Instituto Juan March
de Estudios e Investigaciones

24 CENTRO DE REUNIONES
INTERNACIONALES SOBRE BIOLOGÍA

Workshop on

Deterioration, Stability and Regeneration of the Brain During Normal Aging

Organized by

P. D. Coleman, F. Mora and M. Nieto-Sampedro

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Y.-A. Barde
P. D. Coleman
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INTRODUCTION

F. Mora

Aging is a universal and deleterious process ocurring in every vertebrate. As a result of this process decay in function occurs. This implies severe behavioural deficits in the individual. Nature has solved the problem of aging in wild animals. Simply, they do not survive long enough to experience the deterioration associated with aging. In man, however, aging is present with all its personal, social and economical consecuences. The advances of medical research in western societies have brought with them a rapid increase in the mean age of the population. Its consecuences seem obvious.

Aging of the brain is, without doubt, responsable for the main behavioural deficits that acompany aging: Loss of memory, reasoning, emotional disturbances and also motor and sensory processing. Because of that, research in this field has increased considerably in its efforts to understand the mechanisms by which aging of the brain ocurrs.

One of the aims of this workshop was to update the information on the changes brought about by normal aging in the human brain. Special emphasis of this workshop was on one of the most fascinating aspects of this process, that of plasticity and regeneration. Accordingly, an important part of the wokshop was devoted to neurotransmitters, growth factors and plasticity, as well as to the increasing role of glia and its relation to the neuronal changes that occur during the aging process.

In summary, this workshop dedicated to full sessions to neuronal changes, two sessions to Neurotransmitters, second messengers and neurotrophic factors and a final session to the neuron-glia interaction during aging.

Neuronal Changes

The Role of Microtubule-Associated Proteins in Neuronal Structure.

Andrew Matus, Jacqueline Ferralli and Beat Ludin, Friedrich Miescher Institute, P.O.Box 2543, 4002, Basel, Switzerland.

Microtubule-associated proteins (MAPs) are particularly abundant in neurons where they are present in both axons and dendrites (Matus, 1988). The two most abundant forms, MAP2 and tau, are the products of related genes and bind to microtubules via a domain containing 3 or 4 repeats of an 18 amino acid sequence motif. When either of these proteins are expressed in cultured non-neuronal cells by transfection their microtubules are stabilized and are rearranged in the cytoplasm. Our experiments with MAP2 have identified several characteristic features of these changes (Matus, 1994). First microtubules in MA2 or tau transfected cells exist independently of the centrosomal microtubule-organizing centre, from which microtubules normally arise in non-neronal cells (Weisshaar et al., 1992). In this respect microtubules in MAP-transfected cells resemble those in neuronal processes, which also arise without any distinct initiating organelle. Second, MAP2-stabilized microtubules are longer than those in control cells, or in cells whose microtubules have been stabilized by chemical reagents such as taxol. This property probably arises because MAP2 is a poor initiator of microtubule assembly inside living cells. Third, when MAP2 or tau is added to microtubules either inside cells (Weisshaar et al., 1992) or in vitro (Dye et al., 1993) they become stiffer and are then capable of supporting process outgrowth (Edson et al., 1993). The stiffness of such processes can be directly visualized by video microscopy (B. Ludin and A. Matus, unpublished observations).

To investigate the molecular mechanism of these changes we have transfected cells with mutated versions of MAP2 containing only part of the protein sequence. These experiments show that one particular region of the molecule is responsible for all the effects of MAP2, binding to microtubules, stabilization, polymer elongation and stiffening (Ferralli et al., submitted for publication). The part of MAP2 required for these properties contains the repeats of 18 amino acids, which have been shown to constitute the core of the tubulin-binding domain in the neuronal microtubule-associated proteins MAP2 and tau (Lewis et al., 1988). These repeats are spaced along the length of the MAP2 and tau molecules so that they are able to bind to neighbouring tubulin subunits in the wall of the microtubule (Butner and Kirschner, 1991; Edson et al., 1993). Since MAPs are very abundant in neuronal processes and effectively coat the microtubules, this multimeric binding domain can tether the tubulin subunits to one another and effectively restrict their freedom of movement. We hypothesise that it is this that produces the stiffening effect of MAPs on microtubules and makes them capable of supporting process outgrowth either in transfected non-neuronal cells or in neuronal processes where they normally occur (Edson et al., 1993; Matus, 1994).

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PHOSPHORYLATION OF AXONAL MICROTUBULE ASSOCIATED PROTEINS IN ALZHEIMER'S DISEASE IS SIMILAR TO THAT FOUND DURING NEURONAL DEVELOPMENT

J. Avila, M.D. Ledesma, L. Ulloa, I. Correas, M.A. Moran†, P. Gómez Ramos† and J. Díaz-Nido, Centro de Biología Molecular (CSIC-UAM) 28049-Madrid. †Dpto. Morfología Facultad de Medicina, UAM.

Alzheimer's disease results in the appearance of cytoskeletal disorders yielding pathological structures such a neurofibrillary tangles or dystrophic neurites. Neurofibrillary tangles consit of aggregated paired helical filaments. It has been previously described that the microtubule-associated protein, tau, modified by phosphorylation in serines adjacent to prolines, is a major component of these structures. In an attempt to isolate a soluble precursor of this aberrant tau protein, tau from cytosolic fractions obtained from normal and Alzheimer's disease brains have been fractionated by iron-chelated affinity chromatography to discriminate between isoforms phosphorylated to different extents. Tau isoforms were eluted from the column by an increasing step pH gradient. Both the apparent molecular weight and the amount of protein eluting at the higher pH tested increased in the case of the Alzheimer's disease-soluble fraction compared to the normal-soluble one suggesting an enrichment in abnormally phosphorylated tau isoforms in the former. Immunoblot analysis of the tau fractions eluted at the different pH values shows that SMI 31, an antibody which binds to a phosphorylated epitope present in paired helical filament-tau, recognizes just the tau isoforms isolated in the eluted fraction at higher pH from the Alzheimer's disease-soluble sample. Interestingly, these tau isoforms are not recognized by an antibody which binds to an unmodified epitope, Tau-1, unless the sample is pretreated with alkaline phosphatase. Phosphorylation

experiments performed with proline-directed protein kinases indicate that the fraction isolated at higher pH from Alzheimer's disease-soluble tau may be completely phosphorylated at the corresponding target sites by those kinases.

Thus, a fraction of Alzheimer's disease-soluble tau isolated by iron-chelated affinity chromatography is extensively phosphorylated by proline-directed protein kinases compared to normal soluble tau. This phosphorylation includes the sites constituting the SMI 31 (serines-396 and -404) and Tau-1 (serines-199 and -202) and/or threonine-205) epitopes; these modifications may be considered as a prior event to tau aggregation into paired helical filaments (PHF), although it is not sufficient to promote the polymerization of tau into PHF, since hyperphosphorylated tau can be found in soluble form.

Also, it has been found that another microtubule associated protein, MAP1B, phosphorylated by a proline-dependent protein kinase, is a component of neurofibrillary tangles or dystrophic neurites. Thus, a possible common phosphorylation of axonal MAPs such as tau or MAP1B may correlate with their association with those aberrant cytoskeletal structures present in AD.

Are the Neurodegenerative Mechanisms Associated With Alzheimer's Disease the Same as Those Associated With Normal Aging?

Mark J. West, Stereological Research Laboratory and Department of Neurobiology, University of Aarhus, Denmark

Alzheimer's disease (AD) is a progressive degenerative disease of the central nervous system characterized by changes in personality and decreases in cognitive functions, including memory. The neuropathological signs of the disease, neuron loss and the presence of a relatively large numbers of senile plaques and neurons with neurofibrillary tangles, are particularly pronounced in limbic and temporal lobes of the brain and likely to be responsible for much if not all of the behavioral alterations associated with the disease. Neither palliative nor curative treatments are presently available due to an incomplete understanding of the degenerative mechanisms by which AD progresses. Most findings regarding these mechanisms fall into two categories; those that suggest that AD is accelerated aging and those that indicate that AD is a disease that involves degenerative processes which are qualitatively different from those associated with normal aging. The resolution of this issue has important implications for the design of therapeutic strategies. Toward this end, recently developed, design based, stereological methods for making precise estimates of the total number of neurons in localized regions of the hippocampal region of the brain, have been used to test the hypothesis that AD is accelerated aging by determining whether or not the specific subsets of neurons lost during normal aging are the same as those that are lost with AD. Although the patterns of neuron loss were similar, in that age related and AD related losses were observed in two of the five subdivisions of the region, the AD patients suffered a marked loss in CA1, a subdivision which did not show evidence of age related loss. The unique loss in CA1 of AD patients indicates that there are qualitative differences in neuro-degenerative processes involved in aging and AD and that the hypothesis that the AD is an accelerated form of normal aging is not tenable.

ALTERATIONS IN HUMAN PREFRONTAL CORTEX NEURONS FROM EARLY DEVELOPMENT INTO NORMAL AND DEMENTED AGING

H.B.M. Uylings, M.J.T. Koenderink and R.J.K. Kramers
Netherlands Institute for Brain Research
Graduate School Neurosciences Amsterdam
Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands

Degeneration of cortical neurons is a natural phenomenon which occurs from early development. This is related during the cortical development with the transient cortical organization as especially noticeable in the subplate zone (Kostović and Rakic, 1990; Mrzljak et al., 1990). In previous years it was generally thought (Brody, Terry etc.) that neuronal loss occurred frequently in the late adulthood and senile dementia. This is disputed by a study which used the new stereological methods (Regeur et al., 1994).
The processes of surviving cortical neurons growth during development in size in general. An example of an exception is the reduction in size and reorientation of dendritic trees of subplate neurons in the first year after birth, but afterwards their size is rather stable until late adulthood.
We have examined the prenatal and postnatal development of the prefrontal pyramidal neurons. Initially layer V pyramidal neurons are more mature than layer III neurons, but the basal dendrites of both groups of neurons reach 'adult' values in the same period (i.e. the first 3 years of postnatal life).
Reports of Coleman's group show that in normal human aging depending on cortical area and cell type, the dendritic extent in cortical neurons can increase (in parahippocampal gyrus, Buell and Coleman, 1981), decrease (in granule cells in dentate gyrus, Flood and Coleman, 1990) or remain stable (pyramidal neurons in CA2, CA1 and subiculum, Flood and Coleman, 1990).
In our study on aging we found layer V pyramidal dendritic regression in adulthood in the prefrontal cortex before this was apparent in the size of the somata at about 60 years of age. Due to the dendritic regression in normal aging the size differences with layer V neurons from patients with Alzheimer's disease are not detectable from about 65 years. We noticed a large-interindividual variability, as has been described in magnetic resonance imaging (MRI) studies (e.g. Gur et al., 1991). In this study a volumetric

reduction of brain volume is noted from about 74 year of age.
Apparently different cortical regions react differently in normal human aging and in Alzheimer's disease.

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MAINTAINED NEURONAL PLASTICITY IN NORMALLY AGING BRAIN AND DECREASED PLASTICITY IN ALZHEIMER'S DISEASE ASSOCIATED WITH NEUROFIBRILLARY TANGLES

COLEMAN, PAUL D. AND CALLAHAN, LINDA

Alzheimer's Disease Center, University of Rochester Medical Center, Rochester, NY 14642, USA.

In selected brain regions neurons die in both normal aging and in Alzheimer's disease (AD). We have been concerned with the responses of the surviving, remaining neurons to the death of their neighbors. Our earlier morphological data obtained from Golgi studies indicated that in the normally aging human brain there was an age-related increase in dendritic extent of surviving neurons. In Alzheimer's disease this age-related increase in dendritic extent did not take place. These data led us to propose continuing neuronal plasticity in the aging human brain, with defective neuronal plasticity in Alzheimer's disease. These findings also raised several new questions including: 1) Is the new dendritic material formed in the normally aging brain integrated into the circuitry of the brain? 2) Can the conclusions regarding neuronal plasticity that were based on increased dendritic extent be confirmed by an alternate marker of neuronal plasticity?

To determine whether the new dendritic material formed in the normally aging brain is integrated into the circuitry of the brain we utilized the olfactory bulb of the Sprague-Dawley rat as a model system. Hinds and McNelly (1977) had shown that dendritic extent of mitral cells in this region increased with increasing age, concomitant with neuronal loss. In very old age dendritic extent decreased. In a Golgi-e.m. study we serially reconstructed dendritic tips of mitral cells of Sprague-Dawley rats, and counted and sized synapses at dendritic tips as a function of age. The density of synapses per surface area remained constant whether at an age when dendrites were proliferating or regressing. The sizes of synapses declined with advancing age. These data indicate that newly formed dendritic material forms synapses within the neuropil. Since the formation of synapses requires proliferation of axon terminals as well as the post-synaptic dendrite, these data also imply axonal, as well as dendritic, plasticity in aging brain.

As an alternate marker of neuronal plasticity in aging and AD human brain we utilized the axonal growth-associated protein, GAP-43. Message levels for GAP-43 were determined as a function of age and AD. GAP-43 message was detectable at all ages, indicating some degree of plasticity. However, GAP-43 message level did show an age-related decline in normal brain. In AD brain GAP-43 message level was reduced in proportion to the density of neurofibrillary tangles (NFT), but was unrelated to the density of senile plaques. Combined immunocytochemistry and *in situ* hybridization studies confirmed the neurons containing NFT as being largely responsible for the reduced GAP-43 message in AD. Similar results were also obtained with regard to synaptophysin message level. Some neurons with no evidence of frank NFT also showed greatly reduced synaptophysin message. Potential pathologies in these neurons will be discussed.

These data indicate the existence of neuronal plasticity in the normally aging brain, with a failure of plasticity and synaptic structure in the neurofibrillary tangle-bearing neurons in Alzheimer's disease.

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MITOCHONDRIAL INJURY IN BRAIN AGING, J. Miquel, E. de Juan, M. Martinez, Instituto de Neurociencias, Universidad de Alicante, and M. L. Ferrandiz, Depto. de Farmacología, Facultad de Farmacia, Universidad de Valencia.

In contrast to the abundance of publications on the pathogenesis and pharmacological treatment of Alzheimer's disease and other types of dementia, there is a relative lack of interest in the fundamental mechanisms of the progressive involution that occurs with age in human subjects and animals. A better understanding of these mechanisms, that are related to both "normal" and "pathological" brain aging, may contribute not only to the progress of experimental gerontology but also to the development of a more rational geriatric meuropharmacology.

On the basis of our own electron microscopic observations on neurons of aged animals, we have proposed that the senescent involution of this cell type is linked to an oxygen-radical caused mitochondrial injury, probably involving the mitochondrial genome (1-5). The high rate of mitochondrial oxygen utilization in this cell type may be associated with accumulating damage to the mitochondrial DNA (mtDNA) that these organelles would be unable to repair because, in contrast to nuclear DNA, mtDNA does not have efficient repair. Thus deprived of the ability to renew the hydrophobic proteins of the inner mitochondrial membranes (wich are coded by mtDNA), an increasing number of mitochondria would degenerate, with concomitant declines in bioenergetic competence and function.

The above concept is supported by the finding that a key component of the mitochondrial electron transfer chain, namely cytochrome aa_3, shows an age related decrease in the rat brain. Moreover, data from our laboratory show a considerable impairment in mitochondrial oxidative phosphorylation in the brain of aged mice. This is in agreement with previously reviewed work (4) from other authors on neurons and other fixed postmitotic cells suggesting that in these cells aging is accompanied by changes in the structure, respiration, ATP synthesis and genome stability of their mitochondrial populations.

The fact that there is an age dependent decrease in the number of mitochondria per volume of synaptic structure (6) further supports our early hypothesis that senescence impairs the ability of mitochondria to rejuvenate themselves through the process of organellar replication.

Mitochondrial changes linked to damage to mtDNA, may play a role not only in normal brain aging but also in such age related processes as Parkinson's and Alzheimer's disease. Therefore, as pointed out by Wallace (7), metabolic therapies presently being developed for mtDNA preservation may have some usefulness in the prevention and treatment of those diseases.

Another approach to pharmacological treatment of brain aging and related

diseases could be based on the stimulation of the bioenergetic competence of mitochondria. In agreement with this concept, choline compounds (8) and acetyl-L-carnitine (9) have shown favorable results in the treatment of cognitive impairment and depression in the aged.

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REGULATION OF CELL NUMBERS IN THE NERVOUS SYSTEM BY NEUROTROPHINS

Y.-A. BARDE
Department of Neurobiochemistry Max-Planck Institute for Psychiatry 82152 Martinsried, Germany

During normal development of the vertebrate nervous system, many cells initially generated are eliminated when long lasting relationships are first established, for example when neurons contact their target cells, or oligodendrocytes their axons[1]. It is becoming apparent that cell numbers can be regulated by the limited availability of well defined molecules, some of which belonging to a gene family the neurotrophins. These small, basic, secretory proteins are strongly related in structure and all of them can prevent the death of a variety of cultured neurons. Less is known about their exact roles in vivo, but also for the recently discovered neurotrophins, there are now encouraging indications to suggest that they play essential functions during development[2]. In the case of nerve growth factor (NGF), there is strong experimental evidence (based on very early experiments, later confirmed using a variety of approaches) to suggest that NGF regulates cell numbers by preventing the death of sympathetic and neural crest-derived sensory neurons. Also, brain-derived neurotrophic factor (BDNF) saves motoneurons both during development and after axotomy. However, recent results indicate that neurotrophin-3 (NT-3) regulates neuronal numbers in vivo before normally occurring cell death is seen in NT-3-responsive ganglia. Already during gangliogenesis, the limited availability of NT-3 regulates neuronal proliferation and/or the differentiation of neuronal progenitor cells. In addition, a direct mitogenic effect of NT-3 has been observed on oligodendrocyte precursor cells, and NT-3 is necessary in vivo to reach normal numbers of oligodendrocytes in the optic nerve[3]. It is thus becoming apparent that neurotrophins control cell numbers in the developing nervous system of more than one cell type by more than one mechanism.

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ALTERATIONS IN TAU PROTEIN METABOLISM DURING NORMAL AGING

Mukaetova-Ladinska EB, Harrington CR, Wischik CM
Cambridge Brain Bank Laboratory, Department of Psychiatry, University of Cambridge

Tau protein is normally involved in maintaining the axonal viability (Binder et al, 1985). Although tau protein is a major constituent of PHFs in Alzheimer's disease (Wischik et al, 1988), little is known about changes in tau distribution that occur in normal aging. We have examined the distribution of tau protein in the brain tissue from 15 cognitively unimpaired individuals (mean age at death of 57.27 ± 5.12 years; 19-88 years). Of these cases, 8 constitute a young subgroup of control cases (≤65 years), and 7 an old control subgroup (≥65 years). For the biochemical analysis, the neocortical tissue deriving from the frontal (BA 10), temporal (BA 21 and 22), parietal (BA 7) and occipital (BA 18 and 19) lobes and cerebellum were subdissected into grey and white matter, while only the somato-dendritic domain of the hippocampus and entorhinal cortex were analysed. The biochemical analysis of the tau content was performed using methods described in Wischik et al (1988), Harrington et al (1990, 1991) and Mukaetova-Ladinska et al (1993); tau protein was quantified by ELISA using mAbs 423 and 7.51.

The low level of PHF content, measured using mAb 423, did not increase with aging in any of the analysed brain regions, despite the presence of a limited number of neurofibrillary tangles restricted to the medial temporal lobe in the elderly control cases. PHF-tau content detected in the controls was not correlated with either the density of amyloid deposits or neurofibrillary changes. The levels of normal, soluble tau protein decreased with aging ($r=-0.3234$, $p=0.0001$). This age-related loss of soluble tau protein did not affect all brain regions uniformly. The most affected areas were the frontal grey matter and hippocampus, where the levels of tau protein were decreased by 90% in the elderly individuals, followed by the somato-dendritic compartments of occipital, temporal and parietal lobe (77%, 68% and 59% respectively). The reduction observed in the axonal compartment was more prominent in the fronto-temporal and occipital white matter domains. The levels of soluble tau protein in the parietal and cerebellar axonal compartments were reduced by only 22% and 28%, respectively. The depleted levels of soluble tau protein were not found to be a result of the accumulation of neuropathological changes in these individuals. These findings suggest that the

changes in tau metabolism in normal aging are distinct from those reported previously for AD (Mukaetova-Ladinska et al, 1993).

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THE MECHANISM OF ACTION OF INTERLEUKIN-1β IN ASTROGLIAL CELLS. Amandip K. Utal, James Hale, and Paul D. Coleman, Dept. of Neurobiology and Anatomy, University of Rochester, 601 Elmwood Ave., Rochester, NY 14642, USA.

One of the changes in the course of the normally aging brain is an increase in the number of astrocytes (1). A number of cytokines are known to affect astroglial cells in various ways. We have chosen to focus on the effect of the cytokine interleukin-1β (IL-1β) on astrocytes, since IL-1β is known to cause astrocytes to become more reactive and in some instances to proliferate.

We are investigating the mechanism of action IL-1β on astrocytes in tissue culture, particularly the mitogenic effect of IL-1β and the transduction of the IL-1 signal via its receptor in astrocytes. Our studies are being conducted on two types of astroglial cells, namely U373 cells which are a human astrocytoma cell-line, and on primary rat astrocytes. U373 cells stained uniformly for the astrocytic marker glial fibrillary acidic protein (GFAP) indicating the astrocytic nature of these cells.

The proliferative effect of IL-1 was assayed by tritiated thymidine incorporation. Recombinant human IL-1β induced mitogenesis of U373 cells in a dose-dependent fashion but was unable to cause a similar effect in primary rat astrocytes. 10% fetal bovine serum in the culture medium was able to induce tritiated thymidine incorporation in the rat astrocytes, an affect that IL-1 was unable to modulate.

Stimulation of tritiated choline incorporation into the cellular lipids of astrocytes by IL-1 was also investigated as a potential signal transduction pathway involving phosphatidylcholine (PC) hydrolysis. IL-1 was unable to stimulate the PC pathway in either U373 cells or rat astrocytes. Both cells types, however, incorporated tritiated choline into cellular lipids in response to the phorbol ester, tetradecanoylphorbol acetate, indicating the ability of these cells to operate the PC pathway of signal transduction.

A recent report has shown that IL-1 activates sphingomyelin hydrolysis in lymphocytes (2). We are presently investigating this pathway of IL-1 signal transduction in astrocytes.

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Neurotransmitters, Second Messengers and Neurotrophic Factors

DOPAMINE-GLUTAMATE INTERACTIONS WITH AGE: STUDIES IN NEOSTRIATUM AND PREFRONTAL CORTEX

F. Mora and A. Porras
Department of Physiology, Faculty of Medicine.
University Complutense, 28040 Madrid, SPAIN.

An interaction between dopamine and glutamate has been postulated to exist in both neostriatum and medial prefrontal cortex. In this presentation we will address this type of interaction through the effects of a D1-D2 dopamine receptors agonist, apomorphine, on the release of glutamate in neostriatum and medial prefrontal cortex of rats of 2-3 (young), 12 (middle), and 24-30 (aged) months of age. These studies have been performed in the conscious animal using an intracerebral perfusion system.

In neostriatum and in young rats apomorphine produced a dose-related release of glutamate which was significantly attenuated by previous injections of haloperidol, a dopamine receptors blocker. In middle aged rats apomorphine induced a delayed release of GLU while in aged rats apomorphine had no effects. These results would indicate an age-related deterioration of the dopamine-glutamate interaction in this area of the brain. Discussion will be centered in the light of the stability of the corticostriatal glutamatergic system and the degeneration of the nigrostriatal dopaminergic system with age. These data could be important to understand the motor deficits found in man during aging.

In medial prefrontal cortex apomorphine releases glutamate although in a different profile to that found in the neostriatum. Thus, only at a single intermediate dose, apomorphine released glutamate but had no effects at other doses. The dopamine receptors blocker, haloperidol, also attenuated the release of glutamate produced by apomorphine in the prefrontal cortex. No response to apomorphine was found in middle aged and aged rats. The significance of the altered dopamine-glutamate interactions in the prefrontal cortex could be relevant to understand changes of responses to stress, reward or cognition with age.

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SOME CHANGES IN BRAIN CHEMISTRY AND MORPHOLOGY DURING NORMAL AGING IN HUMANS

Edith G. McGeer, Kinsmen Laboratory of Neurological Research, University of British Columbia, 2255 Wesbrook Mall, Vancouver, B.C., V6T 1Z3, Canada

During normal aging, there are many changes in chemistry and morphology including decreases and increases in neurotransmitters, related enzymes and receptors, changes in glucose metabolism, dendritic shrinkage, loss of neurons, and increased gliosis. It is important to note that these changes are very region-specific, and probably, to some extent, species specific. Thus changes found in one region or species should not be assumed to occur in other regions or species.

In early work on neurotransmitter enzymes in humans, we measured the activity of tyrosine hydroxylase (TH), choline acetyltransferase (ChAT) and glutamate decarboxylase (GAD), the key enzymes in the syntheses of dopamine (DA), acetylcholine and GABA, in some sixty regions of human brain. The purpose was to determine any deficits which might occur in diseases such as Parkinson's (PD) or Huntington's disease (1). Somewhat to our surprise, age seemed to be the major factor in influencing enzyme activity in the neurologically normal controls (2). The greatest effect of age was noted on TH activity in nerve ending regions such as the caudate, putamen and nucleus accumbens, with some 70% of the apparent activity at birth being lost by age 25, after which there was a much slower but continued decline. ChAT and GAD either showed no significant or a much slower rate of decline in these regions. Significant semi-log correlations of ChAT with age were, however, found in many cortical regions, while GAD showed its highest rate of decline with age in the thalamus. Subsequent literature on regional changes in neurotransmitters and their synthetic enzymes in normal human aging is somewhat controversial (much reviewed in 3), but the losses in the basal ganglial DA system (4) and cortical ChAT are probably the best established changes. The marked loss of TH in the striatum at a relatively young age may not be functionally important since we are probably born with considerable excess enzyme capacity. However, continued losses in both the pre- and post-synaptic DA system may account for the increasing difficulty in movement as one ages. The loss of ChAT in the cortex and hippocampus may also explain the usual small memory loss in "normal" aged individuals, as well as the tolerance of children for doses of anti-cholinergics which produce confusion and memory loss in adults (5).

The mechanisms underlying these losses are generally unknown, although cell death, and/or dendritic shrinkage coupled with loss of synapses (which might be consequent on decreases in axonal transport) have been suggested (3). Significant losses of DA neurons in the substantia nigra (SN) (6) and of cholinergic neurons in the basal forebrain (the source of cortical ChAT) (7) have been reported and confirmed by other groups (3), although the exact rate of cell loss found has been variable. The fact that the steepest rates of decline are often in nerve ending areas of long axon neurons (as in the striatum for TH and in the cortex for ChAT) would support a role for decreased axonal transport.

Changes in the receptor systems may also be of great importance and there may be losses in plasticity even in cases where significant decreases in number of binding sites have not been found. Perhaps the best established such loss in humans is in D2 binding sites in the striatum (3, 4). And, again, there are sharp regional differences; actual increases in D2 binding sites have been reported in the retina (3).

It should be noted that not all neurotransmitter related enzymes show decreases with age. Some, indeed show sharp increases. But those appear to be enzymes, like MAO (3), which are largely localized in glia, and the increases probably reflect the increased gliosis seen in the aging brain.

The cortical cholinergic system which appears to show decreases in normal aging is more severely affected in most cases of Alzheimer disease (AD). The noradrenergic system which is pathologically affected in AD is also known to show losses with normal aging (3). However, there are many qualitative differences which indicate that AD is certainly not merely accelerated aging. For example, the serotonin and cortical somatostatin systems are affected in AD but do not appear to change significantly in normal aging. On the other hand, the nigrostriatal DA system is not more severely affected in the majority of AD cases than in controls of comparable age, although some 25% of AD cases do appear to have concomitant PD pathology.

Another aspect of brain chemistry worth noting is the slow but significant drop in cortical glucose metabolism with age. This may be largely a reflection of brain atrophy with aging, although slow drop-out of cortical neurons may play a part. The much steeper fall in cortical glucose metabolism with time in AD cases, which has been revealed by positron emission tomography (PET) (8), appears to be primarily a result of the rapid death of cortical neurons in this disease, particularly of the large pyramidal neurons which probably use glutamate as a transmitter (9).

The increasing evidence for the importance of neurotrophic factors in preserving neurons from death and of immune system elements in many degenerative neurological diseases (10) suggests that these aspects of brain chemistry should receive more attention by scientists interested in the effects of normal aging. As one example of work in this field, we investigated possible changes in basic fibroblast growth factor (bFGF) in the SN in normal aging in humans. This factor had been shown immunohistochemically to be in DA neurons of the SN in a number of species and to have protective effects on these neurons in culture or in lesioned animals (11). We reported (12) immunohistochemical studies suggesting that loss of bFGF precedes death of DA neurons in PD. In more recent work (11), a count of pigmented neurons per mm^3 in sections of the SN at the level where the oculomotor nerve emerges in 11 neurologically normal controls aged 15 to 82 showed the expected slow loss of such neurons with age. Most (82±3.8%) of the pigmented neurons showed immunoreactivity for bFGF, and this percentage was uaffected by age. This is in marked contrast to the case in PD where only some 12.7±2.6% of the remaining dopaminergic neurons showed bFGF-like immunoreactivity, providing further evidence against the hypothesis that PD is due to some early insult followed by age-related attrition of the remaining neurons.

Much research remains to be done on the effects of normal aging on the brain, particularly in humans, and it is to be hoped that the many new techniques that are emerging in brain imaging during life and in nucleic acid and protein biochemistry will help to define these effects.

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ALTERATIONS IN GABAERGIC AND CHOLINERGIC SYNAPTIC TRANSMISSION IN THE AGED RAT HIPPOCAMPUS

LAMOUR Y., POTIER B., BILLARD J.M., DUTAR P.

INSERM U 161, 2 rue d'Alésia, 75014 Paris, France.

It is known that learning and memory deficits are observed in a sub-population of aged rodents. For instance some aged rats do have a deficit in the performance of a spatial learning task such as the "water maze". There is some evidence that this deficit is linked to alterations in the functions of the hippocampal formation. In other words, if aged rats have a spatial memory deficit, it might be due to changes in hippocampal neuronal circuitry. However the study of age-related alterations in hippocampal neuronal networks have yielded conflicting results. Some evidence of enhanced calcium-related events has been obtained, such as an increased duration of the afterhyperpolarizing potentials and of the calcium spike in the CA1 pyramidal neurons (1). These observations suggest that subtypes of neuronal calcium currents might be increased in the aged rat. These observations, however, have not been reproduced in all types of hippocampal neurons.

We studied the properties of the CA1 hippocampal pyramidal neurons in the aged (26-28 month-old) Sprague-Dawley rat, as compared to young (2-3 month-old) adult rats, using intracellular recordings in the hippocampal slice preparation. Many neuronal properties (membrane potential, input resistance, amplitude of sodium or calcium spikes, amplitude and duration of afterhyperpolarization -AHP-) were not altered in the aged rat. In contrast neuronal excitability was decreased, and the spike duration was increased. Synaptic events, as well as the pharmacological properties of the hippocampal pyramidal neurons were also altered. The amplitude of the cholinergic slow EPSP induced by electrical stimulation of the afferent cholinergic fibers was dramatically decreased. The effects of the cholinergic agonist carbachol were reduced in the aged rats. The amplitude and the duration of the slow IPSP, due to the action of GABA on GABA-B receptors, were also dramatically reduced (2).

In a subsequent series of experiments we sought to determine if the alterations observed in the aged Sprague-Dawley rats were also present in other strains of rats. The strain comparison (Sprague-Dawley,Wistar and Fischer 344) revealed that several age-related alterations were found in the three strains (decrease in membrane excitability, decrease in the effects of the cholinergic agonists, decrease in the amplitude and the duration of the slow IPSP). In contrast no consistent

age-related changes in calcium-dependent events (calcium spike or **AHP)** were observed among strains (3).

We then investigated the mechanism of the age-related changes in the slow IPSP recorded from CA1 pyramidal neurons. The amplitude of the baclofen-induced hyperpolarization (GABA-B mediated) was not modified in the aged rats, and therefore the post-synaptic mechanisms are probably intact. The alternative possibility is a presynaptic alteration: the release of GABA from the GABAergic interneurons may be decreased, or the interneurons may even be lost. The first possibility was tested using a protocol of paired-pulse depression. At interstimulus intervals below 1 second the inhibitory components following the second stimulus are depressed, and the degree of depression was used as an index of GABA release. The paired-pulse depression of the slow IPSP was significantly reduced in the aged rat. The paired-pulse depression of the fast IPSP was however not altered, suggesting that an alteration in GABA release is not the most likely explanation. We then used immunohistochemistry to identify alterations in the interneurons responsible for the slow IPSP. There is some evidence that these interneurons contain the calcium-binding protein calbindin. We counted the number of calbindin-positive interneurons in the fields CA1 to CA4 of the hippocampus. The number of calbindin-positive interneurons was significantly decreased in the aged rat. These observations are consistent with either a true neuronal loss, or a decrease in gene expression (4).

In summary our results provide evidence that alterations in the properties of the hippocampal pyramidal neurons are consistently found in the aged rat: decreased neuronal excitability, decreased sensitivity to cholinergic agonists and decrease in the amplitude and duration of the slow, GABA-B mediated, IPSP. Our results suggest that the alteration of the slow IPSP might be due to a functional impairment or a loss of a specific population of GABAergic interneurons containing the calcium-binding protein calbindin.

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CHANGES IN NEUROTRANSMITTER SIGNAL TRANSDUCTION DURING AGING. George S. Roth, National Institute on Aging, NIH, Francis Scott Key Medical Center, Baltimore, MD 21224 U.S.A.

Changes in hormone/neurotransmitter signal transduction are responsible for altered control of many physiological and behavioral functions during aging. Such changes occur at both the receptor and post-receptor levels.

An example of the former is the striatal dopaminergic system. Loss of dopaminergic motor control during aging is al least partially due to 30-50% reductions in concentrations of striatal D_2-dopamine receptors. Such decrements have been demonstrated in species ranging from rodents to humans and result from both loss of receptor containing neurons and reduced receptor synthesis in the surviving neurons. The latter is, in turn, due to age related reductions in D_2 receptor mRNA levels and decreased rates of D_2 receptor mRNA transcription.

An approximately 20% loss of receptor containing neurons accounts for roughly half the D_2 receptor loss. these cells have now been directly quantiated using oligonucleotide probes for the D_2 receptor mRNA. Neurons of all sizes are lost with age with greatest decrease in those $<100\ \mu m^2$ in area. Relative D_2 mRNA content per cell also decreases such that the number of neurons with low mRNA concentrations actually increases with age. Neuronal loss is largely associated with cells susceptible to kainic acid toxicity in vivo. These neurons can be cultured from striata of neonatal rats and demonstrate similar selective vulnerability to kainate as well as the endogenous neurotransmitter, dopamine. Such cell death is believed to result from oxygen radical damage. Thus, D_2 receptor loss during aging may be due to altered transcriptional regulation and the toxic effects of normal neurotransmission, mediated by free radicals.

Post receptor age changes in signal transduction of G protein linked receptor systems seem to occur mainly in the coupling/uncoupling of receptors and G proteins. Examples include alpha-adrenergic stimulation of salivary gland secretion and muscarinic-cholinergic stimulation of striatal dopamine release. Both are dependent on IP_3 generation and calcium mobilization. If receptors are bypassed by direct stimulation of G proteins, phospholipine C or calcium mobilization, no age deficits occur. Normal shift from high to low affinity receptor states in response to GTP and its analogues are deficient in aged parotid and striatum and respective stimulation of GTPase activity by alpha-adrenergic and muscarinic-cholinergic agonists is greatly reduced as well. Both affinity shift and stimulation of GTPase are indices of receptor-G protein uncoupling and coupling.

Coupling/uncoupling deficits appear to be secondary to changes in the membrane environment since it has been possible to modulate responsiveness in the above systems by treatment with membrane active agents including detergents, alcohols, hydrogen peroxide and s-adenosyl methonine. Such plasticity may offer a potential therapeutic strategy to ameliorate age associated dysfunctions in physiological and behavioral regulation by hormones and neurotransmitters.

EXCITOTOXIC MECHANISMS AND CEREBROPROTECTION BY GLUTAMATE ANTAGONISTS

Brian S Meldrum
Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF

Activation of glutamate receptors contributes critically to the pathological outcome in cerebral ischaemia, *Status epilepticus* and cerebral trauma. The relative involvement of NMDA and non-NMDA receptors varies according to the stress and the brain region involved. The sequence of events leading to cell death usually includes an early increase in cytosolic calcium concentration. This activates a variety of enzymes including proteases acting on structural proteins, various phosphatases and protein kinases controlling enzyme activity and receptor and ion channel function, phospholipases which among other things liberate free fatty acids including a arachidonic acid which serves as a precursor for various active compounds, and nitric oxide synthase which by the formation of nitric oxide influences vascular tone and may also produce direct neuronal toxicity (Meldrum & Garthwaite, 1990).

Antagonists acting on glutamate receptors protect against nerve cell loss or cortical infarction in models of global and focal ischaemia (Meldrum 1990, 1992). In severe transient global ischaemia (20 minutes of 4 vessel occlusion) NMDA receptor antagonists are not protective. In less severe global ischaemia or in intermittent global ischaemia powerful protective effects are seen. Non-NMDA antagonists such as NBQX and GYKI 52466 are protective with delayed post-ischaemic administration provided the ischaemic stress is close to threshold for damaging that brain region. They are not protective against hippocampal CA1 damage, for example, when the global ischaemia lasts 20 min. In focal models of ischaemia, such as middle cerebral artery occlusion in the rat, NMDA receptor antagonists of all types (ie, competitive acting on the glutamate recognition site, competitive acting on the glycine recognition site, non-competitive acting on the channel, and polyamine site antagonists), (Meldrum 1992) and non-NMDA antagonists are potently protective against cortical infarction provided they are given within 60-90 min of onset of focal ischaemia (Smith & Meldrum 1992, 1993). Compounds of the lamotrigine type that act on sodium channels and block the ischaemia-induced release of glutamate and aspartate are protective in global ad focal models of ischaemia (Meldrum et al., 1992). They are protective with delayed administration in global ischaemia under circumstances where they do not decrease glutamate release. The therapeutic prospects for compounds acting on glutamate release or receptors appear good in head injury and cerebral ischaemia.

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"GOOD" AND "BAD" SIGNAL TRANSDUCTION CASCADES IN THE AGING AND DISEASED BRAIN. Mark P. Mattson, Sanders-Brown Research Center on Aging and Department of Anatomy & Neurobiology, University of Kentucky, Lexington, KY 40536, U.S.A.

Alterations in brain structure and chemistry that occur in aging and neurodegenerative conditions are a combination of the neurodegenerative process and neuroprotective response mechanisms. It is therefore important to determine which changes in the brain represent "good" changes and which are "bad". For example, despite the important progress in understanding the molecular alterations present in the neurofibrillary tangles of Alzheimer's disease (AD), it is unclear which of the alterations contribute to the neurodegenerative process and which alterations represent neuroprotective mechanisms. The microtubule-associated protein tau is the major component of the straight and paired-helical filaments in the neurofibrillary tangles. Tau is excessively phosphorylated in tangles and recent findings indicate that MAP kinases are likely candidates for eliciting the specific phosphorylation pattern of AD tau (4). Interestingly, MAP kinases can be activated by an array of environmental signals which may have either neurotoxic (glutamate and calcium) or neuroprotective (neurotrophic factors and tyrosine kinase activity) properties (1). If MAP kinases are responsible for tau hyperphosphorylation in AD, then it is clearly important to establish whether MAP kinase activation is part of an excitotoxic mechanism of neurofibrillary degeneration (6,9,10) or a neuroprotective signaling cascade (studies in progress).

Recent findings indicate that the brain possesses a remarkeable array of signaling mechanisms that have evolved to protect neurons against environmental insults. In response to injury, numerous growth factors and cytokines are mobilized including FGFs, IGFs, NGF, BDNF, TNFs and others. Cell culture and in vivo studies have shown that such "neuroprotection factors" protect neurons against metabolic, excitotoxic, and free radical-mediated insults (see ref. 15 for review). At least some of these neuroprotection factors can attenuate neurofibrillary tangle-like alterations in cytoskeletal proteins (tau, MAP-2, spectrin, ubiquitin) induced by overstimulation of excitatory amino acid receptors (2,9). Whereas many of these factors seem to affect particular subsets of neurons during developmental plasticity, they may be recruited to influence a more diverse array of neurons in response to brain injury. The signal transduction pathways for these different growth factors involve cascades of protein tyrosine kinases and, interestingly, several bacteria-derived alkaloid compounds activate similar kinase pathways and mimick the neuroprotective actions of the endogenous growth factors (3).

Increases in levels of protein oxidation have been shown to occur in normal aging and to a greater extent in AD (22). Although the cause of the increased oxidative damage is not firmly established (e.g, reduced antioxidant enzyme levels, increased ischemic conditions), it likely contributes to the pathogenesis of a number of neurodegenerative conditions in which age is a major risk factor. In addition to free radicals, calcium is believed to play a major role in neurodegenerative conditions (14,15). Age-related metabolic impairment may lead to failure of energy-dependent calcium extrusion systems, excess glutamate release, overstimulation of glutamate receptors, and prolonged elevation of intraneuronal calcium levels. In light of the increasing evidence that elevated levels of intracellular calcium and free radicals play major roles in the neuronal injury and death that occurs in a variety of age-associated neurodegenerative conditions, we have begun to examine the actions of neuroprotection factors on neuronal systems that regulate calcium and free radicals. In essentially every case studied the neuroprotection factors promote maintenance of calcium homeostasis and/or enhance antioxidant enzyme systems (15). For example, bFGF can suppress the expression of an NMDA receptor protein, increase the expression of the 28 kDa calcium-binding protein calbindin, and increase levels of the antioxidant enzymes superoxide dismutase and glutathione reductase in cultured neocortical neurons. Interstingly, several different neuroprotection factors (NGF, BDNF, NT-3, and IGF-1) upregulate the antioxidant enzyme levels in this same neuronal population.

In AD, the amyloid β-peptide (Aβ) accumulates as diffuse (soluble Aβ) and compact (aggregated Aβ) plaques, only the latter of which are associated with degenerated neurons displaying neurofibrillary pathology. Recent in vitro studies have shown that while soluble Aβ seems innocuous to neurons, aggregated Aβ can be directly neurotoxic and can render neurons vulnerable to excitoxicity (11,14,20). The mechanism of Aβ toxicity involves actions at the plasma membrane that disrupt cellular Ca^{2+} homeostasis and promote free radical production (7,14). Studies of Aβ suggest that a "seeding" process may occur in AD. Using EPR analysis we recently discovered that Aβ itself can be a source of free radicals generated during the process of transformation from a soluble to an aggregated state (8). In vitro studies showed that radicals arising from Aβ can directly damage both soluble cellular enzymes and membrane-associated proteins. This mechanism of neurotoxicity is not unique to Aβ because other amyloidogenic peptides including human amylin, β2-microglobulin, and prion protein appear to be neurotoxic by a similar mechanism (18 and M. P. Mattson, unpublished data). Moreover, the infectivity of the prion protein is apparently due the fact that the mutated prion protein readily aggregates and can induce endogenous cellular prion protein to aggregate. The increased levels of oxidation that occur in the aging brain probably contribute to aggregation of Aβ since free radicals can promote aggregation of Aβ (5).

Despite the increasing evidence that, as the source of Aβ, the β-amyloid precursor protein (βAPP) plays a "bad" role in the pathogenesis of AD, recent findings suggest that βAPP itself serves a neuroprotective function. βAPP is a transmembrane glycoprotein with a large extracellular N-terminus that contains several biologically active domains (see refs. 16 and 21 for review). A major processing pathway of βAPP involves an enzymatic cleavage in the middle of the Aβ sequence; this pathway precludes Aβ deposition and results in release of secreted forms of βAPP (APP^S) from the cell surface. Recent cell culture and in vivo studies have shown that APP^S can protect hippocampal neurons against excitotoxic/metabolic insults (12,23). The increased expression of βAPP in brain injury is likely part of a protective mechanism. APP^S attenuate calcium responses to glutamate, an action which may play roles in dendritic plasticity during development and at synapses in the mature nervous system (17). Mutations of βAPP which are linked to inherited forms of AD may lead to increased deposition of Aβ, as well as compromising a normal beneficial activity of APP^S.

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ß-AMYLOID AS A RISK FACTOR IN ALZHEIMER'S DISEASE: INDUCTION OF APOPTOTIC NEURONAL DEATH. Carl W. Cotman, Ph.D., Professor of Psychobiology & Professor of Neurology, University of California, Irvine

It has been clear for some time that during development massive numbers of neurons, perhaps as many as one half, degenerate by a pathway of programmed cell death (apoptosis). It has only recently been recognized that apoptosis may also contribute to neurodegenerative diseases such as Alzheimer's disease (AD). In AD, a peptide called ß-amyloid (Aß) accumulates in the AD brain. We and others have shown that Aß causes cultured neurons to degenerate, raising the intriguing possibility that Aß may contribute to neurodegeneration in AD. Further, we have hypothesized and obtained evidence to support the notion that Aß causes neurons to die by an apoptotic pathway. That is, Aß-treated neurons maintained in culture display hallmarks of apoptosis including membrane blebbing, chromatin condensation, and DNA fragmentation into oligonucleosome-length fragments. Initiation of Aß induced apoptosis appears to depend on the assembly state of Aß. Aß spontaneously assembles into small aggregates over time. When Aß 1-42 aggregates into small visible particles, neurons bind these particles and a sequence of events is initiated which eventually destroys the neurons. A series of active structural analogues all share the common feature that they must assemble into aggregates (ß structure) in order to initiate degeneration. Thus a particular form (assembly state) of Aß appears to be the primary risk factor. In cultured hippocampal neurons, one of the earliest neuronal changes after Aß treatment is the loss of neuritic processes and the generation of somal membrane blebs. The blebbing process probably accounts for the visible reduction in cell body size. Polyribosomal breakdown and the appearance of large cytoplasmic vesicles are also a prominent feature of Aß induced degeneration. Following the initial cytoplasmic changes, a set of nuclear events become evident. Within the first several hours the chromatin begins to condense, the nuclear membrane invaginates, and in some cells the nucleus fragments into multiple bodies. Along with these morphological changes, DNA fragmentation occurs resulting in a distinct DNA ladder. Throughout the stages of the process, mitochondria do not swell and appear to show little, if any, change. These *in vitro* data support the hypothesis that Aß induces neurons to undergo apoptosis and predict that apoptosis may occur in the human brain during AD. We will discuss the various lines of evidence that suggests that apoptosis may indeed occur *in vivo* during aging, AD, and perhaps other neurodegenerative diseases.

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PURINERGIC MECHANISMS IN GLIA-NEURONAL INTERACTIONS AFTER BRAIN INJURY AND IN NEURODEGENERATIVE DISORDERS.

Michel P. Rathbone, Pamela J. Middlemiss, John W. Gysbers,
Shaun Hindley and Bernhard H.J. Juurlink*.

Departments of Biomedical Sciences and Medicine, McMaster University Health Sciences Center,
1200 Main Street West, Hamilton, Ontario, L8N 3Z5 Canada,
and *Department of Anatomy, University of Saskatchewan, Saskatoon, S7N 0W0 Canada.

Purine nucleotides, (*e.g.* AMP, ADP, ATP, GMP, GDP, GTP) and nucleosides, (*e.g.* adenosine, guanosine and inosine) are released from neurons and act as neurotransmitters and neuromodulators(1). Additionally, after injury or cell death large quantities of purine nucleosides and nucleotides are released extracellularly(2). These purines interact with specific purinergic receptors on the surface of several types of cells, including neurons, astrocytes and microglia, to produce a variety of physiological effects. We have reported that purine nucleosides also had trophic effects on glia, stimulating proliferation of astrocytes and microglia *in vitro* (3,4,5). Guanosine, GMP, GDP and GTP each stimulated astrocyte and microglial proliferation to a greater extent than did the corresponding adenine-based compounds. This was surprising since previously-described purine receptors respond preferentially to adenosine and its derivatives.

Both microglia and astrocytes become reactive after central nervous system (CNS) injury, at a time when extracellular purine concentrations are high. Therefore we proposed that extracellular purine nucleosides and nucleotides might play a role in the responses of these cells to brain or spinal injury(2). After CNS injury, including diffuse cell death in degenerative diseases, both microglia and astrocytes release protein growth factors that affect several cell types, including the surviving neurons. Therefore we determined whether the extracellular purines stimulate elaboration of such factors by astrocytes. Our initial data demonstrate that both guanosine and GTP stimulate cultured astrocytes to release large amounts of a NGF-like immunoreactive substance into the culture medium. We are currently investigating which of the neurotrophins (*e.g.* NGF, NT-3, BDNF) or pleiotrophins (*e.g.* bFGF, S-100) are released in response to guanosine or GTP. These data indicate that purines may affect neurons indirectly by stimulating release of trophic factors from glia.

We next determined whether extracellular purines might also affect surviving neurons directly. We tested the effects of extracellular purines on PC12 cells, a rat pheochromocytoma cell line, that contains no contaminating glia. When treated with nerve growth factor (NGF) PC12 cells stop dividing, and differentiate into sympathetic-like neurons(6). Addition to the culture medium of either guanosine or GTP, but not GMP or GDP, stimulated outgrowth of neurites from PC12 cells. GTP and guanosine also synergistically enhanced the neuritogenic effects of NGF. Surprisingly, either adenosine or ATP suppressed NGF-stimulated neurite outgrowth. The neurites formed in response to either guanosine, GTP or NGF had the immunohistochemical characteristics of axons; they did not stain for MAP2, normally found in dendrites, but did stain with SMI 31 antisera against phosphorylated epitopes on neurofilaments found predominantly in axons.

Inhibitors of nucleoside uptake, nitrobenzylthioinosine and dipyridamole failed to abolish the neuritogenic effects of guanosine, indicating that it likely acted at the cell surface. Inhibitors of purine nucleoside (P_1) receptors did not reduce the neuritogenic effects of guanosine, indicating that it did not act through known P_1 receptors. We questioned whether the effects of guanosine were transduced by alterations in intracellular cyclic nucleotide levels. Extracellular guanosine stimulated an increase in intracellular cyclic GMP in PC12 cells from 0.6 to 1.25 pmol/mg protein. The neuritogenic activity of guanosine on PC12 cells appeared related to its effects on cGMP since Methylene Blue, an inhibitor of soluble guanylate cyclase, reduced the neuritogenic effects of guanosine, but not those of NGF. In many cases, extracellular

effectors that elevate intracellular cGMP do so through stimulating nitric oxide synthase. This releases nitrogen monoxide (NO), a diffusible unstable gas, that can affect several enzymes. Notably NO activates soluble guanylate cyclase thereby stimulating cGMP synthesis. Substances that released NO into the culture medium such as sodium nitroprusside and sodium nitrite, mimicked the effects of guanosine by stimulating neurite outgrowth. Hemoglobin (Hb) binds NO avidly. When added to the culture medium it reduced the neuritogenic effects of guanosine but not those of NGF. But addition to the PC12 cell cultures of Nω-nitro-L-arginine, an NO synthase inhibitor, did not reduce the neuritogenic effects of guanosine. Therefore we questioned whether the effects of guanosine might be mediated by stimulation of heme oxygenase (HO) to synthesize carbon monoxide (CO), a gas similar to NO. In support of this the heme oxygenase inhibitor zinc protoporphyrin IX (ZnPP) abolished the neuritogenic effects of guanosine but not those of NGF.

The neuritogenic effects of GTP were mediated by different mechanisms from those of guanosine. If GTP was simply converted to guanosine by ectonucleotidases, then GDP and GMP would also have been active. They were not. Moreover, GTP and guanosine appeared to employ different signal transduction mechanisms. The neuritogenic effects of GTP were not affected by Hb, Methylene Blue or ZnPP. Since extracellular nucleotides are not readily internalized by cells, GTP likely exerted its effects at the cell surface. The purine nucleotide (P_2) receptor antagonist Reactive Blue 2 did not inhibit the effects of GTP. Moreover, ATP, which is usually a much more effective P_2 receptor agonist than GTP, inhibited rather than stimulated neurite outgrowth. These data indicated that the effects of GTP were not mediated by hitherto-identified P_2 receptors. Analogs of GTP resistant to hydrolysis, including GTPγS, did not stimulate neurite outgrowth. This may imply that the neuritogenic action of GTP involves hydrolysis of its γ phosphate by ecto-GTPases, possibly by GTP-dependent kinases.

The neuritogenic effects of guanosine and GTP were not limited to PC12 cells. These compounds each stimulated neurite outgrowth from primary cultures of hippocampal neurons that, unlike PC12 cells, are not NGF-responsive. Histochemical analysis of the cultures demonstrated that most of the neurons were GABA-positive. When treated with GTP or guanosine the response of hippocampal neurons differed from that of PC12 cells in three ways. First, the concentrations of GTP or guanosine required to produce neurite outgrowth were 1 to 10 μM - 1 to 2 orders of magnitude lower than those required to produce neurite outgrowth from PC12 cells. Second, the extent of neurite outgrowth in response to guanosine or GTP was much greater in hippocampal neurons than in PC12 cells. Finally, the neurites from hippocampal neurons had characteristics of dendrites, whereas those from PC12 cells had characteristics of axons.

These data demonstrate that extracellular guanosine or GTP, released extracellularly after cell injury or death in the nervous system, may interact with astrocytes and microglia to stimulate their proliferation and to release protein growth and trophic factors. GTP and guanosine may stimulate collateral sprouting of neurites in surviving neurons (i) by direct action on the neurons, (ii) by enhancing the action of growth factors produced by the glia and (iii) by enhancing release of trophic factors from glia.. Guanosine and GTP each produce these effects through distinct biochemical mechanisms.

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Downregulated p140^{trk} expression, reduced NGF transport and increased vulnerability of forebrain cholinergic neurons in aged rats

J.D. Cooper[1,2], D. Lindholm[1] and M.V. Sofroniew[2].
[1]Max-Planck-Institute for Psychiatry, Department of Neurochemistry, Am Klopferspitz 18A, 82152 Martinsried, Munich, Germany.
[2]Department of Anatomy, University of Cambridge, Downing Street, Cambridge, CB2 3DY, UK.

Aging of the central nervous system (CNS) is accompanied by neurodegenerative changes and functional deterioration the underlying cellular and molecular causes of which are not well understood. Administration of exogenous nerve growth factor (NGF) can reverse both the atrophy of basal forebrain cholinergic neurons and an associated cognitive deficit in behaviourally impaired aged rats, suggesting that loss of target-derived neurotrophic support plays a causative role in these changes. Since the target regions of these neurons do not show an age-related reduction in NGF production we looked for neural changes which might compromise neurotrophic support in aged animals in spite of unchanged target neurotrophin levels. Here we report a 43% decline in relative levels of mRNA encoding high affinity (p140^{trk}) but not low affinity (p75^{NGFR}) NGF receptor, as well as a 31% reduction in the number of cholinergic neurons which take up and retrogradely transport ^{125}I-labelled NGF from the hippocampus to the septal region in aged rats. Cholinergic neurons not transporting NGF were severely atrophied. In addition, septal cholinergic neurons in aged rats showed an increased vulnerability to partial loss of target tissue by markedly down-regulating intracellular levels of the transmitter-synthesizing enzyme, choline acetyltransferase (ChAT) in a manner which did not occur in young adult rats with equivalent target lesions. Together these findings suggest that the age-related atrophy and dysfunction of forebrain cholinergic neurons may result from an intrinsic reduction in their capacity to sustain receptor mediated uptake and retrograde transport of target-derived neurotrophin.

REGULATION OF BASIC (bFGF or FGF-2) AND RECEPTOR IN BRAIN PLASTICITY.

Fernando Gómez-Pinilla, Eeke A. van der Wal and Carl W. Cotman. Dept. of Neurology and IRU in Brain Aging, University of California, Irvine, California.

Increasing evidence indicates that FGF-2 is a potent neurotrophic factor for neurons and astrocytes throughout life. Studies were centered on the anatomical distribution and mechanism of regulation of FGF-2 in normal brain function and during pathology. Double staining immunohistochemistry shows that FGF-2 is abundant in the brain primarily localized in astrocytes. A small lesion in the entorhinal cortex which triggers a powerful sprouting in the hippocampus, also up-regulates astrocytic FGF-2 within the sprouting areas. Parallel studies in the hippocampus from Alzheimer's disease (AD) victims, which also exhibit a partial deafferentation of the hippocampus, showed an increase of FGF-2 in sprouting regions of the dentate gyrus. The strongest FGF-2 immunoreactivity in AD was in senile plaques surrounded by FGF-2 positive astrocytes. These astrocytes appear to be the source of FGF-2 to plaques and sprouted axons. These studies indicate that FGF-2 is regulated by injury, that up-regulated FGF-2 may be involved in axonal sprouting, and that FGF-2 may facilitate plaque formation in AD pathology.

In order to further investigate the mechanism of regulation of FGF-2 and its receptor, we tested the possibility that the FGF system could be modulated by neural activity. We induced seizure activity in rats by kainic acid injection and studied the time course of FGF-2 and FGFR-1 expressions in the hippocampus and cerebral cortex. Brain tissue was processed in parallel for immunohistochemistry and *in situ* hybridization. Following 6 h after KA injection, seizure rats showed an up-regulation of FGF-2 and FGFR-1 and their mRNAs in particular hippocampal regions. The main region involved was the dentate gyrus of the hippocampus. Our results show that the FGF-2 system is regulated by brain injury and by afferent activity, suggesting that it may play an important role in brain plasticity in normal brain function and disease.

Neuron-Glia Interaction

Structural and quantitative changes in glial cells in the aging mouse brain.

R. R. Sturrock, Department of Anatomy and Physiology, University of Dundee, DD1 4HN

Different types of glial cells show different types of age related structural changes. Oligodendrocytes appear to be the least affected with age with lipofuscin being present only in small quantities and being more common in satellite oligodendrocytes than in oligodendrocytes in white matter. Astrocytes contain variable amounts of lipofuscin which is usually of the pigment predominant type in contrast to microglial cells where the lipid predominant type of lipofuscin is more common!

The most striking age related structural feature is the presence of large honeycomb bodies in the fluorescent granular perithelial cells which are found in close association with arterioles or venules. Structurally similar granular cells are found in the pia mater and also contain honeycomb bodies in the ageing brain.

Microglial cells show no evidence of phagocytic activity in the normal ageing mouse brain in contrast to the closely related intraventricular macrophages. From 25 months of age many phagocytic macrophages are present on the surface of the choroid plexus and in some cases their cytoplasm contains very large amounts of phagocytosed material. Subarachnoid space macrophages rarely show any evidence of phagocytosis. Apart from an occasional lipofuscin granule leptomeningeal cells exhibit no structural change with age.

Choroid plexus epithelial cells have a shrunken appearance in aged brains but it is not clear if this is in part artefactual. In light microscopic sections stained with Lapham's stain there is a thin layer on the ventricular surface of the choroid plexus with similar staining properties to that of the basement membrane. This layer largely disappears between 22 and 25 months of age although the staining of the basement membrane remains unchanged.

Quantitative studies of age related changes in neuroglia have been carried out in a number of regions of the ageing mouse brain using the same sets of serial sections. No obvious pattern of age related change in number has been found. In both the motor nucleus of the trigeminal and facial nerve nucleus neuroglial number remained constant from 6 to 31 months of age despite a decrease in neuron number between 28 and 31 months. Neuroglial number declined in the retrofacial nucleus at an even greater rate than loss of neurons. A decline in neuroglial number was also found in the neostriatum between 25 and 28 months of age.

In contrast there was a substantial increase in neuroglial number with age in both the indusium griseum and anterodorsal thalamic nucleus despite neuron number remaining constant. Both neuron and neuroglial number remained constant from 6 to 31 months of age in the lateral mammillary nucleus, the supraoptic nucleus, the parabigeminal nucleus and the abducent nucleus.

Intraventricular macrophages showed a dramatic increase in number between 22 and 25 months of age which correlated with the light microscopic change in structure of the choroid plexus.

Detailed studies of cell division and cell death in the anterior commissure, indusium griseum, neostriatum, parabigeminal nucleus and subependymal layer showed that mitotic and pyknotic cells were present in all these regions at all ages. The greatest amount of mitotic activity was found in the subependymal layer and this was the only region where mitotic cells outnumbered pyknotic cells. The mitotic index was lowest in white matter. Intraventricular macrophages also undergo mitosis throughout life but although every serial section containing the choroid plexus was examined in three brains at 6, 15, 22, 25, 28 and 31 months no mitotic choroid plexus epithelial cell was ever observed.

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The Microglial Response to Central Nervous System Aging

V. Hugh Perry, Department of Pharmacology, University of Oxford, Mansfield Road, Oxford OX1 3QT

In the developing central nervous system (CNS) many cells, both neurons and macroglia, undergo natural cell death. During this time the CNS is invaded by monocytes from the blood. These macrophages are phagocytic cells and are involved in the removal of the degenerating cells and their processes. Ultrastructural analysis of these macrophages and immunocytochemical analysis of molecules expressed by them reveals that they express many features typical of other tissue macrophages. However, during CNS maturation the macrophages take on a distinctive morphology as they differentiate to become microglia, the resident macrophages of the CNS. The morphological differentiation to microglia is accompanied by a pronounced alteration in phenotype and many cell surface and cytoplasmic antigens are greatly reduced or downregulated. The factors in the CNS microenvironment that are responsible for this phenotypic downregulation are poorly understood. We have established that exclusion of plasma proteins by the blood brain barrier is involved (Perry et al, 1992). In addition, recent evidence showing that microglia may be dramatically activated and induced to proliferate via the complement type 3 receptor suggests that adhesion to a ligand within the CNS parenchyma is involved (Reid et al, 1993).

In young adult rodents the downregulated phenotype is a consistent feature and there is a notable lack of MHC class I and Class II antigens. We have studied the microglia in healthy but aged rats. In the CNS of these animals, raised under the same standard laboratory conditions as their young counterparts, there is evidence for activation and upregulation of the resident microglia. They are found to express readily detectable levels of MHC antigens and in addition they express lysosomal antigens at higher levels than in young animals (Perry et al, 1993). These observations on the antigenic phenotype are consistent with ultrastructural observations showing that microglia in the CNS of aged rodents and primates have phagocytosed debris and appear to be more active cells than in immature animals (Peters et al, 1991). It is known that there is cell loss and neuronal degeneration in the aging rodent CNS but the regions of microglial activation were not limited to

these structures (Perry et al, 1993). It is possible that repeated subclinical infections may lead to microglial activation. In the human CNS the situation is less clear. Whether microglia in human tissue are constitutively more activated than those in rodents or whether this is an age related phenomenon is debatable.

The mechanisms leading to the activation of microglia in the aged brain deserve investigation since this may not only shed light on the mechanisms by which the microglia are normally regulated but also may have consequences for the initiation of an inflammatory response in the CNS. It has been shown that the inflammatory response in the CNS following acute neuronal degeneration or challenge with various inflammogens is quite unlike that in other tissues (Perry et al, 1993). We have proposed that the unusual kinetics of the myelomoncytic response in the CNS parenchyma is in part related to the downregulated phenotype of the microglia. Activation of the resident cell population, such as is seen in aging may influence the outcome of an inflammatory response within the CNS parenchyma.

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Glial Environment and CNS Degeneration and Regeneration

Manuel Nieto-Sampedro, José Abad, Isabel Fernaud-Espinosa and Paola Bovolenta.
Department of Neural Plasticity, Instituto Cajal CSIC, 28002-Madrid, Spain

The adaptive response of the central nervous system (CNS) to perturbations, is usually described as CNS plasticity. CNS plasticity arises from cellular and molecular interactions in glia-neuron ensembles[1] and, experimentally, is most reliably evoked by neural damage. Depending on whether or not the *glia limitans* is disrupted, two general types of lesion may occur. Anisomorphic lesions, occur when the *glia limitans* is locally destroyed, typically by mechanical damage. Invasion of the CNS by blood components ensues, quickly followed by astrocyte activation and division, whereas endogenous microglial cells seem little affected . In contrast, isomorphic lesions, e.g. neurotoxic or ischemia lesions, do not affect directly the *glia limitans* and evoke microglial activation and division. Microglial cells contributed to a large extent and for prolonged periods of time to isomorphic gliotic tissue. Astrocytes became reactive around the site of isomorphic neuronal loss, but not at the lesion site itself, which was invaded by reactive astrocytes only about two weeks postlesion[2].

These differences in lesion response were independent of the brain region damaged and correlated with the injury-evoked increase in CNS growth factor activities. The time-course and magnitude of the increase in CNS neurotrophic and neuritogenic activities evoked by injury, was strongly influenced by the age of the subject and the type of injury, and seemed to depend on astrocyte proliferation[1]. Suppression of astrocyte division, prevented the increase in trophic activity. Thus, trophic response to injury may be under the control of inhibitors of glial proliferation. The existence in brain of inhibitors of astrocyte division, the concentration of which strongly diminished after injury, was demonstrated[3] and was recently purified and characterized[4]. It is a cytostatic glycolipid, capable of inhibiting the proliferation of both astrocytes and microglia with ID50's ranging from 0.1 to 0.2 μM. Its effect on trophic factor production by glia remains to be examined.

Isomorphic gliotic tissue contained increased amounts of a neurite outgrowth inhibitory proteoglycan[5]. This inhibitor, probably located in reactive microglial membranes[6], prevented sprout initiation and repelled or caused the collapse of initiated sprouts. Both, neurite outgrowth promoters and inhibitors were present concomitantly in both normal and gliotic tissue. However, inhibition was favored in isomorphic gliosis, whereas promotion was primed in normal tissue or after anisomorphic damage.

The changing properties of neuron-glia ensembles after a sufficient proportion of glial cells have become reactive, may be used to explain Alzheimer's disease (AD) progression[7]. AD neurodegeneration is formally an isomorphic lesion and would cause the corresponding gliosis response. We assumed that AD is initiated by axonal transport failure subsequent to anomalous tau phosphorylation[7], such failure leading to arrest of synaptic transmission at the affected nerve endings Axon sprouting and reactive synaptogenesis cannot restore the lost synaptic contact, being prevented by the neurite outgrowth inhibitor present in reactive microglial membranes. Synapse loss would become chronic, eventually extending to a sufficient proportion of the nerve endings, until synaptic output of the neuron becomes negligible. This situation, formally similar to axotomy and called "functional axotomy"[7], would be signaled to microglia surrounding input synapses on the axotomized neuron. Reactive microglial pseudopods would interpose themselves between the pre- and postsynaptic moieties and "strip" the axotomized neuron of synaptic input[8]. These microglial cells, capable of producing APP in amounts comparable to neurons[9] and preferentially processing it through an amyloidogenic pathway, would favor the formation of amyloid plaques with microglial core. New β amyloid deposits, left by reactive glia now one synapse relay further, would result in new nerve ending disfunction[7]. The process would be repeated, sequentially affecting connected brain regions. After AD is initiated, reactive glial cells may have a major role in promoting self-sustaining neurodegeneration cycles. Preventing microglia activation and/or proliferation may interfere with development of the disease.

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POSTERS

ENRICHMENT AND EARLY HANDLING PROTECT AGAINST AGE-RELATED DEFICITS. A BEHAVIORAL AND HISTOLOGICAL STUDY IN RHA/VERH AND RLA/VERH RATS.

B. González, B. Castellano, J. Vela, P. Fábregas, I Dalmau, R.M.. Escorihuela*, A. Tobeña* and A. Fernández-Teruel*.

*Unit of Histology, Dept. of Cell Biology and Physiology and *Unit of Medical Psychology, Dept. of Pharmacology and Psychiatry. Faculty of Medicine. Autonomous University of Barcelona, Spain.*

It has been well established that as age advances, the brain undergoes different structural changes leading to a slow but progressive deterioration of the different cerebral functions. This impairment is due to an important loss of functional neurons and connections in different brain areas. Nevertheless in this work we present evidences suggesting that this deleterious and apparently irreversible process, could be prevented by means of the appropriate stimulation during infancy.

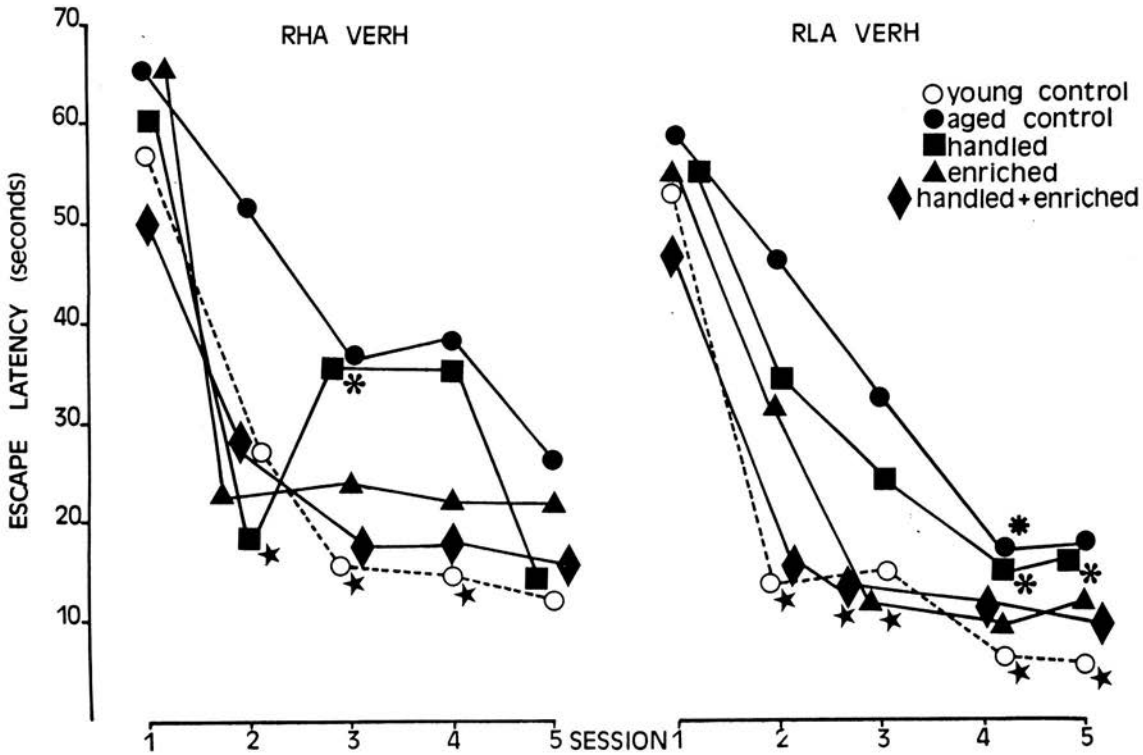
Our studies have been performed on two rat lines (RHA/Verh and RLA/Verh) which have been genetically selected and bred for good (RHA/Verh) vs poor (RLA/Verh) ability to acquire a two-way active avoidance task. We investigated the long-lasting effects of postnatal handling and/or environmental enrichment in both rat lines. Handling treatment was administered between postnatal days 1 to 21, and consisted of removing (twice daily) the pups from the nest and individually placing them in plastic cages lined with paper towel for a total period of ten minutes. Control animals were left undisturbed. Enrichment treatment started at weaning and consisted of placing the animals into large cages containing playthings which were changed every two days for a period of 6 months. After weaning non-enriched animals were housed in pairs in standard laboratory cages. At the age of 24 months, rats were tested for spatial learning in the Morris water test. They performed five 4-trial sessions spaced 24 hours apart. In each trial the rat was placed in the pool for a maximum of 120 seconds to locate the platform submerged in a fixed position. Two additional groups of 5-month-old rats from both strains were used as a young control groups. The results, presented in Fig. 1, indicated that there were differences between RHA/Verh and RLA/Verh rats in spatial learning (ANOVA, 'line' effects: $F(1,52)=12,7$, $P<0.01$) and more interestingly the latencies to find the platform were reduced as a result of handling (ANOVA, $F(1,52)=4.6$, $P<0.05$) and enrichment treatment (ANOVA, $F(1,52)=17.5$, $P<0.0001$), although the later was more effective. In fact the performance of enrichment and handling+enrichment animals was similar to the behavior observed in young rats.

The histological study of aged brains revealed clear-cut differences among rats receiving postnatal treatment and untreated rats. Microscopic observations showed that in normal rats (without postnatal treatment) aging induced a significant loss of functional neurons and a manifest alteration of the cytoarchitecture in some cerebral areas. These changes were particularly noteworthy in those cerebral areas specifically related to memory and learning as the hippocampal formation. In this location the major part of surviving neurons showed signs of atrophy by displaying a dark appearance, whereas only a low proportion of functional neurons (light appearance) was observed. However, aged brains of rats receiving postnatal treatments showed a cytoarchitecture very similar to young rats. The major part of neurons seen in the hippocampal formation displayed a light aspect and only few dark neurons (atrophic) were found (see Fig. 2). In addition it should be noted that in enriched animals, a considerable increase in the number of glial cells was observed. Recent research indicates that astrocytes and microglial cells are intrinsic brain elements involved in the synthesis and release of different growth factors. The suitable activation of these glial cell populations could facilitate the survival of neurons and maintenance of cerebral circuits during aging.

In conclusion, this parallelism between behavioral and histological results suggest that some types of infantile stimulation may prevent to some degree the neuronal degeneration and loss of cognitive abilities taking place normally during aging.

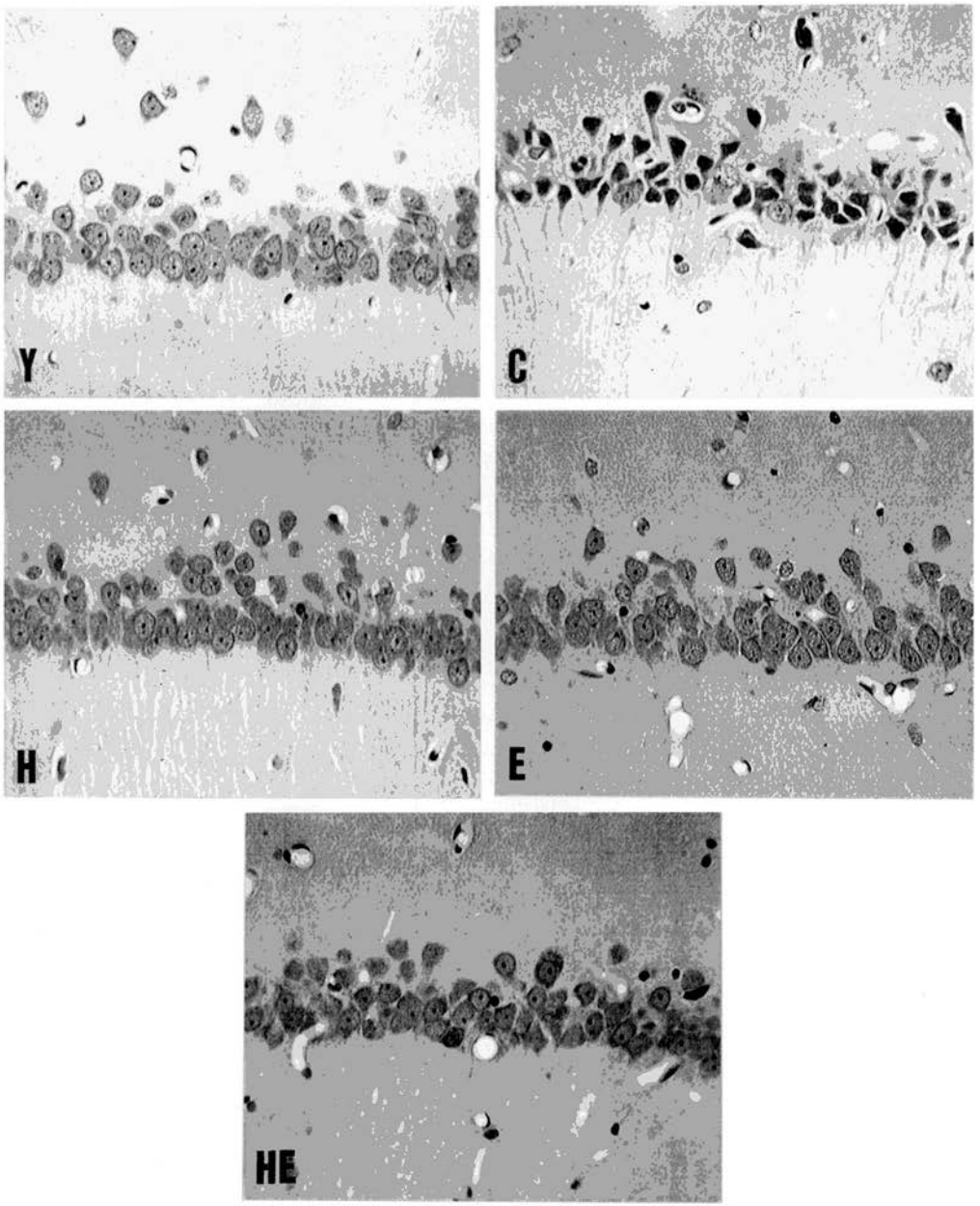
Work partially supported by the FISSs (92/0712)

Figure 1. Performance of RHA/Verh and RLA/Verh rats in the Morris water test



*P<0.05 vs. aged control group of the same rat line and session;
*P<0.05 vs. young control group of the same rat line and session;
*P<0.05 vs. the respective RHA/Verh group (6-10 animals per group).

Figure 2. Pyramidal neurons in the CA1 area of the hippocampus in young animals (Y), untrated old animals and treated old animals (H, E, HE).



AGED ASTROCYTES OF THE MOUSE HYPOTHALAMUS.

R.VERDUGA, C.FERNANDEZ-VIADERO, J.VILLEGAS & D.CRESPO.
Dept. Anatomy & Cell Biology. University of Cantabria. 39011-Santander. SPAIN.

The magnocellular neurosecretory system is formed by the supraoptic nucleus (SON) neurons together with the magnocellular neurons of the paraventricular nucleus (PVN) of the hypothalamus. These neurons are committed to perform the synthesis, transport and release of the neurohormones oxytocin or vasopressin. Quantitatively the main glial cell type in the SON are astrocytes. Most astrocytes are intermingled with SON neurons either alone or forming small clusters.

The SON was studied in senescent mice (26-month-old), and compared to those of young ones in order to assess ultrastructural morphologic correlates of cellular glial activity with aging (*). In aged mouse SON astrocytes there is a conspicuous increment in the number of secondary dystrophic lysosomes filled with a lipidic component. Cytoplasmic organelles termed glial concentric bodies (GCBs) were frequently observed, and in several cases these organelles were so numerous that they occupied most of the glial cell cytoplasm. They were formed by the regular arrangement of concentric layers of unfenestrated arrays of cisterns around a central hyaloplasmic core. Due to the coronal orientation of the SON sections these GCBs appeared always rounded in shape. The number of cisterns forming every GCB was variable (between two to eleven, the average was seven). Lumen obliteration was frequent due to the flattened shape of these cisterns.

The glial placement of these inclusions was confirmed by the fact that, in some astrocytes, GCBs were observed in close relation with bundles of gliofilaments. Images suggesting GCBs formation were present all over the astrocytic bodies and prolongations. At first stage a circular cistern appeared limiting a cytoplasmic area that would constitute the central core of these inclusions. This was followed by the addition of a variable number of new rings of concentric cisterns around the first one, forming typical mature GCBs.

We suggest that GCBs are formed by RER cisterns avoided of functional activity. Mentioned loss in glial cells is a result of the decrease in enzymatic activity of the lysosomal system which normally processes the membrane cell remnants in order to allow a cell-recycling of organelles.

(*) D.Crespo, et al. *Mechanisms of Ageing and Development*.62:223(1992).
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CENTRAL NORADRENERGIC MECHANISMS INVOLVED IN PHARMACOLOGICAL AND PSYCHOLOGICAL INTERVENTIONS USED TO RESTORE COGNITIVE DEFICITS IN MIDDLE-AGED RATS

M. Dierssen, A. Fernández-Teruel, A. Tobeña, N. M. Vivas, F. Màrmol, A. Badia, J. Sallés, R.M. Escorihuela. Dep. de Fisiología y Farmacología, Universidad de Cantabria, Santander, Spain. Dep. de Farmacología y Psiquiatría, Universidad Autónoma de Barcelona, Bellaterra, Barcelona, Spain.

The role of the central noradrenergic system in learning and memory processes seems to be important, but has not been clearly defined. Alterations in the function of central noradrenergic system participate in age-related cognitive deficits; in some circumstances, the behavioral effects of cholinergic degeneration are known to be alleviated by a concurrent reduction in noradrenergic function. The present study was aimed at elucidating the possible involvement of the hippocampal ß-adrenoceptor system in the effectiveness of psychological or pharmacological manipulations that improve age-sensible cognitive tasks.

The effects of a cognitive enhancer, 9-amino-1,2,3,4-tetrahydroacridine (THA) and two types of early stimulation (postnatal handling and/or enriched environment) were evaluated in 18-20 months old Sprague-Dawley rats. Postnatal handling was given between 1 and 22 postnatal days. In the enriched environment procedure, the pups were maintained in enriched conditions from weaning until postnatal day 100. An object recognition test was used to explore working memory, and retention was evaluated in a standard one-trial passive avoidance task. In the neurochemical studies, basal and stimulated cyclic AMP accumulation was determined in cortical and hippocampal structures in every experimental group.

A significant age-related difference in test latencies (STLs) was observed between young and middle-aged rats ($P<0.001$) in the passive avoidance task. Post-training administration of THA (0.1-2.5 $mg.Kg^{-1}$) improved retention of a previously learned aversive habit in a dose related manner in middle-aged but not in young rats. This improving post-training effect of THA reflects an action on retention since the elevated STLs produced by THA are different from nonshocked rats ($P<0.01$). Regarding to environmental manipulations, animals reared in the enriched conditions performed better in the working memory test than did controls (Median=9.0; p=0.0154). There were no improving effects of handling in retention. The number of animals scoring over the median were: controls (C), 1/6; postnatally handled (H), 1/7; exposed to enriched environment (E), 5/7 and combined handling and enriched environment (HE) 7/9. The lack of effects of postnatal handling agrees with earlier suggestions that it seems not to have important effects in learning tasks not directly affected by emotional factors.

The neurochemical studies analyzed the effects of age on adenylate cyclase activity in cerebral cortex and hippocampus. Basal levels of cyclic AMP remained unchanged with age, but prevention of degradation (IBMX, 1 mM) did not increase cyclic AMP accumulation in the hippocampus of middle-aged rats, suggesting an alteration of the metabolic pathway in this region. In isoprenaline (10 µM)-stimulated conditions the increase in cyclic AMP production was higher in middle-aged rats ($p<0.01$), indicating a region-specific supersensitive response of adenylate cyclase to catecholamines with increasing age.

In animals submitted to the behavioral test, footshock experience itself was unable to modify basal cyclic AMP accumulation, but reduced the ability of isoprenaline to stimulate adenylate cyclase activity in the hippocampus of young ($P<0.05$) and middle-aged ($P<0.02$) rats. Post-training injection of THA (0.1-2.5 mg.kg-1) resulted in a significant reduction of basal cyclic AMP levels with respect to untreated animals in cortex and hippocampus of young rats ($p<0.01$) and in the hippocampus ($P<0.02$) of middle-aged animals. When degradation was prevented, the decreasing effect attained in adult rats was not significant, suggesting an action of THA on cyclic AMP metabolic processes in conditions where such proccesses are altered, as occurs with aging. Finally, THA reduced isoprenaline-stimulated cyclic AMP synthesis in hippocampus ($P<0.001$) and cortex ($P>0.05$).

In the groups submitted to environmental manipulations, those presenting better performance in the object recognition test had a decreased ability to stimulate adenylate cyclase activity with isoprenaline in hippocampus (HE $P<0.01$ and E $P<0.05$), suggesting that enviromental enrichment in early life was able to modify the equilibrium in response of the hippocampal noradrenergic system. Postnatal handling and its combination with exposure to enriched environment significantly increased basal cyclic AMP accumulation in cerebral cortex ($p<0.01$), but it did not affect isoprenaline-stimulated cyclic AMP formation in either anatomical structure. However, the net increase in isoprenaline-stimulated cyclic AMP accumulation (stimulated minus basal value) attained a similar reduction (23%) in every experimental group as compared to controls in cortex (H, $p<0.02$; E, $p<0.001$ and HE, $p<0.01$). In the hippocampus, the net increase in isoprenaline-stimulated cyclic AMP production was also reduced compared to untreated animals (E, 28.6% ($P<0.05$) HE, 20.8% ($P<0.02$) and H, 11.9%).

In conclusion, the data suggest that subtle manipulations of the environment early in life, and pharmacological treatments that produce improvements of age-sensible cognitive tasks induce stable modifications in the responsiveness of ß-adrenoceptor in the hippocampus, an effect that might prevent its age-related supersensitivity. A common mechanism is suggested for the cognitive consequences of both psychological and pharmacological interventions which converge on the hippocampal ß-adrenergic system. This leads to the suggestion that a correct equilibrium in the response of the ß-adrenoceptor transduction system is crucial for the maintenance of an accurate memory processing.

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The M22 antibody identifies a subset of astrocytes responding to CNS disease - reactive astrocytosis as a region-specific response to injury. Michael Eddleston, Juan Carlos de la Torre & Michael B.A.Oldstone, Division of Virology, Dept Neuropharmacology, The Scripps Research Institute, La Jolla, California, USA.

Astrocytes constitute a substantial proportion of the CNS, participating in a number of important physiological processes. They also vigorously respond to diverse neurological insults, a response termed reactive astrocytosis, by upregulating the expression of many molecules including glial fibrillary acidic protein (GFAP), the prototypic marker of astrocytosis. Reactive astrocytosis, as revealed by increased GFAP expression and astroglial hypertrophy, is a common feature of aging in human brains. Debate continues whether this process benefits or injures the brain, whether the reponse itself results in functional deficits of the CNS, or acts to limit damage during aging or after brain injury. However, it is possible that the response consists of activated astrocyte subpopulations, some of which are beneficial, whilst others are toxic. Few studies have addressed the possibility of reactive astrocyte heterogeneity. Using a novel immunohistochemical marker of reactive astrocytes, termed M22, together with anti-GFAP antibodies, we have analysed both region- and insult-specific variation in reactive astrocytosis.

Scrapie-infected mouse brains exhibited an $GFAP^+$ reactive astrocytosis throughout the brain. In contrast, the astrocytosis identified by M22 was limited to those areas that displayed the most intense GFAP staining, such as the hippocampus. No M22 reactivity was apparent in the cerebellum or brain stem. Wild mouse retrovirus-infected mouse brains exhibited $GFAP^+$ reactive astrocytes limited to these caudal regions. Again no M22 staining was apparent in this area, even though the spongiform pathology was similar to that found in the hippocampus of scrapie-infected mice. Two transgenic models that expressed either HIV gp41 or IL-6 in astrocytes under the control of the GFAP promoter, and which resulted in reactive astrocytosis, were then studied. The expression of HIV gp120 resulted in astrocytosis concentrated rostrally around the hippocampus, while that of IL-6 was concentrated in the cerebellum. M22 was expressed in the hippocampus of the GFAP-gp120 mice; however, M22 was also expressed by reactive Bergmann glia and some reactive astrocytes in the cerebellum of the GFAP-IL6 mice. This indicated that M22 could be induced in caudal parts of the brain but probably required more extensive damage. This suggests that astrocytosis can be induced to varying levels (as measured by M22 expression) by similar stimuli in different parts of the brain. To further analyse this hypothesis, we injured mouse brains with needles through the cortex, hippocampus and cerebellum. M22 expression was only co-induced with GFAP in the hippocampus.

In conclusion, these studies suggest that the reactive astrocyte response to injury is not homogenous but is instead induced to varying levels by different insults and in different regions of the brain. The M22 antibody should prove to be a useful marker of highly activated astrocytes responding to CNS damage. As further subset markers are identified, reactive astrocytosis will probably be shown to be a highly complex response to injury.

Phosphorylated MAP1B in sites of neurofibrillary degeneration: ultrastructural observations

P. Gómez-Ramos[1], L. Ulloa[2], J. Avila[2] and M.A. Morán[1]
[1] Departamento de Morfología. Facultad de Medicina. Universidad Autónoma de Madrid. [2] Centro de Biología Molecular. Universidad Autónoma de Madrid. Spain.

In Alzheimer's disease, immunochemical evidence suggests that phosphorylated MAP1B is associated with neurofibrillary tangles (Hasegawa et al., 1990). Moreover, this phosphorylated MAP1B has been implicated in the regeneration process, postulated to occur concomitantly with neuronal degeneration. Supporting this regeneration hypothesis, it has also been described an altered distribution of non-phosphorylated MAP1B immunoreactivity, in vulnerable as well as non-vulnerable neurons of Alzheimer's disease (Geddes et al, 1991). The MAP1B immunostaining pattern of neurofibrillary degeneration, is similar to that obtained with a tau antibody (Takahashi et al., 1991), despite the lack of cross-reactivity between antibodies recognizing both proteins (Riederer et al., 1986). To further analyze the association of MAP1B with neurofibrillary degeneration, we have ultrastructurally studied the immunostaining of neurofibrillary degeneration with a novel monoclonal antibody against phosphorylated MAP1B. This MAP1B immunostaining has been compared with that obtained using a monoclonal antibody against abnormally phosphorylated tau (5E2) (Kosik et al., 1988). Our results demonstrate that both antibodies label neurofibrillary degeneration in a very similar way. In addition to the labeling of neurofibrillary degeneration, MAP1B stained faintly the nucleus and diffusely the cytoplasm of positive neurons.

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ß-Adrenoceptors and subtypes in normal aging and Alzheimer's Disease: an autoradiographic study.

Bernardo Grijalba[1], Joaquín González-Gil[1], Julio Pascual[1,2] and Angel Pazos[1].
[1]Dpt. Physiol. & Pharmacol., Univ. de Cantabria and [2]Serv. Neurol. Univ. Hosp. "Marqués de Valdecilla", Santander, Spain.

Both in aging and neurodegenerative disorders related to aging, mainly Alzheimer's disease, noradrenalin levels are decreased in several human areas. Functional noradrenergic neurotransmission depends not only on the amount of noradrenalin but also on the state of the adrenergic receptors and their second messenger systems.

The density of ß-adrenergic receptors was studied by means of autoradiography in post-mortem human brain. Sections of representative brain areas were incubated with ^{125}Iodocyanopindolol. ICI-89406 and ICI-118551 were used to define $ß_2$ and $ß_1$-receptor estimates respectively. After exposure to ^{3}H-Hyperfilms (Amersham,UK), autoradiograms were quantified using a computer-assisted image analysis system (Microm España, Barcelona).

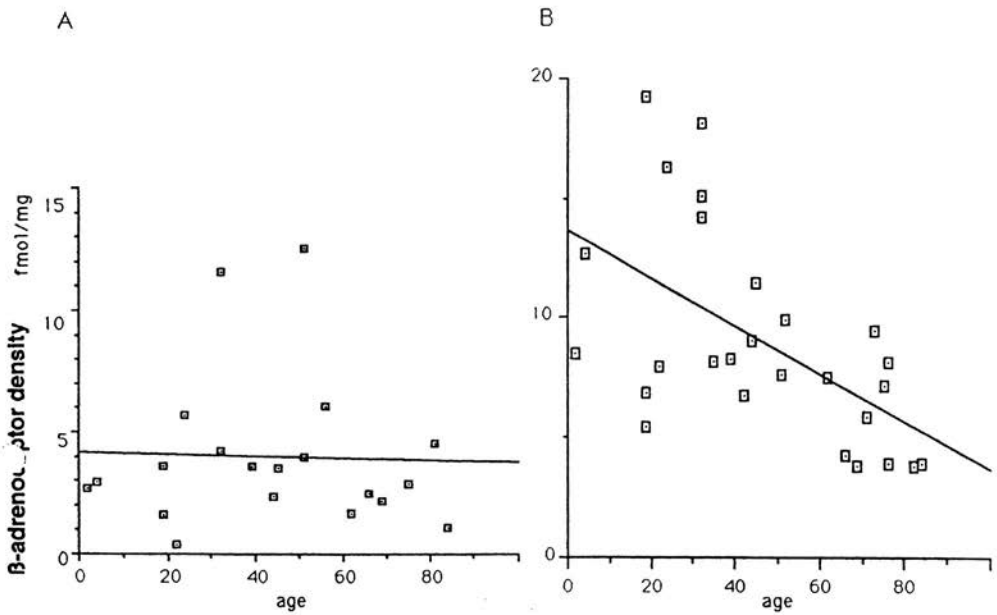
In order to study ß-adrenoceptor levels in normal aging, brains of control cases representing every life-decade were used.
Although the results showed that there is a general tendency of ß-receptors to decrease with age -except for the cerebellum-,this decrease was only statistically significant in telencephalic areas, such as basal ganglia and visual cortex, but not in other areas studied such as the mesencephalon and the cerebellum (see fig. 1). This decrease was far noticeable in the $ß_1$-subtype.

In order to study ß-adrenergic receptors in brains of subjects who suffered from Alzheimer's disease (AD), brain samples of AD-patients and age-matched controls were obtained at autopsy. Results revealed a general tendency to decrease in total receptor binding, in all areas studied: frontal, temporal and visual cortex, basal ganglia, hippocampus and cerebellum. This decrease was statistically significant in all of the subcortical regions and in some of the cortical and hippocampal layers (see fig. 2). While in the neostriatum the receptor loss was due to a decrease of $ß_1$-receptor binding, in the cerebellar cortex and hippocampus the receptor loss was secondary to a decrease of $ß_2$-receptors.

Our findings show a decrease of ß-adrenoceptor density in human brain during normal aging and in Alzheimer´s disease. This receptor loss can be explained by the neuronal loss, known to occur in these processes.

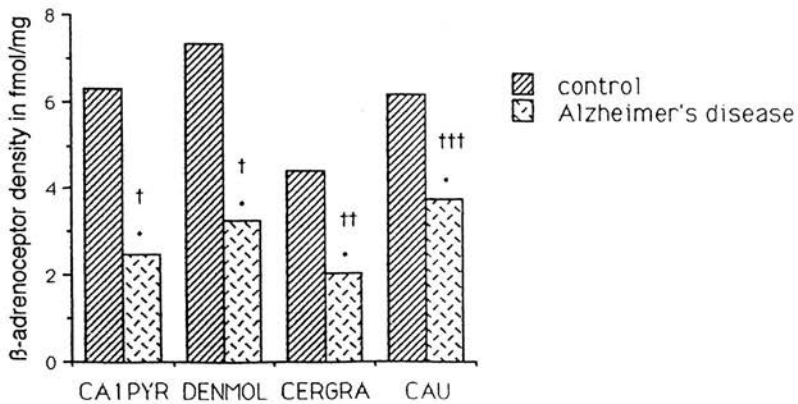
In normal aging, the brainstem is spared from this receptor loss, as compared to the telencephalic structures studied here. These results suggest that aging is not a non-specific process throughout the brain, but that it follows differential patterns and degrees depending on the region of the human brain.

Figure 1



ß-adrenoceptor densities in A: cerebellar cortex (molecular layer) and in B: caudate nucleus

Figure 2



CA1PYR=Pyramidal layer of CA1
DENMOL=Molecular layer of dentate gyrus
CERGRA=Granular layer of cerebellar cortex
CAU=Caudate Nucleus

• p<0,05 in a Student's t-test
† n=10 †† n=12 ††† n=6

SHRINKAGE OF MEMORY-RELATED CENTERS WITH AGE AND ALZHEIMER'S DISEASE. HOW MUCH AND WHERE?

R. Insausti. Department of Anatomy. University of Navarra. Apdo. 273, 31080 Pamplona, Spain.

An earlier and common phenomenon of normal aging as well as Alzheimer's disease is the forgetfulness for facts, events and spatial relationships. The frontier between benign forgetfulness associated with normal aging and the devastating amnesic symptoms of patients with Alzheimer's disease is easy to draw when symptomatology is flourished. However, it remains obscure whether one leads to the other and under what circumstances does it happen. No doubt, it would be of considerable interest for health authorities to single out the population at risk for developing full Alzheimer's disease.

In the past few years we have been working on the quantitative assessment of centers related to memory processing, both in humans and in an animal model of aging such as rodents (rat). Two approaches were designed: one based in cell counting and quantitative measurement of the neuronal nuclear size in paraffin sections through the centers and comparatively in rat and human samples; the other one, performed only in humans, consisted on cytoarchitectonic analysis of cortical areas closely related to the hippocampal formation, and quantitative measurement of the cortical extension of these areas.

Our results can be summarized as follows:

A) Cell count and neuronal nuclear size. The structures examined comprise the dentate gyrus, CA1-CA3 of the hippocampus proper, subicular complex, (encompassing subiculum, presubiculum and parasubiculum), amygdala, mammillary complex, anterior thalamic complex and basal nucleus of Meynert. All these structures show a common pattern of decrease in neuronal density with age, beginning either in the fifth or sixth decade in the hippocampus and basal nucleus of Meynert or more uniformly, in the case of amygdala, mammillary complex and anterior thalamic complex. These changes are accompanied with an increase in neuronal nuclear size from the sixth decade and a final decrease in the extreme ages. Both parameters correlate and they were confirmed in aging rats.

B) Areal extension. The cortical areas studied so far are entorhinal cortex, posterior parahippocampal gyrus (areas TH and TF), posterior cingulate cortex and perirhinal cortex (areas 35 and 36, in progress). All of them (excluding the entorhinal cortex itself) have in common heavy direct projections to the entorhinal cortex, as a previous step to the hippocampus, according to our own anatomical data in the nonhuman primate brain. After an evaluation of the main morphologic features of the different cortical areas and their correlation with the known nonhuman primate cytoarchitectonics, boundaries were drawn onto plots of thionin-stained serial sections. Two-dimensional maps were constructed and measured. The results obtained showed a 10% decrease of the extent of both entorhinal and posterior parahippocampal cortices, while posterior cingulate cortex had a 20% decrease. Alzheimer cases displayed a heavier atrophy that reached to 40% in posterior parahippocampal gyrus and posterior cingulate cortices, and 20% in entorhinal cortex, compared to age-matching controls. Assuming that excessive brain atrophy precede clinical symptoms, it can be probably assessed by non-invasive methods in the general population for the identification of individuals at risk of Alzheimer disease.

Cholinergic innervation of the human hippocampal formation
S. de Lacalle et al.

The cholinergic innervation of the hippocampal formation is thought to play an important role in memory processes, but its organization in humans has not been described in detail. We studied the cholinergic innervation of the human hippocampal formation by means of immunohistochemistry with polyclonal antisera directed against acetyl cholinesterase (AChE), choline acetyltransferase (ChAT) and the low affinity (p75) nerve growth factor receptor (NGFR). The density of ChAT-like immunoreactive fibers differed substantially among the various regions, in general paralleling the pattern of AChE immunoreactive staining. One notable exception was the presence of AChE immunoreactive cell bodies. In contrast, ChAT immunoreactivity was associated only with fibers and terminals. NGFR immunoreactive staining corresponded closely to the ChAT immunoreactive fiber pattern.

ChAT immunoreactive fibers in the CA fields diffusely filled the stratum pyramidale and extended into the stratum oriens and radiatum as well. The highest density was consistently observed in CA4 and CA3 subfields. Staining decreased from CA4 to CA1 and was substantially less dense in the subicular complex. In the entorhinal cortex, the ChAT- and NGFR- immunoreactive fiber innervation displayed a laminar pattern, most intense over the nests of cells in layer II.

There was a trend towards an age-related reduction in the density of ChAT- and AChE immunoreactive fibers and terminals. Nonetheless, we also found a surprisingly conserved NGFR immunoreactive innervation and the presence of occasional NGFR immunoreactive pyramidal cells, providing evidence of a plastic response in the brains of the elderly patients.

ISOLATION OF A HYPERPHOSPHORYLATED TAU PROTEIN FRACTION PRESENT IN SOLUBLE FORM IN ALZHEIMER'S DISEASE BRAIN CYTOSOL

M.D. LEDESMA, J.AVILA and I. CORREAS.Centro de Biología Molecular. Universidad Autónoma . 28049 Madrid. Spain.

Alzheimer's disease neurofibrillary tangles consist of aggregated paired helical filaments which contain, as a major core component, an insoluble and hyperphosphorylated form of tau protein.In an attempt to isolate a soluble precursor of this aberrant tau protein, tau from cytosolic fractions obtained from normal and Alzheimer's disease brains have been fractionated by iron-chelated affinity chromatography. Tau isoforms were eluted from the column by an increasing step pH gradient comprising pH values from 7.0 to 8.5. Both the apparent molecular weight and the amount of tau isoforms eluting at pH 8.5 are increased in the case of the Alzheimer's disease-soluble fraction compared to the normal-soluble one suggesting an enrichment in abnormally phosphorylated tau isoforms in the former. Immunoblot analysis of the tau fractions eluted at the different pH values show that the only fraction recognized by an antibody which binds to a phosphorylated epitope, SMI 31, is that isolated at pH 8.5 from the Alzheimer's disease-soluble sample. This fraction is not recognized by an antibody which binds to an unmodified epitope, Tau-1, unless the sample is pretreated with alkaline phosphatase. Tau-1 recognizes fractions eluting at intermediate pH values, in contrast to antibody SMI 31.

Phosphorylation experiments performed with proline-directed protein kinases indicate that the fraction isolated at pH 8.5 from Alzheimer's disease-soluble tau may be completely phosphorylated at the corresponding target sites.

PERIPHERAL NERVOUS SYSTEM FUNCTION WITH AGING IN THE MOUSE

Xavier Navarro, Enrique Verdú, Miquel Butí.
Depto. Biología Celular y Fisiología, Universidad Autónoma de Barcelona.

The influence of aging on peripheral nerve and target organ function was investigated in 6 groups of mice aged 2, 6, 9, 12, 18 and 24 months. Sudomotor, motor and sensory functions mediated by the sciatic nerve were evaluated by silicon imprints, electromyographic and neurographic recordings from the distal hindpaw. The number of sweat glands reactive to pilocarpine averaged 325, 314, 331, 315, 335 and 338 in mice 2, 6, 9, 12, 18 and 24 month old respectively. The cMAP of plantar muscles, evoked by electrical stimulation of the sciatic nerve, decreased in amplitude with age, from mean values higher than 9.0 mV in mice aged 2 and 6 month to less than 6.5 mV in the oldest groups. The motor nerve latency decreased from 2 (mean 2.14 ms) to 6 (1.80 ms) months, was unchanged until 12 months, and increased thereafter (to 2.16 ms at 24 months). Similar patterns were observed in sensory nerve conduction. NAP amplitude decreased from 42 μV in mice aged 6 months to 30 μV in mice aged 18 and 24 months; sensory nerve latency initially decreased (1.89 ms in 2 month, 1.64 ms in 6 month old mice) and increased progressively in older mice (1.97 ms in 24 month old mice).

These results indicate that aging affects differently motor, sensory and autonomic sudomotor functions. Neurophysiological responses mediated by large diameter nerve fibers deteriorated with age, while those dependent of small fibers were preserved.

CHANGES IN DOPAMINE-GLUTAMATE-GABA INTERACTIONS IN THE STRIATUM WITH AGE

A. Porras and F. Mora
Department of Physiology, Faculty of Medicine.
University Complutense, 28040 Madrid, SPAIN.

The striatum receives glutamatergic terminals from the cerebral cortex and dopaminergic terminals from the substantia nigra, pars compacta (1). The striatum also contains GABA neurons (2). In previous studies we have shown an interaction between glutamate (GLU), GABA and dopamine in the striatum of the rat (3,4,5,6). In the present study we tried to investigate further this type of interaction during aging.

The effects of intrastriatal infusion of apomorphine, a D1-D2 dopamine receptors agonist, on the extracellular concentrations of GLU, GABA, and their precursor, glutamine (GLN) were investigated in the striatum of 3 (young), 12 (middle-aged), and 24 (aged) months old rats. A continuous push-pull perfusion system was performed at a flow rate of 20 µl/min. After obtaining steady concentrations of amino acids, 10 min samples were collected for 100 min. The concentration of amino acids in samples was analyzed by HPLC with fluorometric detection.

In young rats apomorphine (5, 10, and 20 µM) produced a dose-related increase in the extracellular [GLU] and [GABA]. In middle-aged rats, apomorphine only at 10 µM induced a delayed release of GLU, while had no effect on striatal [GABA]. In aged rats apomorphine did not induce changes in striatal [GLU] and [GABA]. The extracellular [GLN] did not change during the total time of experiments. These data indicate an age-related deterioration of dopamine-GLU-GABA interactions in the striatum of the rat.

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THE MODULATION OF NEUROPEPTIDES ON IMMUNE FUNCTION DECREASES DURING AGING

De la Fuente M. & Del Rio M.
Depto. Biología Animal II (Fisiología Animal). Facultad de Ciencias Biológicas. U.C.M. Madrid.

Functional age-dependent changes have been described in the immune system. Although it is well known that immune function can decline with age in humans as well as in many other mammalian species, our results show that this occurs mainly in T cell-dependent immune functions (1), whereas phagocytic cells do not seem to be affected in the functions implicated in the phagocytic process; moreover, these functions can be increased in old animals (2). There is increasing evidence about the existence of a neuroimmune axis, suggesting that the interaction and communication between the nervous and immune system have to be multidirectional. This communication between nervous and immune system is mediated by neuropeptides and cytokines via specific membrane receptors (3). We have studied the effects in vitro of several neuropeptides (GRP and other member of bombesin-related peptides, NPY, neurotensin, VIP and CCK-8) on immune functions (4-8). There is the idea that with aging there exists a decrease in the neuroimmune regulation (9). In this work we have studied the effect in vitro of 3 neuropeptides (GRP, NPY and CCK-8) on several representative functions of phagocytes and lymphocytes (peritoneal macrophages and lymphocytes and lymphocytes from spleen, thymus and axillary nodes) from young (15±2 weeks old) and old (75±2 weeks old) BALB/c mice. The adherence to substrate, chemotaxis, production of superoxide anion and lymphoproliferative response (spontaneous and induced by the mitogen Con A) were analized. The results have indicated that in aging there is an increase in the functions of macrophages (adherence, chemotaxis and anion superoxide production) as well as in the chemotaxis of lymphocytes. However, a decrease in the proliferative response was shown. The modulatory effects of neuropeptides were diminished with age. Moreover, in old age the activation of protein kinase C, a representative intracellular effector of these immune responses, was increased, while the stimulation of the enzyme's activity by the neuropeptides was decreased.

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BRAIN-DERIVED NEUROTROPHIC FACTOR INFUSIONS IN THE RODENT CENTRAL NERVOUS SYSTEM: RETROGRADE TRANSPORT AND CODISTRIBUTION WITH P75 AND TRK RECEPTORS

T. Sobreviela[1], J. S. Kroin2, R. D. Penn[2], D. Clary[3], L. F. Reichardt[3], and E. J. Mufson[1]. Depts. of Neurol. Sci.[1], Neurosurgery[2], Rush Presbyterian Med. Ctr. Chicago, IL 60612. and Howard Hughes Med. Ctr[3], University of California, San Francisco, CA 94143 USA

Brain-derived neurotrophic factor (BDNF) mRNA distribution differs from that of other members of the NGF family of neurotrophins within the mammalian central nervous system (CNS). Neuroregeneration and developmental investigations suggest that BDNF may influence both cholinergic basal forebrain and dopaminergic mesencephalic neurons. In order to determine those neuronal populations which may require BDNF, human BDNF was infused at a rate of 3 µg/h with an Alzet 2002 minipump into frontal cortex, amygdala, hippocampus, striatum and lateral ventricle in rats to examine its distribution within the CNS. After 7 days animals were perfused and immunohistochemically processed using turkey anti-BDNF (Amgen). Cortical infusion revealed numerous retrogradely labeled neurons within anterodorsal, laterodorsal, mediodorsal, reticular thalamic nuclei, nucleus basalis, lateral hypothalamic/lateral preoptic area and endopiriform nucleus. Amygdaloid infusion retrogradely labeled neurons in the paraventricular, reuniens, mediodorsal thalamic nuclei, diagonal band, nucleus basalis, medial and anterior cortical amygdaloid nuclei and piriform cortex. Hippocampal infusion labeled only a few neurons in septal/diagonal band complex, supramammillary region and peri-and entorhinal cortex. Labeled pyramidal neurons were seen in the ipsilateral and contralateral CA3-CA2. Intense bands of BDNF immunoreactivity were seen within stratum oriens and radiatum. Striatal infusion of BDNF revealed retrograde labeling in frontal cortical, parafascicular thalamic and substantia nigra, pars compacta neurons. Colocalization experiments revealed that only 28% of the BDNF positive substantia nigra neurons coexpress tyrosine hydroxylase, a marker for dopaminergic neurons. Following intracerebroventricular injection, BDNF immunoreactivity was concentrated within the ependymal layer throughout the ventricular system and did not diffuse within the adjacent parenchyma. To begin to unravel the receptor phenotype of those neurons containing retrogradely transported BDNF adjacent sections were processed with antibodies directed against the low affinity p75 nerve growth factor receptor (NGFR; Oncogene), the high affinity signal transducing trk A receptor (made against the extracellular domain) as well as a pan-trk antibody (Oncogene). Within the basal forebrain (i.e., medial septum, vertical limb of the diagonal band and nucleus basalis) numerous neurons expressed p75, trk A and pan trk immunoreactivity. p75 NGFR neuronal staining was restricted to these regions. In contrast, trk A and pan-trk immunopositive neurons was also found in the striatum, paraventricular thalamus, lateral preoptic region, interpeduncular, solitary and raphe nuclei. In addition, pan-trk staining was observed in the nucleus accumbens, supraoptic nucleus and ventral tegmental area and substantia nigra. In conclusion, intraparenchymal infusions of BDNF resulted in widespread retrograde neuronal labeling within the CNS. Immunostaining for p75 NGFR and trk receptors revealed a discordance between the BDNF labeling and receptor staining patterns. The most striking codistribution between BDNF staining and the various receptor subtypes was seen within the basal forebrain. Further studies employing specific trk B antibodies are needed to more clearly determine the receptor phenotype associated with BDNF retrograde transport in the CNS. Neuronal subgroups which selectively transport BDNF may respond specifically to this neurotrophin in conjunction with a currently known trk receptor or an as yet unknown BDNF/trk receptor complex under normal or pathologic conditions.

CHANGES WITH AGING IN REGENERATION AND REINNERVATION AFTER NERVE INJURY

Enrique Verdú, Miquel Butí, Xavier Navarro
Depto. Biología Celular y Fisiología, Universitat Autònoma de Barcelona

Experimental investigations have shown that axon regeneration occurs at a faster rate in young than older animals, although different recovery characteristics have been reported for different types of nerve fibers.

We investigated the influence of age on nerve regeneration and reinnervation of target organs in mice aged 2, 6, 12, 18 and 24 months, after a complete section and repair by epineurial suture of the sciatic nerve. Reinnervation of muscle, sweat glands (SG) and skin was evaluated over three months after operation by functional methods.

Compound muscle action potentials (MAP) after sciatic nerve stimulation were first recorded from plantar muscles at 26 days postoperation in mice aged 2 and 6 months, and at 33-41 days in those aged 12 and 18 months. The MAP amplitude increased to approximately 50% of control baseline values in young mice and to 35% in old mice by 90 days. SGs reactive to pilocarpine reappeared by 21 days in the 2 and 6 month old mice and by 28 days in older groups, and their number increased up to 93, 85, 83,75 and 78% of control counts. Positive responses to skin pinprick were first observed by 19 days in proximal areas of the hindpaw of all groups and present in all areas at the end of the study. The plot of functional reinnervation against time followed a slower slope of the recovery curve as the age increased for the three types of nerve fibers evaluated.

These results show that, after section and suture repair, axonal regeneration and reinnervation in the peripheral nervous system is maintained during youth and adulthood, but tends to be more delayed and slightly less effective with aging.

AN ULTRASTRUCTURAL STUDY OF THE OLIGODENDROCYTES IN THE TELENCEPHALON OF THE ADULT LIZARD

C.Yanes, M. Monzón Mayor & R. R. Sturrock

The present study set out to investigate the ultrastructure of oligodendrocytes in the telencephalon of the adult lizard. In comparison to mammals few studies of glial cells have been carried out in reptiles. This investigation is complementary to an immnuhistochemical study of myelination in the same species (Yanes,et al., 1992).

The three types of oligodendrocytes (light, medium and dark) first classified by Mori and Leblond (1970) in the rat are also found in the lizard midbrain (Monzon et al., 1990). In the telencephalon light oligodendrocytes have a cytoplasmic density similar to that of myelinated axon. Medium oligodendrocytes have a moderately electrodense cytoplasm with microtubules particularly evident in their processes. Medium oligodendrocytes predominate in the lizard telencephalon. Dark oligodendrocytes with a very electron dense cytoplasm are also present but are less numerous the medium variety.

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SUMMARY

M. Nieto-Sampedro

The greek playwright Menander wrote "those whom the gods love, die young". Of late, with the help of modern medical care, the gods are showing a progressive loss of love for Western world citizens. The Juan March workshop had as dominant background our growing concern with the manifestations of gods' dislike, particularly senile dementia and the hope that, in time, medical research will give everybody the chance of a long life and of a dignified, mentally healthy, old age.

For a long period, most of the research on aging was an inventory of the deficits in elderly people. Then, with the obvious recognition that healthy aging is possible, research emphasis has shifted to a comparison of the differences between the mentally healthy and mentally ill elderly people. The papers presented in the Juan March workshop, indicate that we are getting close to the end of the inventory. The emerging consensus is that in the brain of normal, healthy elders, overall neuronal loss is not frequent and that plastic compensatory mechanisms take place . In contrast, selective neuronal and synaptic loss, as well as a deficit in neuronal plasticity, seem to be major problems in pathological aging.

A most important concern in brain aging studies, is to ascertain whether or not dementia and old age are inseparable; that is, whether dementia would be the unavoidable result of prolonging anybody's life, long enough. This question has not yet a definitive answer. There are good indications that the two processes , although they tend to converge, can be separated. Age is, indeed, a powerful risk factor. As years pass, our chances of being exposed to events capable of initiating deleterious cascades that can progress to dementia, increases. Reactive glia is emerging as an important player and models explaining its possible intervention in pathological aging, were presented in the General Discussion.

Our understanding of the processes at the basis of pathological aging is rapidly increasing. Furthermore, there are already some indications that these processes can be arrested. Concomitantly, increasing information on the neural plasticity mechanisms, encourages us to believe that longer life, free from disastrous mental decay, is within reasonable hope of reach in the not too far future.

List of Invited Speakers

Workshop on

DETERIORATION, STABILITY AND REGENERATION OF THE BRAIN DURING NORMAL AGING

List of Invited Speakers

J. Avila — Centro de Biología Molecular "Severo Ochoa", (CSIC), Universidad Autónoma, Cantoblanco, 28049 Madrid (Spain).
Tel.: 34 1 397 50 70
Fax : 34 1 397 47 99

Y.-A. Barde — Department of Neurobiochemistry, Max-Planck Institute for Psychiatry, Am Klopferspitz 18A, 82152 Planegg-Martinsried, (Germany).
Tel.: 49 89 8578 3614
Fax : 49 89 8578 3749

P.D. Coleman — Department of Neurobiology & Anatomy, Box 603, University of Rochester Medical Center, 601Elmwood Avenue, Rochester, NY. 14642 (USA).
Tel.: 1 716 275 28 51
Fax : 1 716 442 87 66

C.W. Cotman — Department of Psychobioloy and Neurology, Irvine Research Unit in Brain Aging, University of California, Irvine, CA. 92717 (USA).
Tel.: 1 714 865 58 47
Fax : 1 714 725 20 71

Y. Lamour — INSERM, U161, 2 Rue D'Alésia, 75014 Paris (France).
Tel.: 33 1 45 89 36 62
Fax : 33 1 45 88 13 04

M.P. Mattson — Sanders Brown Research Center on Aging and Department of Anatomy & Neurobiology, University of Kentucky, Lexington, KY. 40536 (USA).
Tel.: 1 606 257 60 40
Fax : 1 606 323 28 66

A. Matus — Friedrich Miescher Institut, P.O. Box 2543, CH-4002 Basel (Switzerland).
Tel.: 41 61 697 66 95
Fax : 41 61 697 39 76

E.G. McGeer — Kinsmen Laboratory Neurological Research, University of British Columbia, 2255 Westbrook Mall, Vancouver B.C. V6T 1Z3 (Canada).
Tel.: 1 604 822 83 77
Fax : 1 604 822 70 86

B.S. Meldrum — Department of Neurology, Institute of Psychiatry,, De Crespigny Park, Denmark Hill, London, SE5 8AF (U.K.).
Tel.: 44 71 70 35 411
Fax : 44 71 70 35 796

J. Miquel — Instituto de Neurociencias, Universidad de Alicante, Campus de San Juan, Ap. Correos 374, 03080 Alicante (Spain).
Tel.: 34 6 565 98 11
Fax : 34 6 594 07 25

F. Mora — Departamento de Fisiología, Facultad de Medicina, Universidad Complutense, Ciudad Universitaria, 28040 Madrid (Spain).
Tel.: 34 1 394 14 37
Fax : 34 1 394 16 28

M.Nieto-Sampedro — Departamento de Plasticidad Neuronal, Instituto Cajal, (CSIC), Avda. Doctor Arce 37, 28002 Madrid (Spain).
Tel.: 34 1 585 41 50
Fax : 34 1 585 47 54

V.H. Perry — Department of Pharmacology, University of Oxford, Mansfield Road, Oxford OX1 3QT (U.K.).
Tel:. 44 865 27 18 50
Fax : 44 865 27 18 53

M.P. Rathbone — Departments of Biomedical Sciences and Medicine, McMaster University Health Sciences Center, 1200 Main Street West, Hamilton, ON. L8N 3Z5 (Canada).
Tel.: 1 416 574 86 30
Fax : 1 416 575 73 20

G.S. Roth — National Institutes of Health, National Institute on Aging, Francis Scott Key Medical Center, Baltimore, MD. 21224 (USA).
Tel.: 1 410 558 81 78
Fax : 1 410 558 83 23

R.R. Sturrock — Department of Anatomy & Physiology, University of Dundee, Dundee, DD1 4HN, Scotland (U.K.).
Tel.: 44 382 34 42 17
Fax : 44 382 24 117

H.B.M. Uylings — Netherlands Institute for Brain Research, Graduate School Neurosciences Amsterdam, Meibergdreef 33, 1105 AZ Amsterdam (The Netherlands).
Tel.: 31 20 566 55 00
Fax . 31 20 696 10 06

M.J. West — Stereological Research Laboratory and Department of Neurobiology, University of Aarhus, 8000, Aarhus, C. (Denmark).
Tel.: 45 86 12 55 55
Fax : 45 86 19 12 17

List of Participants

Workshop on

DETERIORATION, STABILITY AND REGENERATION
OF THE BRAIN DURING NORMAL AGING

List of Participants

B. Castellano — Unidad de Histología, Departamento de Biología Celular y de Fisiología, Facultad de Medicina, Universidad Autónoma, 08193 Bellaterra, Barcelona (Spain).
Tel.: 34 3 581 18 75
Fax : 34 3 581 20 04

P. Cazorla — Centro de Bioquímica y Biología Molecular, (CSIC), Facultad de Ciencias, Universidad Autónoma, Cantoblanco, 28049 Madrid (Spain).
Tel.: 34 1 397 84 71
Fax : 34 1 397 47 99

J.D. Cooper — Max-Planck-Institute for Psychiatry, Department of Neurochemistry, Am Klopferspitz 18A, 82152 Martinsried, Munich (Germany).
Tel.: 49 89 8578 36 32
Fax : 49 89 8578 37 49

D. Crespo — Departamento de Anatomía y Biología Celular, Facultad de Medicina, Universidad de Cantabria, Avda. Cardenal Herrera Oria, s/nº, 39011 Santander (Spain).
Tel.: 34 42 20 19 20
Fax . 34 42 20 19 03

M. Dierssen — Departamento de Fisiología y Farmacología, Facultad de Medicina, Universidad de Cantabria, Avda. Cardenal Herrera Oria, s/nº, 39011 Santander (Spain).
Tel.: 34 42 20 19 80
Fax . 34 42 20 19 03

M. Domínguez — Laboratorio de Neurobiología, Facultad de Medicina, Universidad Complutense, Ciudad Universitaria, 28040 Madrid (Spain).
Tel.: 34 1 394 14 37
Fax : 34 1 394 16 28

M. Eddleston — Department of Medicine, University of Cambridge School of Clinical Medicine, Level 5, Addenbrooke's Hospital, Hill Road, Cambridge CB2 1QQ (U.K.).
Tel.: 44 223 33 68 44
Fax : 44 223 33 68 46

M.L. Plaza — Departamento de Morfología, Facultad de Ciencias Médicas y de la Salud, Universidad de Las Palmas de Gran Canaria, Avda. Marítima de Sur s/nº, 35016 Las Palmas de Gran Canaria (Spain).
Tel.: 34 28 45 34 25
Fax : 34 28 45 14 13

A. Porras — Departamento de Fisiología, Facultad de Medicina, Universidad Complutense, 28040 Madrid (Spain).
Tel.: 34 1 394 14 37
Fax : 34 1 394 16 28

M. del Río — Departamento de Biología Animal II (Fisiología Animal), Facultad de Ciencias Biológicas, Universidad Complutense, Ciudad Universitaria, 28040 Madrid (Spain).
Tel.: 34 1 394 49 86
Fax : 34 1 394 49 35

J. Sallés — Departamento de Farmacología y Psiquiatría, Facultad de Medicina, Universidad Autónoma,08193 Bellaterra, Barcelona (Spain).
Tel.: 34 3 581 19 52
Fax : 34 3 581 20 04

C. Sáchez Martín — Centro de Biología Molecular, (CSIC), Facultad de Ciencias, Universidad Autónoma, Cantoblanco, 28049 Madrid (Spain).
Tel.: 34 1 397 84 43
Fax : 34 1 397 47 99

J. Satrústegui — Centro de Biología Molecular "Severo Ochoa", (CSIC), Facultad de Ciencias, Universidad Autónoma, Cantoblanco, 28049 Madrid (Spain).
Tel.: 34 1 397 50 70
Fax : 34 1 397 47 99

T. Sobreviela — Department of Neurological Sciences, Rush Presbyterian Medical Centre, Chicago, IL. 60612 (USA).
Tel.: 1 312 633 15 50
Fax : 1 312 633 15 64

A.K. Utal — Department of Neurobiology and Anatomy, University of Rochester, 601 Elmwood Ave., Rochester, NY. 14642 (USA).
Tel.: 1 716 275 25 91
Fax : 1 716 442 87 66

E. Verdú — Departamento de Biología Celular y Fisiología, Facultad de Medicina, Universidad Autónoma, 08193 Bellaterra, Barcelona (Spain).
Tel.: 34 3 581 19 66
Fax : 34 3 581 20 04

F. Gómez-Pinilla — Department of Neurology and IRU in Brain Aging, University of California, Irvine, CA. 92717 (USA).
Tel.: 1 714 856 60 71
Fax . 1 714 725 20 71

P. Gómez-Ramos — Departamento de Morfología, Facultad de Medicina, Universidad Autónoma, Arzobispo Morcillo s/nº, 28029 Madrid (Spain).
Tel.: 34 1 397 53 22
Fax : 34 1 315 00 75

B. González de Mingo — Unidad de Histología, Departamento de Biología Celular y Fisiología, Facultad de Medicina, Universidad Autónom, 08193 Bellaterra, Barcelona (Spain).
Tel.: 34 3 581 18 75
Fax : 34 3 581 20 04

B. Grijalba — Departamento de Fisiología y Farmacología, Facultad de Medicina, Universidad de Cantabria, Avda. Cardenal Herrera Oria, s/nº, 39011 Santander (Spain).
Tel.: 34 42 20 12 22
Fax . 34 42 20 19 03

R. Insausti — Departamento de Anatomía, Facultad de Medicina, Universidad de Navarra, Apartado 273, 31080 Pamplona (Spain).
Tel.: 34 48 25 21 50 Ext. 397
Fax : 34 48 17 55 00

S. de Lacalle — Department of Neurology, Bldg. B1 - Room 153, Harvard Medical School, Boston, MA. 02115 (USA).
Tel.: 1 617 432 45 31
Fax : 1 617 432 45 29

M.D. Ledesma — Centro de Biología Molecular, (CSIC), Facultad de Ciencias, Universidad Autónoma, Cantoblanco, 28049 Madrid (Spain).
Tel.: 34 1 397 84 43
Fax : 34 1 397 47 99

A. Morán — Departamento de Morfología, Facultad de Medicina, Universidad Autónoma, Arzobispo Morcillo s/nº, 28029 Madrid (Spain).
Tel.: 34 1 397 53 22
Fax : 34 1 315 00 75

E.B. Mukaetova-Ladinska — Department of Psychiatry, Cambridge Brain Bank Laboratory, University of Cambridge, Cambridge CB2 2OH (U.K.)
Tel.: 44 223 21 73 36
Fax : 44 223 40 24 87

X. Navarro — Departamento de Biología Celular y Fisiología, Facultad de Medicina, Universidad Autónoma, 08193 Bellaterra, Barcelona (Spain).
Tel.: 34 3 581 19 66
Fax : 34 3 581 20 04

J. Verhaagen — Rudolf Magnus Institute for Pharmacology, University of Utrecht, Vondellaan 6, 3521 GD Utrecht (The Netherlands).
Tel.: 31 30 88 05 21
Fax : 31 30 89 60 34

C. Yanes — Departamento de Microbiología y Biología Celular, Facultad de Biología, Universidad de La Laguna, 38206 La Laguna, Tenerife (Spain).
Tel.: 34 22 60 37 53
Fax : 34 22 25 33 44

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Organized by D. Kolakofsky and J. Ortín. Lectures by A. K. Banerjee, M. A. Billeter, P. Collins, M. T. Franze-Fernández, A. J. Hay, A. Ishihama, D. Kolakofsky, R. M. Krug, J. A. Melero, S. A. Moyer, J. Ortín, P. Palese, R. G. Paterson, A. Portela, M. Schubert, D. F. Summers, N. Tordo and G. W. Wertz.

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263 **Lecture Course on the Polymerase Chain Reaction.**
Organized by M. Perucho and E. Martínez-Salas. Lectures by D. Gelfand, K. Hayashi, H. H. Kazazian, E. Martínez-Salas, M. Mc Clelland, K. B. Mullis, C. Oste, M. Perucho and J. Sninsky.

264 **Workshop on Yeast Transport and Energetics.**
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22 Workshop on Molecular Bases of Ion Channel Function.
Organized by R. W. Aldrich and J. López-Barneo. Lectures by R. W. Aldrich, C. M. Armstrong, P. Ascher, K. G. Beam, F. Bezanilla, S. C. Cannon, A. Castellano, D. Clapham, A. Ferrús, T. Hoshi, A. Konnerth, R. Latorre, J. Lerma, J. López-Barneo, R. MacKinnon, G. Mandel, A. Marty, C. Miller, B. Sakmann, B. Soria, W. Stühmer and W. N. Zagotta.

23 Workshop on Molecular Biology and Ecology of Gene Transfer and Propagation Promoted by Plasmids.
Organized by C. M. Thomas, E. M. H. Wellington, M. Espinosa and R. Díaz Orejas. Lectures by J. C. Alonso, F. Baquero, P. A. Battaglia, P. Courvalin, F. de la Cruz, D. K. Chattoraj, E. Díaz, R. Díaz Orejas, M. Espinosa, J. C. Fry, K. Gerdes, D. R. Helinski, B. Hohn, E. Lanka, V. de Lorenzo, S. Molin, K. Nordström, R. W. Pickup, C. M. Thomas, J. D. van Elsas, E. M. H. Wellington and B. Wilkins.

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