227P METABOTROPIC GLUTAMATE RECEPTOR SUBTYPES INFLUENCE NEURONAL DEGENERATION THROUGH DIFFERENT MECHANISMS

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Metabotropic glutamate (mGlu) receptors form a family of eight subtypes (mGlu1 to –8), classified into three groups (mGlu1 and –5; mGlu2 and –3; and mGlu4, -6, -7 and –8) on the basis of their primary structure, pharmacological profile and transduction pathways.

All these subtypes (with the exception of mGlu6) have been implicated in the control of neuronal degeneration and survival, but the underlying mechanism is still unclear. Agonists of mGlu1 and –5 receptors (such as DHPG) can either facilitate or reduce neurodegeneration in culture depending on the paradigm of toxicity, the subunit composition of NMDA receptors, and the proportion between neurons and astrocytes. Interestingly, both effects can be observed when cultured cortical cells receive two consecutive applications of DHPG. While a single application of the drug amplifies NMDA toxicity, the second application consistently produces neuroprotection. This suggests the existence of an experience-dependent switch from facilitary into inhibitory group-I mGlu receptors in the control of excitotoxic death.

In contrast, mGlu1 and –5 receptor antagonists are consistently neuroprotective, but through different mechanisms. mGlu1 antagonists (such as CPCCOEt or LY367385) attenuate NMDA toxicity by enhancing GABAergic transmission, as shown by combining these drugs with GABA receptor antagonists or by measuring GABA release in freely moving animals. mGlu5 receptor antagonists (such as MPEP, SIB1757 or SIB1893) would instead prevent postsynaptic mGlu5 receptors from amplifying NMDA currents through a mechanism of receptor-receptor interaction. Neuroprotection by mGlu2/3 receptor agonists involves a novel form of glial-neuronal interaction mediated by an enhanced glial production of TGF-ß. In particular, activation of glial mGlu3 receptors induces the *de novo* synthesis of TGF-ß1 through the activation of the MAP-kinase pathway. The MEK inhibitor, PD98059, prevents the neuroprotective activity of mGlu2/3 receptor agonists against NMDA toxicity. Finally, the use of cultures prepared from knock-out mice suggests that activation of mGlu4 receptors mediate a large component of the neuroprotective action of group-III mGlu receptor agonists, such as L-AP4 and PPG.

Based on these mechanisms, subtype-selective mGlu receptor agonists or antagonists may have differential applications in the experimental treatment of neurodegenerative disorders.

228P METHODOLOGIES AND RESULTS OF STUDIES OF COGNITIVE CHANGES IN OLD AGE

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The main questions in Cognitive Gerontology are "When do Mental Abilities begin to change"? "How rapidly do changes continue?" "Do all mental abilities change together or do some change earlier than others"? and "What factors accelerate and retard cognitive changes"?" Are there simple biological or psychometric indices that can act as markers to predict future rates of change"?

To answer these questions we may either make cross-sectional comparisons between groups of different ages or longitudinal comparisons, tracking the same individuals over a period of many years. Longitudinal studies are more efficient because they also allow initial successive cross-sectional comparisons between groups and so reveal, and can control for cohort effects. However they have two serious and hitherto unacknowledged drawbacks: longitudinal studies suffer from selective drop-out of older and frailer participants so that populations become steadily more "elite"as the study continues. Also they involve repeated administration of the same, or very similar cognitive tests and this results in progressive improvement with practice, even when inter-test intervals range from 2 to 8 years. Practice effects are also counter-intuitive, with older and less able showing greater improvement than younger and more able participants.

We discuss recent statistical techniques for detecting and adjusting for both selective dropout and practice effects. When these techniques are applied the questions with which we began are answered in the following ways: Age related changes begin at a very early age, becoming detectable between the ages of 18 and 35 years and more marked in the range between 49 and 65 years, accelerating rapidly thereafter. However changes in average performance of ageing cohorts are much less interesting than increases in variance between their members. Because of very marked individual differences in trajectories of age-related change differences between the most and least able members of cohorts sharply increase as they age.

Evidently differences in trajectories of ageing are influenced by a variety of factors, among these genetics, general health and presence or absence of specific pathologies, socio-economic advantage education and life-style, gender and lifetime exposure to biological risk factors. We discuss the absolute and relative contributions of these factors to rates of cognitive change, and also new evidence that particular simple measurements of sensory function and balance can account for up to 85% of age-related variance in cognitive status between individuals.

229P MEMORY-IMPAIRED AGED RODENTS AS RELEVANT MODELS :MOLECULAR AND PHARMACOLOGICAL STUDIES.

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Over the past two decades, research on the causes and treatments of dementia of the Alzheimer's type has markedly expanded. However, progress is hampered by inherent difficulties in performing brain aging research in human subjects, normal and pathological. Accordingly, various laboratories have focused on the characterization and development of relevant rodent models, especially in mice and rats which have lifespan short enough to allow for the use of a variety of molecular and pharmacological approaches, chronic drug treatments, etc.

We have particularly focused our attention on the Long Evans aged memory-impaired and -unimpaired rat and transgenic mice over-expressing amyloid peptides. In these models, we investigated the integrity of various neurotransmitter systems known to be affected in the Alzheimer's brain such as certain cholinergic projections originating from the basal forebrain, cortical and hippocampal somatostatin and glutamatergic neurons, etc. In parallel to data derived from brains of Alzheimer's patients, aged memory-impaired rats display major cholinergic dysfunctions not seen in aged memory-unimpaired animals. Moreover, increasing the efficacy of cholinergic neurotransmission by pharmacological treatments (eg M2 blockers) can reverse learning deficits in the aged memory-impaired rat. Interestingly, these beneficial effects of an M2 blocker are long lasting suggesting the modulation of genes involved in learning processes.

We are currently using DNA microarray technology to address this issue. Alterations in cholinergic markers were also observed in aging amyloid transgenic mice and the existence of functional links between cholinergic innervation , APP maturation and amyloid production is currently under investigation.

While it is clear that no rodent models can reproduce all features of the aging and Alzheimer human brain, pertinent information and treatment strategies can certainly be derived from studies performed with the aged rodent.

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230P GALANIN AS A MODULATOR OF BRAIN SEROTONIN AND ACETYLCHOLINE TRANSMISSION: PROSPECTS IN AGING AND DEMENTIAS.

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Galanin (GAL) is a 29 amino acid long neuropeptide with an amidated C-terminal in most species (30 amino acids in humans with a non-amidated C-terminal). Galanin-like immunoreactivity (GAL-LI) was shown to coexist with several "classical" neurotransmitters in the brain known to play a role in cognition, such as acetylcholine (ACh) in the septohippocampal projection, noradrenaline (NA) in the locus coeruleus and serotonin (5-HT) in the raphe nuclei.

Similar to many neuropeptides, GAL displayed an inverted U-shape dose-response learning curve using the Morris swim maze. GAL (3 nmol/rat), infused into the ventral hippocampus (HPC) (mainly the CA3 area), caused an impairment of spatial learning related to a decrease in basal ACh release. Infusions of GAL (1 nmol/rat) into the dorsal CA1 area facilitated spatial learning. In contrast, combined infusions of GAL (3 nmol/rat) into both the dorsal and ventral CA1 regions retarded spatial learning, indicating that the action of GAL depends on an intrahippocampal network. Infusions of GAL (3 nmol/rat) in the dentate gyrus, containing mainly GAL-R2 receptor mRNA and a high degree of GAL–NA coexistence, retarded spatial learning. This suggests that the GAL-R2 receptor subtype has a particularly important role in modulation of afferent input from the cerebral cortex. Analysis of memory performance in a probe trial suggested that intrahippocampal GAL primarily acts via disruption of acquisition mechanisms. However, when there exists a concomitant cholinergic dysfunction, GAL can also play a role in memory.

GAL given icv produced a long-lasting inhibition of 5-HT release *in vivo* in the ventral HPC indicating that GAL is a potent inhibitor of mesencephalic 5-HT neurotransmission. The *in vivo* actions on 5-HT release appears to be mediated by stimulation of GAL receptors at the level of the raphe nuclei. In addition, centrally administered GAL has been shown to attenuate 5-HT_{1A}-mediated responses in the forebrain. These *in vivo* actions occur at surprisingly low doses (3 nmol/rat, icv) suggesting that GAL or possibly GAL fragments infused in the ventricular space can exert long-term physiologic effects. GAL (icv) can also modify the mRNA expression of the 5-HT_{1A} receptors and GAL itself in the dorsal raphe, suggesting that GAL also exerts effects at the prejunctional level.

These results suggest an important role for GAL within the septo-hippocampal cholinergic projection and the ascending 5-HT pathways of importance for learning and memory with potential relevance for Alzheimer's disease and aging.

231P CHOLINERGIC COMPONENTS OF COGNITIVE AND NEUROPSYCHIATRIC FEATURES OF HUMAN BRAIN AGING

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It has long been established that the basal forebrain cholinergic system is involved in disorders of the aging brain such as Alzheimer's disease [AD], and several cholinesterase inhibitors are now licensed for treatment.

More recently it has been apparent that cholinergic neuropathology is more global than originally thought - in terms of the number of disorders in which this system is affected, the cholinergic systems other than basal forebrain involved, and range of associated cognitive and non cognitive symptoms. Thus, in addition to AD, cholinergic abnormalities have been reported in Parkinson's disease and Dementia with Lewy bodies (DLB), Progressive Supranuclear Palsy, Vascular dementia, head injury and Down's syndrome [and also in other developmental disorders such as Rett's syndrome and Autism]. Brain areas affected by cholinergic abnormalities in these disorders include not only hippocampus, neocortex and basal forebrain, but also striatum, thalamus and brainstem.

Symptoms that have been correlated in clinico-pathological studies with cortical abnormalities include: global cognitive function, attentional deficits, visual hallucinations, delusions and disturbances in consciousness. In response to treatment with cholinesterase inhibitors a number of different symptoms are relieved, including delusions, hallucinations, apathy and agitation. These treatment effects are particularly pronounced in patients with DLB in whom cortical Alzheimer-type pathology is variable or absent. In attempting to provide a unitary basis for the diverse range of clinical features associated with cholinergic pathology, it has been suggested that key cholinergic pathways are involved not just in selective attention but in the more specific process of conscious awareness

There has been some debate about how early cholinergic deficits occur in diseases such as AD, with some autopsy and in vivo imaging studies indicating that abnormalities in presynaptic markers such a choline acetyltransferase or the vesicular acetylcholine transporter only at more advance stages of the disease, and other studies indicating changes in acetylcholinesterase and nicotinic receptors at early stages

Most interestingly long term treatment studies suggest that cholinotherapy may be disease stabilizing and possible mechanisms of this effect include nicotinic or muscarinic receptor mediated promotion of the normal as opposed to amyloidogenic processing of the amyloid precursor protein

For the modulatory neurotransmitter system discovered after the major monaminergic systems, acetylcholine has emerged as the most important in relation to sympomatology and possibly also aspects of disease progression in many types of degenerative dementia.

232P THE EFFECTS OF HORMONE REPLACEMENT ON COGNITIVE FUNCTION IN ELDERLY WOMEN

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The attitude towards Hormone Replacement Therapy (HRT) has seen a turbulent change over the last decade. While the early nineties showed a growing enthusiasm for its biological potential to protect the ageing brain, it became gradually apparent that the human studies all had methodological problems and conflicting results (Yaffe *et al.*, 1998). Last year the well designed and long awaited Alzheimer's disease (AD) trial was completed and its results seem clear. The question remains whether HRT can delay the onset of AD. Large controlled studies with healthy women are currently underway and should be able to answer these questions within 6 to 10 years time.

However, both the AD and the healthy women's trials use Premarin, the most widely prescribed HRT consisting mainly of conjugated estrogens. Positive effects of Premarin in AD have been described [see Yaffe *et al.*, 1998] but the studies with healthy women (Goebel *et al.*, 1995; Ditkoff *et al.*, 1991; in Yaffe *et al.*, 1998 and Shaywitz *et al.*, 1999) showed no effects on tests. It is conceivable that other preparations (e.g. containing estradiol, the most potent estrogen) could show effects, especially using the more sensitive within subjects design (Hogervorst *et al.*, 1999).

It is also possible that estrogens only have time-limited effects. Earlier animal work showed that continued treatment with estradiol counteracted its initial beneficial effects (Gibbs RB, 1994). This may be in line with the positive effect of HRT on some of the cognitive measures in the AD study after 8 weeks, which then declined, and the difference favouring the placebo group on other scales after 12 months. Gibbs (1994) suggested that the administration of a progestagen could help maintain estrogen's beneficial effects. However, other positive effects of estrogens may be reversed by progestagens (Hogervorst *et al.*, 1999). Hence, while the above study has clearly shown no beneficial long term effect of Premarin in women with AD, the last word about the effectiveness of HRT on cognitive functions of elderly women has not been said

Gibbs RB. Estrogen and nerve growth factor-related systems in brain. *Ann NY Acad Sci* 1994;**14**:165-199.

Hogervorst E, Boshuisen M, Riedel WJ, *et al.* The effect of hormone replacement therapy on cognitive function in elderly women. *Psychoneuroendocrinology.* 1999;**24**:43-68.

Shaywitz SE, Shaywitz BE, Pugh KR, *et al.* Effects of estrogen on brain activation patterns in postmenopausal women during working memory tasks. *JAMA* 1999;**281**:1197-1202.

Yaffe K, Sawaya G, Lieberburg I & Grady D. Estrogen therapy in postmenopausal women. *JAMA* 1998;**279**:688-695.

233P POSITIVE MODULATORS OF AMPA-RECEPTORS (AMPAKINES)

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Much of my research has been concerned with understanding the relationship between brain activity patterns, synaptic plasticity and learning. Thus, my work has mainly focused on the interaction between the hippocampal theta rhythm, LTP and memory formation. My goals are two-fold: first, to extract the key neurobiological variables that are essential for understanding how memories are stored, and second, to use this information for the development of therapeutic compounds directed at memory disorders associated with aging and disease.

In this symposium I will focus on aspects of my work that deal with the relationship between AMPA receptor modulation, LTP induction and memory formation. Over 80% of receptors in the brain are glutamatergic, roughly 2/3 of which are AMPA receptors, located both on principal cells and interneurons. The AMPA receptor mediates fast excitatory transmission in the forebrain and is crucial for transmission of information within and between brain regions. It is also an essential component of the machinery involved in the induction of long-term potentiation (LTP) under standard physiological conditions.

Specifically, high-frequency afferent stimulation used to induce LTP results in spatial and temporal summation of fast excitatory synaptic responses mediated by the AMPA receptor, the extent of which directly determines the degree of voltage-sensitive NMDA receptor activation and thereby the amount of LTP that occurs. I will describe a series of physiological and behavioral experiments that examined the effect of Ampakines, a class of compounds designed to cross the blood-brain barrier and selectively enhance AMPA receptor-mediated synaptic currents via allosteric modulation. We found that facilitation of AMPA receptor-mediated responses with Ampakines readily promotes LTP induction in hippocampal slices by reducing the amount of afferent stimulation needed to produce maximal LTP, and significantly increases the amount and duration of LTP in area CA1 of freely moving rats. We, as well as other laboratories, further found that Ampakines, by selectively enhancing the operation of the AMPA receptor, reliably promote learning across a broad range of behavioral paradigms in rats. Moreover, studies conducted in humans indicate that Ampakines are effective at enhancing recall in aged subjects and are able to improve memory in various cognitive tests.

234P DIVERSE SIGNALLING BY 5-HT RECEPTORS

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Fourteen different receptor subtypes might be regarded as a diversity that is sufficient to accommodate the wide-ranging physiological roles of 5-hydroxytryptamine (5-HT). However, it is becoming clear that, for 5-HT as for other neurotransmitters, the concept of a receptor as gatekeeper for a specific cellular process or event is too restrictive.

Multiple receptor-mediated biochemical cascades can be activated in cells in response to an agonist by a number of mechanisms. Whereas it is well established that different agonists do not necessarily elicit the same magnitude of response, they may probably also select between various possible signal transduction pathways. Receptor signalling may be diverse *via* a single receptor subtype as a consequence of specific agonist: receptor: G protein interactions.

5-HT receptors are even more heterogeneous when considering that the amino acid sequence of these receptor subtypes may vary from individual to individual, and the increasing number of receptor isoforms due to alternative splicing and RNA editing of 5-HT receptor transcripts. Activation, in particular the constitutive, agonist-independent one, for some of these receptor isoforms has been reported to be altered. This implies that ligands with similar binding affinities may display different pharmacological properties (partial agonist, antagonist or inverse agonist) *versus* these receptor isoforms dependent on their activation state. Therefore, intervention with receptor ligands to modify hampered neurotransmission pathways is a difficult task and needs to consider the growing evidence of diversity by G protein-coupled receptor signalling.