIR appears to be either in the cytosol of the pyramidal neurons or outside adjoining the perikaryal membrane. Similar sized deposits of BIR are hardly seen in the stratum pyramidale of the young adult rat (left). In both, blood capillaries are unstained.

lesser extent in the pyramidal neurons of CA1, CA2 and CA4. Such a distribution of BIR was much less prominent in the sections of the hippocampus of 4-weeks-old rats. As immunocytochemical control we examined sections of the young adult and aged rat hippocampus with two other antisera to immunostain the structural markers myelin and astrocytes. The results did not display marked qualitative differences, in contrast to those of the immunostaining pattern of B-50 (Fig. 3).

At present, the significance of this age-related change in cellular B-50 localization is unclear. It is tempting to conclude that it relates to the diminution of synaptic plasticity as seen in the hippocampus of aged rats (see below). Further work is in

progress to gain insight into the nature and precise cellular localization of these BIR deposits around pyramidal cells in the CA3 layer of the hippocampus of old rats.

Distribution of B-50 determined by radioimmunoassay

In order to study the effect of age on B-50 in a more quantitative manner, we developed a sensitive radioimmuno assay (RIA) using anti-B-50 IgGs to measure B-50 (0.1–10 ng) in small amounts of rat brain tissue (5–400 μg wet weight). The tracer was prepared by phosphorylating purified B-50 with carrier-free [γ-^{32}P]ATP, catalyzed by purified protein kinase C. With this RIA we have determined the B-50 content of various brain regions of the adult Wistar rat. We found the following distribution pattern: septum > periaqueductal gray > cerebral cortex > hippocampus > cerebellum > medulla spinalis. The septum contained 80 μg B-50/g wet weight tissue (Oestreicher et al., 1985). This regional distribution pattern shows a fair agreement with the previously reported pattern of distribution of the endogenous B-50 phosphorylation in SPM isolated from the same brain areas (Kristjansson et al., 1982). Furthermore, these data confirm our earlier suggestion that regional differences in the endogenous B-50 phosphorylation reflect differences in the presence of B-50 rather than that of protein kinase C (Kristjansson et al., 1982). Holmes and Rodnight (1981) reported on the ontogeny of substrate proteins for protein kinases in rat brain membrane fractions. They found that the maximum phosphorylation of the 48-kDa protein γ-5 was reached 15 days after birth and from then declined to the adult level. This decline may arise from several age-related changes. We found that the B-50 content in total brain, measured by RIA during postnatal development of the rat, was high after birth and decreased to a lower adult level. That B-50 levels in rat change with age is shown for specific brain regions in Fig. 4. The B-50 content of the septum, cortex cerebri and hippocampus diminishes by 40–50% in 28-months-old rats, in comparison to

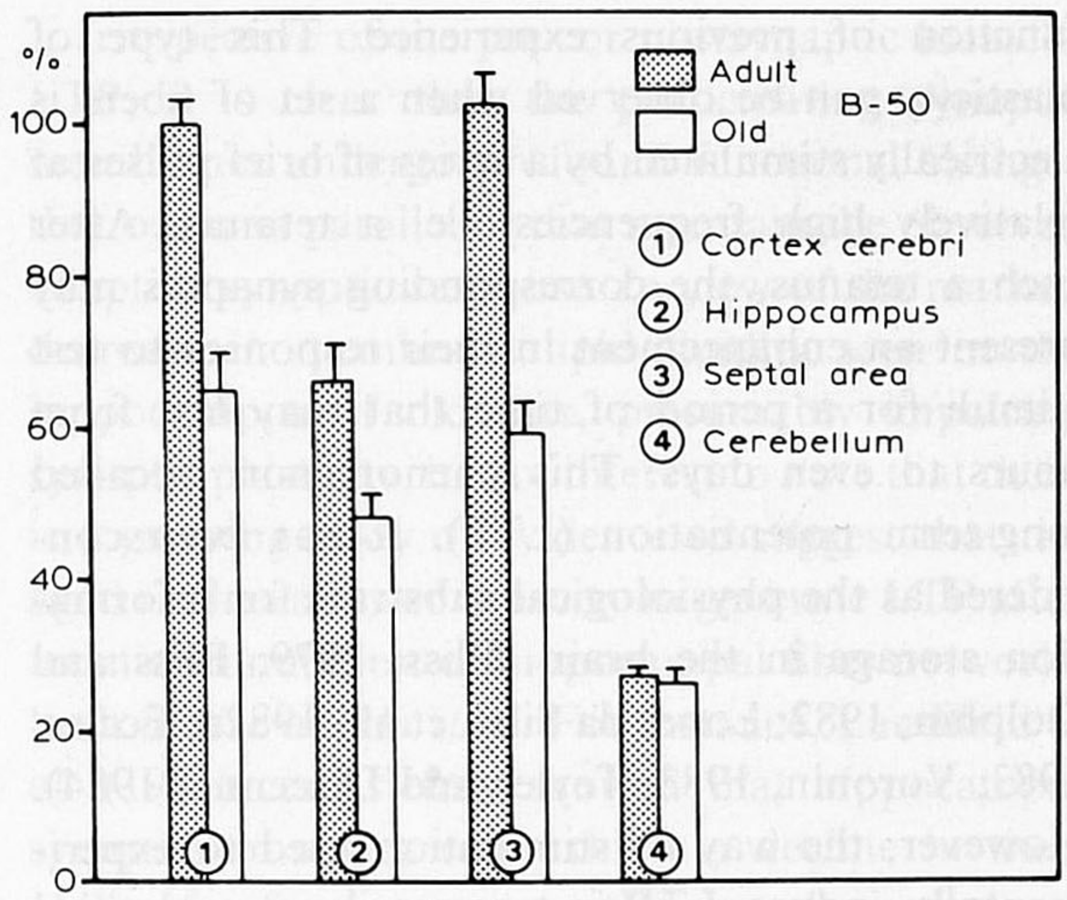

Fig. 4. RIA of B-50 levels in homogenates of various brain regions of young and old rats. The brain regions were dissected from groups of three animals. The B-50 content (84 μg/mg wet weight tissue) of the septum of the young rat has been taken as the reference level (100%). The RIA was carried out as described by Oestreicher et al. (1985). Data represent the mean of triplicate assays, SEM < 5%. Student's t test was applied for the comparison of young versus old rats. The differences found in brain regions: 1, cortex cerebri; 2, hippocampus and 3, septal area, were significant to $p < 0.05$.

that of 2-months-old, young adult rats. Surprisingly, the content of the cerebellum remained unaltered. The causes and consequences of the changes of the levels of B-50 in various brain regions with the age of the rat are unknown. From another field of study concerned with outgrowth and sprouting of axons (Pfenninger et al., 1983; Katz et al., 1985) the suggestion came that protein F_1 may be a growth-associated protein, since its synthesis is stimulated by growth and regeneration. A decrease of B-50, probably identical to F_1, during maturation and aging might reflect a diminished synthesis of some membrane proteins in response to altered requirements for growth associated with synaptic plasticity.

Synaptic plasticity and membrane fluidity

A synaptic system shows plasticity when its response to a certain electrical stimulus is a

function of previous experience. This type of plasticity can be observed when a set of fibers is electrically stimulated by a series of brief pulses at relatively high frequencies, i.e. a tetanus. After such a tetanus, the corresponding synapses may present an enhancement in their responses to test stimuli for a period of time that may last from hours to even days. This phenomenon is called long-term potentiation (LTP). It has been considered as the physiological substrate for information storage in the brain (Bliss, 1979; Bliss and Dolphin, 1982; Lopes da Silva et al., 1982a; Eccles, 1983; Voronin, 1983; Teyler and Discenna, 1984). However, the way of stimulation used to experimentally induce LTP is necessarily a prolonged synchronous volley of impulses applied to a relatively large number of fibers. Yet, it may be assumed that in the normal functioning brain similar types of processes, although in a much less widespread way, take place: the scale at which this would occur, however, is so minute that such processes cannot be put in evidence by using the methods available. It may be pointed out that the consolidation of cognitive memory involves repeated recall; in a similar way one may assume that enduring LTPs may be established as a consequence of repeated reinforcements of the type of synaptic processes described here (Eccles, 1983).

LTP is essentially a synaptic phenomenon since it has been clearly demonstrated that it does not correspond to a generalized increase in the excitability of the neurons involved. Indeed, LTP stays restricted to the synapses being stimulated and is not transferred heterosynaptically (Andersen et al., 1977). However, some studies report that under certain conditions generalized changes in the excitability of the post-synaptic membrane may occur (Lynch et al., 1977).

There is still controversy on whether LTP consists of a pre- or a post-synaptic phenomenon, or a combination of both. Evidence in favor of a presynaptic locus is mainly based on experimental findings of transmitter release during LTP and on changes in the phosphorylation and activity of pyruvate dehydrogenase (PDH; Browning et al., 1982) and in the phosphorylation of B-50/F_1 and the 52-kDa protein (De Graan et al., 1985b; Routtenberg et al., 1985). Skrede and Malthe-Sörensen (1981) found that LTP in the CA1 field of the hippocampal slices is accompanied by a long-lasting increase in the resting release of previously accumulated D-[^{3}H]aspartate. *In vivo*, using the push-pull cannula technique, Dolphin et al. (1982) and Lynch et al. (1984) have shown that LTP in the fascia dentata of the rat hippocampus is accompanied by a prolonged release of glutamate. Voronin (1983) reported that LTP studied in hippocampal slices is related to an increase in the mean number of transmitter quanta released per presynaptic action potential. More experimental evidence for a presynaptic locus is provided by the recent investigation of Dolphin (1983), who showed that a tetanus to the perforant path *in vivo*, during a period where the glutamate antagonist γ-D-glutamylglycine was applied, was able to induce LTP, whose expression was masked until the antagonist was removed.

Against a presynaptic locus of LTP appear to be the experiments of Dunwiddie et al. (1978), who used the glutamate antagonist D,L-2-amino-4-phosphonobutyric acid (APB) to block synaptic transmission in the CA1 field in hippocampal slices, and showed that a tetanus applied under these conditions did not cause LTP even after the antagonist has been washed out.

Regarding the postsynaptic hypothesis of LTP, the main argument emerges from the work of Baudry and Lynch (1979; Lynch and Baudry, 1984), who reported that the number of glutamate receptor binding sites increased during LTP and that this increase was dependent on calcium. They interpret these findings as indicating that a membrane-bound enzyme, calpain, is activated by the influx of calcium provoked by the tetanization, and acts on the cytoskeletal protein fodrin which lines the inner surface of the neuronal membrane. In this way a fodrin lattice would be disrupted which would uncover glutamate receptors on the post-synaptic membrane. Such a mechanism could explain the morphological changes in the shape of

dendritic spines as reported in the CA1 field, on *in vitro* hippocampal slices (Lee et al., 1981) and on *in vivo* hippocampus (Lee et al., 1980), and by Chang and Greenough (1984). Fifková and Van Harreveld (1977) found an increased spine surface area after potentiation in fascia dentata, but could not determine a change in shape. Desmond and Levy (1983) reported an increase in the number of large spine profiles in the region of high synaptic activation in the fascia dentata. There remain discrepancies between the morphological data related to LTP obtained from fascia dentata and CA1.

The possibility of both pre- and postsynaptic events being responsible for LTP has been put forward (cf. Lopes da Silva et al., 1982a). Eccles (1983) proposed a unifying hypothesis which assumes that the primary event is an increased sensitivity of the postsynaptic membrane and that, secondary this would induce an increased output of transmitter from the presynaptic terminals by means of a trophic action across the synaptic cleft. A further clarification of pre- and postsynaptic events in LTP may be expected by combining physiological, biochemical and electron microscopical techniques. It may be advanced that LTP is most likely a phenomenon of the whole synaptic system and that it corresponds to changes at both sides of the synapse (De Graan et al., 1985b).

Irrespective of the pre- or postsynaptic locus of LTP, it is clear that the participating synaptic membranes undergo profound functional changes that occur in parallel or that maintain the observed adaptive synaptic response. In view of the marked decrease in membrane lipid fluidity seen in the hippocampus of old rats, presumably impairing synaptic plasticity, it is of interest to note that there is a growing body of evidence to suggest that the aged rat has a lower capacity to generate LTP after tetanic stimulation of hippocampal fiber networks both *in vivo* and *in vitro*. For instance, Landfield et al. (1981) consistently showed that hippocampal synapses in aging rats exhibit a decline in their ability for frequency potentiation, whereas many, but not all, other neurophysiological properties appear normal. It now seems clear that this potentiation deficit is due to specific impairment of synaptic function (Landfield, 1983). Similar age-related impairments after repetitive stimulation were reported by Tielen et al. (1983). They reported that in slices obtained from the hippocampus of senescent rats tetanic stimulation did not result in the generation of LTP as normally seen in slices from hippocampus of young rats. As shown in Table I, there is no change in the amplitude of the population spike characteristics for LTP in senescent rats, during the time after the tetanus that the LTP develops in adult rat

TABLE I

LTP of the population spike of CA1 in young and senescent rats. Population spikes are expressed as percentages (mean ± SEM) of the amplitude of pre-tetanus period (−1′–0′). The two-tailed significance levels of the percentages with respect to the general pre-tetanus mean are given in column *p* (Student's *t* test)

Pre-tetanus ($n = 33$, periods: 10′–9′, 5′–4′, 1′–0′) 101 ± 2

Post-tetanus	young ($N = 17$)	*p*	senescent ($N = 16$)	*p*
1′–2′	121 ± 11	ns	99 ± 3	ns
3′–4′	137 ± 16	0.05	100 ± 5	ns
5′–6′	133 ± 13	0.05	98 ± 5	ns
7′–8′	131 ± 15	ns	99 ± 6	ns
9′–10′	139 ± 17	0.05	100 ± 6	ns

From Tielen et al., 1983.

hippocampal slices (Lopes da Silva et al., 1982b). It is tempting to assume that impaired functioning of the membranes of those synapses that are affected by repetitive stimulation, is causing this impaired synaptic plasticity.

Summary and conclusions

In the present paper some aspects of neuronal membrane function that are affected by aging have been reviewed. There is increasing evidence to suggest that the age-related increase in the microviscosity of SPM may cause diminution of transmembrane signal transduction and synaptic plasticity. Especially the protein kinase C substrate protein B-50 seems of interest as its degree of phosphorylation exerts a feedback control in receptor-mediated membrane events. Its degree of phosphorylation, its brain localization and content seem to undergo specific alterations during the lifespan of the rat. As an example of impaired synaptic plasticity, the reduced responsiveness of hippocampal networks of aged rats to tetanic stimulation was discussed. Although the precise molecular mechanism of action of LTP is unknown, the impaired lipid fluidity as found in these membranes most likely negatively influences the capacity of these membranes to generate the post-tetanic response.

As shown previously, *in vivo* and *in vitro* induced decreases in membrane lipid fluidity can be restored by *in vivo* and *in vitro* treatment with membrane fluidizers concomitantly normalizing some of the impaired membrane functions originating from the decrease in membrane lipid fluidity. These observations suggest that certain diseases that are parallelled by increases in membrane microviscosity may be therapeutically approached at the level of the membrane lipid fluidity of the cells that are involved in the disease process. Likewise, it may be that part of the loss of plasticity of brain tissue in old age may effectively be counteracted by treatment with membrane fluidizers. Further research should address the efficacy of such treatment in improving brain function in the elderly.

References

Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K. and Watson, J. D. (1983) *Molecular Biology of the Cell*, Garland Publ. Inc., New York, London, pp. 256–314.

Aloyo, V. J., Zwiers, H. and Gispen, W. H. (1982) B-50 protein kinase and kinase C in rat brain. In W. H. Gispen and A. Routtenberg (Eds.), *Brain Phosphoproteins: Characterization and Function*, *Progress in Brain Research*, *Vol. 56*, Elsevier Biomedical Press, Amsterdam, pp. 303–315.

Aloyo, V. J., Zwiers, H. and Gispen, W. H. (1983) Phosphorylation of B-50 protein by calcium-activated, phospholipid-dependent protein kinase and B-50 protein kinase. *J. Neurochem.*, 41: 649–653.

Andersen, P., Sunderberg, S. H., Sveen, O. and Wigstrøm, H. (1977) Specific-long-lasting potentiation of synaptic transmission in hippocampal slices. *Nature (Lond.)*, 266: 736–737.

Baudry, M. and Lynch, G. (1979) Regulation of glutamate receptors by cations. *Nature (Lond.)*, 282: 748–750.

Berridge, M. J. (1983) Rapid accumulation of inositol trisphosphate reveals that agonists hydrolyse polyphosphoinositides instead of phosphatidylinositol. *J. Biochem.*, 212: 849–858.

Berridge, M. J. and Irvine, R. F. (1984) Inositol trisphosphate, a novel second messenger in cellular signal transduction. *Nature (Lond.)*, 312: 315–321.

Bliss, T. V. P. (1979) Synaptic plasticity in the hippocampus. *Trends Neurosci.*, 2: 42–45.

Bliss, T. V. P. and Dolphin, A. C. (1982) What is the mechanism of long-term potentiation in the hippocampus? *Trends Neurosci.*, 5: 289– 290.

Browning, M., Baudry, M. and Lynch, G. (1982) Evidence that high frequency stimulation influences the phosphorylation of pyruvate dehydrogenase and that the activity of this enzyme is linked to mitochondrial calcium sequestration. In W. H. Gispen and A. Routtenberg (Eds.), *Brain Phosphoproteins: Characterization and Function*, *Progress in Brain Research*, *Vol. 56*, Elsevier Biomedical Press, Amsterdam, pp. 317–338.

Chang, F. L. F. and Greenough, W. T. (1984) Transient and enduring morphological correlates of synaptic and efficacy change in the rat hippocampal slices. *Brain Res.*, 309: 35–46.

Cimino, M., Vantini, G., Algeri, S., Curatla, G., Pezzoli, C. and Stramentinoli, G. (1984) Age-related modification of dopaminergic and β-adrenergic receptor system: restoration to normal activity by modifying membrane fluidity with *S*-adenosylmethionine. *Life Sci.*, 34: 2029–2039.

Cockcroft, S., Gomperts, B. D. (1985) Role of the guanine nucleotide binding protein in the activation of polyphosphoinositide phosphodiesterase. *Nature*, 314: 536–543.

De Graan, P. N. E., Van Hooff, C. O. M., Tilly, B. C., Oestreicher, A. B., Schotman, P. and Gispen, W. H. (1985a)

Phosphoprotein B-50 in nerve growth cones from fetal rat brain. *Neurosci. Lett.*, 61: 235–241.

De Graan, P. N. E., Lopes da Silva, F. H. and Gispen, W. H. (1985b) The role of hippocampal phosphoproteins in long-term potentiation. In R. L. Isaacson and K. H. Pribram (Eds.), *The Hippocampus, Vol. 3*, Plenum Press, New York, in press.

Desmond, N. L. and Levy, W. B. (1983) Synaptic correlates of associative potentiation/depression: an ultrastructural study in the hippocampus. *Brain Res.*, 265: 21–30.

Dolphin, A. C. (1983) The excitatory amino acid antagonist γ-D-glutamylglycine masks rather than prevents long-term potentiation of the perforant path. *Neuroscience*, 10: 377–383.

Dolphin, A. C., Errington, M. L. and Bliss, T. V. P. (1982) Long-term potentiation of the perforant path in vivo is associated with increased glutamate release. *Nature (Lond.)*, 297: 496–498.

Downes, C. P. and Michell, R. H. (1982) Phosphatidylinositol 4-phosphate and phosphatidylinositol-4,5-biphosphate: lipids in search of a function. *Cell Calcium*, 3: 467–502.

Dunwiddie, T., Madison, D. and Lynch, G. (1978) Synaptic transmission is required for initiation of long-term potentiation. *Brain Res.*, 150: 413–417.

Eccles, J. C. (1983) Calcium in long-term potentiation as a model for memory. *Neuroscience*, 10: 1071–1081.

Eichberg, J. and Hauser, G. (1969) Polyphosphoinositide biosynthesis in developing rat brain homogenates. *Ann. NY Acad. Sci.*, 165: 784–789.

Fifková, E. and Van Harreveld, A. (1977) Long-lasting morphological changes in dendritic spines of dentate granular cells following stimulation of the entorhinal area. *J. Neurocytol.*, 6: 211–230.

Gispen, W. H. and Routtenberg, A. (Eds.) (1982) *Brain Phosphoproteins: Characterization and Function, Progress in Brain Research, Vol. 56*, Elsevier Biomedical Press, Amsterdam.

Gispen, W. H., Van Dongen, C. J., De Graan, P. N. E., Oestreicher, A. B. and Zwiers, H. (1985a) The role of phosphoprotein B-50 in phosphoinositide metabolism in brain synaptic plasma membranes. In J. E. Bleasdale, G. Hauser and J. Eichberg (Eds.), *Inositol and Phosphoinositides*, Humana Press, Dallas, pp. 399–413.

Gispen, W. H., Leunissen, J. L. M., Oestreicher, A. B., Verkleij, A. J. and Zwiers, H. (1985b) Presynaptic localization of B-50 phosphoprotein: the ACTH sensitive protein kinase substrate involved in rat brain polyphosphoinositide metabolism. *Brain Res.*, 328: 381–385.

Gonzalez-Sastre, F., Eichberg, J. and Hauser, G. (1971) Metabolic pools of polyphosphoinositides in rat brain. *Biochim. Biophys. Acta*, 248: 96–104.

Gower, H. and Rodnight, R. (1982) Intrinsic protein phosphorylation in synaptic plasma membrane fragments from the rat. *Biochim. Biophys. Acta*, 716: 45–52.

Heron, D. S., Shinitzky, M., Hershkowitz, M. and Samuel, D. (1980) Lipid fluidity markedly modulates the binding of serotonin to mouse brain membranes. *Proc. Natl. Acad. Sci. USA*, 77: 7463–7467.

Heron, D. S., Shinitzky, M. and Samuel, D. (1982) Alleviation of drug withdrawal symptoms by treatment with a potent mixture of natural lipids. *Eur. J. Pharmacol.*, 83: 253–263.

Hershkowitz, M. (1983) Mechanisms of brain aging — the role of membrane fluidity. *Dev. Neurol.*, 7: 85–99.

Hershkowitz, M. (1985) Press Release, The Weizmann Institute of Science, Rehovot, Israel, June 9, 1985.

Hershkowitz, M., Heron, D., Samuel, D. and Shinitzky, M. (1982) The modulation in synaptic protein phosphorylation and receptor binding in synaptic membranes by changes in lipid fluidity: implications for ageing. In W. H. Gispen and A. Routtenberg (Eds.), *Brain Phosphoproteins: Characterization and Function, Progress in Brain Research, Vol. 56*, Elsevier Biomedical Press, Amsterdam, pp. 419–434.

Hirata, F. and Axelrod, G. (1978) Enzymatic methylation of phosphatidylethanolamine increases erythrocyte membrane fluidity. *Nature (Lond.)*, 275: 219–220.

Hokin, M. R. and Hokin, L. E. (1954) Effects of acetylcholine on phospholipids in the pancreas. *J. Biol. Chem.*, 209: 549–558.

Holmes, H. and Rodnight, R. (1981) Ontogeny of membrane-bound protein phosphorylating systems in the rat. *Dev. Neurosci.*, 4: 79–88.

Inoue, M., Kishimoto, A., Takai, Y. and Nishizuka, Y. (1977) Studies on a cyclic nucleotide-independent protein kinase and its proenzyme in mammalian tissues. *J. Biol. Chem.*, 252: 7610–7616.

Jolles, J., Wirtz, K. W. A., Schotman, P. and Gispen, W. H. (1979) Pituitary hormones influence polyphosphoinositide metabolism in rat brain. *FEBS Lett.*, 105: 110–114.

Jolles, J., Zwiers, H., Van Dongen, C. J., Schotman, P., Wirtz, K. W. A. and Gispen, W. H. (1980) Modulation of brain polyphosphoinositide metabolism by ACTH-sensitive protein phosphorylation. *Nature (Lond.)*, 286: 623–625.

Jolles, J., Zwiers, H., Dekker, A., Wirtz, K. W. A. and Gispen, W. H. (1981a) Corticotropin-(1-24)-tetracosapeptide affects protein phosphorylation and polyphosphoinositide metabolism in rat brain. *Biochem. J.*, 194: 283–291.

Jolles, J., Bär, P. R. and Gispen, W. H. (1981b) Modulation of brain polyphosphoinositide metabolism by ACTH and beta-endorphin: structure-activity studies. *Brain Res.*, 224: 315–326.

Jork, R., De Graan, P. N. E., Van Dongen, C. J., Zwiers, H., Matthies, H. and Gispen, W. H. (1984) Dopamine-induced changes in protein phosphorylation and polyphosphoinositide metabolism in rat hippocampus. *Brain Res.*, 291: 73–81.

Katz, F., Ellis, L. and Pfenninger, K. H. (1985) Nerve growth cones isolated from fetal rat brain. III. Calcium-dependent protein phosphorylation. *J. Neurosci.*, 5: 1402–1411.

Kishimoto, A., Takai, Y., Mori, T., Kikkawa, U. and

Nishizuka, V. (1980) Activation of calcium and phospholipid-dependent protein kinase by diacylglycerol, its possible relation to phosphatidyl-inositol turnover. *J. Biol. Chem.*, 255: 2273–2276.

Kristjansson, G. I., Zwiers, H., Oestreicher, A. B. and Gispen, W. H. (1982) Evidence that the synaptic phosphoprotein B-50 is localized exclusively in nerve tissue. *J. Neurochem.*, 39: 371–378.

Krnjevic, K. (1974) Chemical nature of synaptic transmission in vertebrate. *Physiol. Res.*, 54: 418–540.

Kuo, J. F., Andersson, R. G. G., Wise, B. C., Mackerlova, L., Salomonsson, I., Brackett, M. L., Katoh, N., Shoji, M. and Wrenn, R. W. (1980) Calcium-dependent protein kinase: widespread occurrence in various tissues and phyla of the animal kingdom and comparison of effects of phospholipid, calmodulin and trifluoperazine. *Proc. Natl. Acad. Sci. USA*, 77: 7039–7043.

Landfield, P. W. (1983) Mechanisms of altered normal function during aging. *Dev. Neurol.*, 7: 51–71.

Landfield, P. W., Braun, L. D., Pitler, T. A., Lindsey, J. D. and Lynch, G. (1981) *Neurobiol. Aging*, 2: 265.

Lee, K. S., Schottler, F., Oliver, M. and Lynch, G. (1980) Brief bursts of high-frequency stimulation produce two types of structural change in rat hippocampus. *J. Neurophysiol.*, 44: 247–258.

Lee, K. S., Oliver, M., Schottler, F. and Lynch, G. (1981) Electron microscopic studies of brain slices: the effect of high-frequency stimulation on dendritic ultrastructure. In G. Kerkut and H. V. Wheal (Eds.), *Electrical Activity in Isolated Mammalian C.N.S. Preparations*, Academic Press, New York, pp. 189–212.

Lopes da Silva, F. H., Bär, P. R., Tielen, A. M. and Gispen, W. H. (1982a) Plasticity in synaptic transmission and changes of membrane-bound protein phosphorylation. In R. M. Buys, P. Pévet and D. F. Swaab (Eds.), *Chemical Transmission in the Brain, Progress in Brain Research, Vol. 55*, Elsevier Biomedical Press, Amsterdam, pp. 369–377.

Lopes da Silva, F. H., Bär, P. R., Tielen, A. M. and Gispen, W.H. (1982b) Changes in membrane phosphorylation correlates with long-lasting potentiation in rat hippocampal slices. In W. H. Gispen and A. Routtenberg (Eds.), *Brain Phosphoproteins: Characterization and Function, Progress in Brain Research, Vol. 56*, Elsevier Biomedical Press, Amsterdam, pp. 339–345.

Lynch, G. S. and Baudry, M. (1984) The biochemistry of memory: a new and specific hypothesis. *Science*, 224: 1057–1063.

Lynch, G. S., Dunwiddie, T. and Gribkoff, V. (1977) Heterosynaptic depression: a post-synaptic correlate to long-term potentiation. *Nature (Lond.)*, 266: 737–739.

Lynch, M., Errington, M. L. and Bliss, T. V. P. (1984) Sustained increase in release of endogenous glutamate associated with long-term potentiation of the perforant path in the anaesthetized rat. *Neurosci. Lett.*, Suppl. 18, S194.

Mahler, H. R., Kleine, L. P., Ratner, N. and Sörensen, R. G. (1982) Identification and topography of synaptic phosphoproteins. In W. H. Gispen and A. Routtenberg (Eds.), *Brain Phosphoproteins: Characterization and Function, Progress in Brain Research, Vol. 56*, Elsevier Biomedical Press, Amsterdam, pp. 27–48.

Michell, R. H. (1975) Inositol phospholipids and cell surface receptor function. *Biochim. Biophys. Acta*, 415: 81–148.

Michell, R. H. and Kirk, C. J. (1981) Why is phosphatidylinositol degraded in response to stimulation of certain receptors? *Trends Pharmacol. Sci.*, 2: 86–89.

Nestler, E. G. and Greengard, P. (1983) Protein phosphorylation in the brain. *Nature (Lond.)*, 305: 583–588.

Nestler, E. G. and Greengard, P. (1984) *Protein Phosphorylation in the Nervous System*, John Wiley and Sons Ltd., New York.

Nishizuka, Y. (1980) Three multifunctional protein kinase systems in transmembrane control. *Mol. Biol. Biochem. Biophys.*, 32: 113–135.

Nishizuka, Y. (1983) Phospholipid degradation and signal translation for protein phosphorylation. *TIBS*, 8: 13–16.

Nishizuka, Y. (1984a) The role of protein kinase C in cell surface signal transduction and tumor promotion. *Nature (Lond.)*, 308: 693–697.

Nishizuka, Y. (1984b) Turnover of inositol phospholipids and signal transduction. *Science*, 1365–1370.

Oestreicher, A. B. and Gispen, W. H. (1986) Comparison of the immunocytochemical distribution of the phosphoprotein B-50 in the cerebellum and hippocampus of immature and adult rat brain. *Brain Res.*, 375: 267–269.

Oestreicher, A. B., Zwiers, H., Schotman, P. and Gispen, W. H. (1981) Immunohistochemical localization of a phosphoprotein (B-50) isolated from rat brain synaptosomal plasma membranes. *Brain Res. Bull.*, 6: 145–153.

Oestreicher, A. B., Zwiers, H., Gispen, W. H. and Roberts, S. (1982) Characterization of infant rat cerebral cortical membrane proteins phosphorylated in vivo: identification of the ACTH-sensitive phosphoprotein B-50. *J. Neurochem.*, 39: 683–692.

Oestreicher, A. B., Van Dongen, C. J., Zwiers, H. and Gispen, W. H. (1983a) Affinity-purified anti-B-50 protein antibody: Interference with the function of the phosphoprotein B-50 in synaptic plasma membranes. *J. Neurochem.*, 41: 331–340.

Oestreicher, A. B., Zwiers, H., Leunissen, J. L. M., Verkleij, A. J. and Gispen, W. H. (1983b) Localization of B-50 in rat brain studied by immuno light and electron microscopy. *J. Neurochem.*, Suppl. 41, S95.

Oestreicher, A. B., Dekker, L. V. and Gispen, W. H. (1985) A radioimmunoassay (RIA) for the phosphoprotein B-50: distribution in rat brain. *Neurosci. Lett.*, Suppl. 22, S560.

Pfenninger, K. H., Ellis, L., Johnson, H. P., Friedman, L. and Somlo, S. (1983) Nerve growth cones isolated from fetal rat brain: subcellular fractionation and characterization. *Cell*, 35: 573–584.

Prentki, M., Biden, T. J., Janjic, D., Irvine, R. F., Berridge, M. J. and Wollheim, C. B. (1984) Rapid mobilization of Ca^{2+} from rat insulinoma microsomes by inositol-1,4,5-trisphosphate. *Nature (Lond.)*, 309: 562–564.

Roth, G. S., Henry, J. M. and Joseph, J. A. (1986) The striatal dopaminergic system as a model for modulation of altered neurotransmitter action during aging: effects of dietary and neuroendocrine manipulations. In D. F. Swaab, E. Fliers, M. Mirmiran, W. A. Van Gool and F. P. A. J. Van Haren (Eds.), *Aging of the Brain and Alzheimer's Disease, Progress in Brain Research, this volume*, Elsevier Biomedical Press, Amsterdam, pp. 473–484.

Rouser, G., Kitchensky, G., Yamamoto, A. and Baxter, C. F. (1972) Lipids in the nervous system of different species as a function of age: brain, spinal cord peripheral nerves, purified whole cell preparations and subcellular particulates: regulatory mechanisms and membrane structure. *Adv. Lipid Res.*, 10: 26–360.

Routtenberg, A. (1982) Brain phosphoproteins and behavioral state. In W. H. Gispen and A. Routtenberg (Eds.), *Brain Phosphoproteins: Characterization and Function, Progress in Brain Research, Vol. 56*, Elsevier Biomedical Press, Amsterdam, pp. 349–374.

Routtenberg, A., Lovinger, D. M. and Steward, P. (1985) Selective increase in phosphorylation state of a 47 kDa protein (F_1) directly related to long-term potentiation. *Behav. Neural Biol.*, 43: 3–11.

Salway, J. G., Harwood, J. L., Kai, M., While, G. L. and Hawthorne, J. N. (1968) Enzymes of phosphoinositide metabolism during rat brain development. *J. Neurochem.*, 15: 221–226.

Shaik, N. A. and Palmer, F. B. St. C. (1976) Deposition of lipids in the developing central and peripheral nervous systems of the chicken. *J. Neurochem.*, 26: 597–603.

Shinitzky, M. (1984) Membrane fluidity in malignancy: adversative and recuperative. *Biochim. Biophys. Acta*, 738: 251–261.

Shinitzky, M. and Henkart, P. (1979) Fluidity of cell membranes: current concepts and trends. *Int. Rev. Cytol.*, 60: 121–147.

Skrede, K. K. and Malthe-Sörensen, D. (1981) Increased resting and evoked release of transmitter following repetitive electrical tetanization in hippocampus: a biochemical correlate to long-lasting synaptic potentiation. *Brain Res.*, 208: 436–441.

Sörensen, R. G., Kleine, L. P. and Mahler, H. R. (1981) Presynaptic localization of phosphoprotein B-50. *Brain Res. Bull.*, 7: 57–61.

Takai, Y., Kishimoto, A., Iwasa, Y., Kawahara, Y., Mori, T. and Nishizuka, Y. (1979a) Calcium-dependent activation of a multifunctional protein kinase by membrane phospholipids. *J. Biol. Chem.*, 254: 3692–3695.

Takai, Y., Kishimoto, A., Iwasa, Y., Kawahara, Y., Mori, T., Nishizuka, Y., Tamura, A. and Fukii, T. (1979b) A role of membranes in the activation of a new multifunctional protein kinase system. *Biochem. J.*, 86: 575–578.

Teyler, T. J. and Discenna, P. (1984) Long-term potentiation as a cardiate mesmonic device. *Brain Res. Rev.*, 7: 15–28.

Tielen, A. M., Mollenvanger, W. J., Lopes da Silva, F. H. and Hollander, C. F. (1983) Neuronal plasticity in hippocampal slices of extremely old rats. *Dev. Neurol.*, 7: 73–83.

Uma, S. and Ramakrishnan, C. V. (1983) Studies on polyphosphoinositides in developing rat brain. *J. Neurochem.*, 40: 914–916.

Van Dongen, C. J., Hershkowitz, M., Zwiers, H., De Laat, S. and Gispen, W. H. (1983) Lipid fluidity and phosphoinositide metabolism in rat brain membranes of aged rats: effects of ACTH (1-24). *Dev. Neurol.*, 7: 101–114.

Van Dongen, C. J., Zwiers, H. and Gispen, W. H. (1984) Purification and partial characterization of the phosphatidylinositol 4-phosphate kinase from rat brain. *Biochem. J.*, 223: 197–203.

Van Dongen, C. J., Zwiers, H., De Graan, P. N. E. and Gispen, W. H. (1985) Modulation of the activity of purified phosphatidylinositol 4-phosphate kinase by phosphorylated and dephosphorylated B-50 protein. *Biochem. Biophys. Res. Commun.*, 8: 1219–1227.

Voronin, L. L. (1983) Long-term potentiation in the hippocampus. *Neuroscience*, 10: 1051–1069.

Weller, M. (1979) *Protein Phosphorylation. The Nature, Function and Metabolism of Proteins, which contain covalently bound Phosphorus*, PION Ltd., London.

Wiegant, V. M., Zwiers, H., Schotman, P. and Gispen, W.H. (1978) Endogenous phosphorylation of rat brain synaptosomal plasma membranes in vitro: methodological aspects. *Neurochem. Res.*, 3: 443–453.

Zwiers, H., Veldhuis, D., Schotman, P. and Gispen, W. H. (1976) ACTH, cyclic nucleotides and brain protein phosphorylation in vitro. *Neurochem. Res.*, 1: 669–677.

Zwiers, H., Schotman, P. and Gispen, W. H. (1980) Purification and some characteristics of an ACTH-sensitive protein kinase and its substrate protein in rat brain membranes. *J. Neurochem.*, 34: 1689–1699.

Zwiers, H., Verhaagen, J., Van Dongen, C. J., De Graan, P. N. E. and Gispen, W. H. (1985) Resolution of rat brain synaptic phosphoprotein B-50 into multiple forms by two-dimensional electrophoresis: evidence for multi-site phosphorylation. *J. Neurochem.*, 44: 1083–1090.

Discussion

R. D. TERRY: Did the PAP reaction with anti-B-50 display a cytoplasmic or a plasma membrane localization of B-50 in the aged hippocampus?

W. H. GISPEN: Presently we cannot be sure about the exact localization of the B-50 in the hippocampus of aged rats. The data strongly suggest, however, that there occurs an age-related redistribution of B-50-like material over the life-span of the rat. Further ultra-localization experiments are necesssary before final conclusions can be drawn.

R. S. WILLIAMS: Is B-50 also on lysosome membranes? Could the apparently paradoxical increase in cytoplasmic staining with anti-B-50 in region CA3 of hippocampus in very old rats be due to lysosomal staining of lipofuscin pigment as it accumulates there in old age?

W. H. GISPEN: From what we know of the cellular distribution of B-50 in the adult rat brain using subcellular fractionation techniques, B-50 is primarily associated with cell and vesicle membranes and not found in fractions enriched in lysosomes (Sörensen et al., 1981; Kristjansson et al., 1982; Gispen et al., 1985b).

D. F. SWAAB: How can you explain the discrepancy between the drop in B-50 in the assay in the hippocampus of old animals and the accumulation of stained material in this structure as shown by immunocytochemistry?

W. H. GISPEN: The immunocytochemical techniques show profound effects of age on the regional distribution of B-50. However, the local accumulation of B-50 in or around hippocampal pyramidal cells is not in disagreement with a drop in total B-50 content of the whole hippocampus as measured by RIA.

T. SCHUURMAN: How can it be explained (theoretically) that with increased membrane fluidity some receptor types are more available in the membrane, whereas other receptors have a lower availability?

W. H. GISPEN: This depends on the structure and micro-environment of the receptor. Some are pulled out of the membrane, others stay inside with increasing fluidity. There is no general rule. Every receptor type has to be studied separately.

R. RAVID: How do you explain that the changes in fluidity and responses of membranes with age are purely restricted to the protein systems like B-50?

W. H. GISPEN: I do not think that the effect of changes in membrane lipid fluidity will be limited to proteins like B-50 in particular. As more will be known about the function of other membrane proteins more effects of changes in the microviscosity will become apparent as is already the case in many non-neuronal cell types.

J. KORF: Are the coupling proteins located inside the membrane as was shown in the presented models?

W. H. GISPEN: The exact localization of the proteins involved in the signal transduction is not entirely known. It seems reasonable, however, to assume that regulatory proteins that couple receptors to catalytic subunits at the inside of the membrane are in part inverted into the lipid bilayer. Therefore, it can be assumed that lipid fluidity may affect the function of such proteins.

References

Gispen, W. H., Leunissen, J. L. M., Oestreicher, A. B., Verkleij, A. J. and Zwiers, H. (1985b) Presynaptic localization of B-50 phosphoprotein: the ACTH sensitive protein kinase substrate involved in rat brain polyphosphoinositide metabolism. *Brain Res.*, 328: 381–385.

Kristjansson, G. I., Zwiers, H., Oestreicher, A. B. and Gispen, W. H. (1982) Evidence that the synaptic phosphoprotein B-50 is localized exclusively in nerve tissue. *J. Neurochem.*, 39: 371–378.

Sörensen, R. G., Kleine, L. P. and Mahler, H. R. (1981) Presynaptic localization of phosphoprotein B-50. *Brain Res. Bull.*, 7: 57–61.