Instituto Juan March
de Estudios e Investigaciones

149 CENTRO DE REUNIONES INTERNACIONALES SOBRE BIOLOGÍA

Workshop on

Synaptic Dysfunction and Schizophrenia

Organized by

P. Levitt, D. A. Lewis and J. DeFelipe

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Introduction

P. Levitt, D. A. Lewis and J. DeFelipe

The biological substrate of disorders of cognition, emotion and thought remains a central problem of clinical and basic neuroscience. Of the diseases with the greatest negative economic and health impact on the human population, 8 of the top 10 are neuropsychiatric in nature. Indeed, improvements in the treatment and prevention of these disorders are likely to have the greatest impact on reducing disability in the populations of developed countries in the future.

Converging evidence from studies in both humans and animals indicates that dysfunctional communication between specific populations of neurons may be the principal alteration in schizophrenia, a disease that afflicts approximately 1% of the world's population. Our basic understanding of the molecular and cellular mechanisms that mediate synaptic function has never been greater, providing an opportunity to integrate basic biological concepts of synaptic chemistry and physiology with disease-related alterations in the genes and proteins that mediate these functions.

We gathered investigators, who are experts in the fields of neuropsychiatry, synaptic function and neuronal communication, to exchange information that will facilitate a more sophisticated understanding of the biological bases of schizophrenia. While these participants had not before been assembled, we believe their interactions will promote the development of novel, integrative approaches to the study of the etiopathogenesis of schizophrenia and to the identification of new targets for therapeutic interventions.

The meeting was organized around five scientific themes to address the difficult challenge of translating our understanding of the clinical disorder into fundamental biological mechanisms that underlie the etiology and pathogenesis of schizophrenia: 1) *Functional Architecture of Cortical Circuitry and Its Dysfunction in Schizophrenia.* Schizophrenia involves the dysfunction of cortical microcircuits, yet we are still at a very early stage in a detailed understanding of the very cellular and physiological components that altered by the disease. Investigators who focus their studies on the functional attributes of circuitry of the cerebral cortex and thalamus are using technically sophisticated approaches to provide remarkable details of both macro- and microcircuitry. This framework allowed the group to translate the newest concepts towards an understanding of the disrupted circuitry in schizophrenia. 2) *Neuromodulators and Their Alterations in Schizophrenia.* Both basic and clinical research has implicated abnormal functioning of neuromodulatory systems, principally the monoamines, in schizophrenia. To date, the most prevalent and effective pharmacological treatments alter the activity of these neuromodulators. Investigators reported on the use of a variety of technical strategies, to elucidate the roles for these essential neurochemical components in mediating the functions that are abnormal in schizophrenia. Participants were provided with an opportunity to probe the relationship between observations of behavioral and physiological dysfunction in animal models and the functional features of these cortical systems in the context of schizophrenia. 3) *Synaptic Function: Understanding the Pathophysiology of Schizophrenia.* Accumulating evidence suggests that the dysfunction of synapses, the major element through which neurons interact, underlies the pathophysiology that characterizes schizophrenia. Sophisticated molecular and physiological approaches by scientists at the meeting facilitated remarkable progress

defining the cellular and molecular components that are necessary to assemble and maintain mature, functional synapses. The current understanding of the presynaptic elements that control neurotransmitter release, and postsynaptic components that define the responsiveness of neurons in circuits will be placed in the context of new findings that implicate problems of the synaptic machinery in schizophrenia. 4) *Development and Plasticity: Role in the Pathogenesis of Schizophrenia*. The concept of a developmental etiology of schizophrenia was popularized during the last decade, but the theory has been difficult to crystallize from a cellular and molecular perspective of disrupted cortical circuits. In particular, the field wrestles with the polygenic and epigenetic nature of schizophrenia that is characterized by a significant temporal delay in the expression of overt symptoms of the disorder. These issues were addressed by investigators who have made seminal contributions to our understanding of synapse and receptor assembly in cerebral cortex, and the developmental and functional plasticity exhibited by these systems with particular genetic perturbations. This group was challenged with discussing the mechanisms that drive the assembly of dysfunctional circuits and the untapped potential for regulating plasticity as a strategy for intervention and treatment. 5) *Testable Models and Strategies*. The adaptive processes that occur in schizophrenia are likely to be initiated by a combination of genetic susceptibility and environmental perturbation. The issues discussed in the first four sessions provided the framework for the scientists in the last session to elaborate on new concepts of schizophrenia based on disruption of synaptic function. Testable models may provide new approaches to study the pathophysiology and to help define the etiology of the disease.

Thus, these scientists had the opportunity to integrate increasingly complex data from neuroimaging and circuit analyses, which continue to provide important details of the neural networks that are dysfunctional in schizophrenia, with the fundamental molecular and cellular processes of synaptic communication that form the basis for the disease. Rather than segregate investigators according to their specific technical approaches or broader laboratory interests, we purposely organized the sessions in which data on a key topic, extracted from studies of normal and disease states, was presented and discussed in an integrated fashion. Thus, each had broad representation and expertise. This approach facilitated the interactions among scientists with different backgrounds and perspectives, enhanced the cross-fertilization of ideas from different disciplines, and emphasized the importance and opportunities for translating fundamental discoveries in synaptic function and cortical circuitry into strategies for understanding neuropsychiatric disorders.

Pat Levitt, David A. Lewis and Javier DeFelipe

Session 1: Cortical inhibitory circuitry and its dysfunction in schizophrenia
Chair: Patricia S. Goldman-Rakic

Human GABAergic cortical microcircuitry: catecholaminergic interneurons

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In the cerebral cortex, information flows through synapses arranged in a highly organized network of intricate neuronal circuits. Modifications in this circuitry can lead to profound neurological and psychiatric diseases, and a number of alterations in cortical function probably arise from the impairment of specific intracortical circuits. Thus, it is fundamental to understand both normal and altered cortical circuitry for us to gain a better insight into cortical diseases.

Most of what is known about human neuronal circuitry is based on data obtained from the cortex of non-human mammals. More specifically, the majority of studies have been carried out on the primary sensory areas of the monkey, cat, rat and mouse. Although some aspects of neocortical organization appear to be universal across all species, at the microanatomical, molecular and hodological levels, there are significant differences between the primary and non-primary areas, and between homologous cortical areas in different species. Thus, relatively little is known about human neocortical circuitry. Cortical tissue removed during the course of surgery to treat patients with intractable epilepsy or to gain access to tumors located in subcortical structures, represents an excellent opportunity to study the human neocortex. This resected tissue can be immersed immediately in fixative, eliminating the influence of postmortem factors. Furthermore, the development of certain microanatomical tools, and a variety of light and electron immunocytochemical techniques, has now made it feasible to perform detailed studies of cortical microrganization directly in the human cerebral cortex. As a result, novel aspects of neurochemical and microanatomical human cortical organization are being revealed.

Processed information leaves the cortex along the axons of pyramidal cells to reach its destination in other cortical areas or subcortical nuclei. Our current understanding of the synaptic organization of the cerebral cortex, and of how this information flow occurs, depends to a large extent on our knowledge of the synaptic inputs to pyramidal cells. In general, it is considered that the basic cortical microcircuit is composed of a pyramidal cell and its input-output connections. Inhibitory inputs, which mostly originate from GABAergic interneurons, terminate on the dendrites, soma and initial axon segment of pyramidal cells. Amongst the cortical interneurons, one group has been identified that contain tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine synthesis. Because catecholamines have been shown to be involved in schizophrenia, the study of these interneurons may be particularly relevant for this disease. It is particularly interesting that in the human, these TH neurons have

a peculiar laminar distribution and charateristic neurochemical attributes. Notably, they are found almost exclusively in layers V-VI and only 50% of them contain GABA. We found that neurons that contain TH do not contribute to three of the major GABA-ergic interneuron populations: double bouquet cells, chandelier cells and basket cells. We also estimated that 26% of TH neurons also contain nitric oxide synthase. In addition, we observed that TH fibers preferentially form connections with dendrites, and that pyramidal cells filled with Lucifer Yellow and reconstructed from serial confocal microscope images received a remarkable constant number of contacts per neuron from TH axons (15 to 31; average of 23). As previous studies have implicated both nitrergic neurons in deep cortical layers and the cathecolaminergic system in the pathogenesis of schizophrenia, it will be of interest to study these TH neurons and their innervation of pyramidal cells to determine whether they are specifically affected in schizophrenic patients.

Significance of $GABA_A$ receptor heterogeneity in cortical circuits

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$GABA_A$ receptors mediate most of the fast inhibitory neurotransmission in vertebrate CNS by gating Cl^- ions through an integral membrane channel. $GABA_A$ receptors form multimeric complexes assembled from a family of at least 21 constituent subunits (α1-6, β1-4, γ1-4, δ, ρ1-3, θ, π) (Barnard et al., 1998). The subunit composition and stoichiometry of native $GABA_A$ receptors have not been elucidated but evidence favors the existence of pentameric complexes containing 2α/2β/1γ subunit variants. Immunochemical, pharmacological, and functional analyses of $GABA_A$ receptors give convergent results that the majority contains a single type of α and β subunit variant, with the α1β2γ2 combination representing the largest population of $GABA_A$ receptors, followed by α2β3γ2 and α3β3γ2. Receptors containing the α4, α5, or α6 subunit, as well as the β1, γ1, γ3, δ, π and θ subunit, form minor receptor populations.

Pharmacological analysis of $GABA_A$ receptors immunoprecipitated with antibodies against specific α subunit variants allows differentiating between $GABA_A$ receptor subtypes. The α1-, α2-, α3-, and α5-$GABA_A$ receptors correspond to diazepam-sensitive receptors, whereas α4- and α6-$GABA_A$ receptors are insensitive to diazepam (Benson et al., 1998). Functionally, distinct subunit-specific properties have been identified in both recombinant and native receptors, supporting the concept that $GABA_A$ receptor heterogeneity is a major facet determining the functional properties of GABAergic inhibitory circuits. In particular, the type of α subunit determines the kinetics of receptor deactivation and the presence of the δ subunit results in markedly increased agonist affinity and apparent lack of desensitization. Immunohistochemical analyses based on the visualization of α subunit variants revealed a region- and neuron-specific distribution pattern of $GABA_A$ receptor subtypes that is largely conserved across species, suggesting that individual subtypes are present in distinct neuronal circuits. The specificity of subtype expression is underscored by the remarkable selectivity of action of diazepam in knock-in mutant mice carrying "custom-made" diazepam-insensitive $GABA_A$ receptor subtypes. Thus, abolition of diazepam binding on α2-$GABA_A$ receptors in vivo by exchanging the conserved histidine 101 residue with an arginine residue results in selective suppression of the anxiolytic action of this drug, whereas anxiolytic drugs acting by other mechanisms are unaffected (Löw et al., 2000). This finding indicates that α2-$GABA_A$ receptors are strategically located in circuits mediating the anxiolytic action of diazepam. Likewise, the sedative effects of diazepam are abolished, even at high dose, in mice carrying $\alpha1^{H101R}$-$GABA_A$ receptors, while its anxiolytic action is fully retained in these mice (Rudolph et al., 1999; McKernan et al., 2000). Therefore, brain circuits containing α1-$GABA_A$

receptors mediate sedation and activation of these receptors by diazepam is ineffective for relieving anxiety.

The cellular corollary of the specificity of diazepam action is the synapse-specific distribution of $GABA_A$ receptor subtypes, in particular in neurons expressing multiple $GABA_A$ receptor subtypes, such as hippocampal pyramidal neurons. These cells express a high level of $\alpha 1$, $\alpha 2$, and $\alpha 5$ subunit, along with $\beta 1$-3 and $\gamma 2$ subunit, suggesting that they form at least three main $GABA_A$ receptor subtypes. $\alpha 1$-$GABA_A$ receptors are located postsynaptically in a majority of somatodendritic synapses and to a lesser extent in the axon initial segment; in contrast, $\alpha 2$-$GABA_A$ receptors are particularly abundant in the axon initial segment and are only few in somatodendritic synapses (Nusser et al., 1996). Finally, $\alpha 5$-$GABA_A$ receptors have an extrasynaptic localization, being distributed throughout the somatodendritic compartment of hippocampal pyramidal cells without being aggregated at postsynaptic sites. Strikingly, in the soma of hippocampal pyramidal cells, $\alpha 1$- and $\alpha 2$-$GABA_A$ receptor subtypes are segregated to distinct synapses formed by two separate populations of basket cells. Parvalbumin-positive basket cells form GABAergic synapses containing the $\alpha 1$ subunit, whereas cholecystokinin-positive basket cells form GABAergic synapses containing the $\alpha 2$ subunit (Nyiri et al., 2001; Klausberger et al., 2002), suggesting that presynaptic afferents modulate targeting of receptor subtypes to specific types of synapses. Additional evidence, notably the presence of CB1 cannabinoid receptors in CCK-positive terminals (Katona et al., 1999), indicates that these two types of basket cell correspond to distinct neuronal circuits.

Finally, a substantial proportion of $GABA_A$ receptors are found extrasynaptically, diffusely distributed in somato-dendritic membranes. These receptors likely contribute to normal GABAergic inhibition in vivo and are involved in the expression of specific behaviors. This was shown by in mice with targeted mutations of the $\alpha 5$ subunit gene (Collinson et al., 2002; Crestani et al., 2002). For example, a selective role of $\alpha 5$-$GABA_A$ receptors in hippocampal-dependent tasks was reported in a mouse line carrying a H105R point mutation in the $\alpha 5$ subunit gene. Unexpectedly, this mutation caused a profound reduction of $\alpha 5$-$GABA_A$ receptors selectively in the hippocampal formation and was correlated with altered performance in a delay trace-conditioning task (Crestani et al., 2002). In contrast, learning of non-hippocampus-dependent behavioral tasks was not affected, pointing to a selective role of extrasynaptic $GABA_A$ receptors for proper hippocampal function.

In the neocortex, although the details of synaptic circuits remain largely to be worked out, similar arrangements are very likely. In addition, a distinct laminar and areal segregation of $GABA_A$ receptor subtypes is evident, suggesting the contribution of different receptor subtypes in cortico-cortical and cortico-subcortical circuits (Fritschy & Mohler, 1995). Major differences are also seen in the thalamo-recipient layers between primary and secondary sensory areas. The adult pattern is established during the period of synaptogenesis, with major alterations in the expression and cellular distribution of receptor subtypes, indicating

that a given subunit can have distinct functions at various stages of brain development (Paysan et al., 1997).

The significance of $GABA_A$ receptor function is underscored by the multiple neurological and psychiatric diseases for which an alteration in the GABAergic system has been postulated, including epilepsy, anxiety disorders, ethanol dependence, Huntington's disease, Angelman syndrome, and schizophrenia. It is therefore of crucial importance to unravel the mechanisms underlying the selective expression of $GABA_A$ receptor subtypes in specific neuronal circuits under normal and pathological conditions.

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Selective alterations in cortical GABA circuits in schizophrenia

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Critical cognitive deficits in schizophrenia are associated with dysfunction of the dorsolateral prefrontal cortex (DLPFC). In addition, markers of GABA neuron transmission are altered in the DLPFC of subjects with schizophrenia. Understanding the contribution of altered GABA neurotransmission to the cognitive deficits of schizophrenia requires knowledge of the functional circuitry of the different classes of DLPFC GABA neurons, of the changes that these neurons undergo during developmental periods of relevance to the pathophysiology of schizophrenia, and of the identification of the specific populations of GABA neurons and circuits that are altered in schizophrenia. This presentation will describe recent data from these three types of investigations that converge upon the parvalbumin (PV)-containing class of DLPFC GABA neurons as being particularly vulnerable in schizophrenia.

First, in contrast to the calretinin-containing subset of GABA neurons, PV-containing cells receive inputs from dopamine axons, the mediodorsal thalamus, and the axon collaterals of neighboring pyramidal cells, all of which appear to be involved in the working memory processes mediated by the DLPFC. In addition, electrophysiological studies in an *in vitro* slice preparation of monkey DLPFC indicate that these two classes of GABA neurons also differ in their responses to the patterns of sustained excitatory activity recorded *in vivo* in the DLPFC during working memory tasks.

Second, during both the neonatal and adolescent periods of development, substantial changes occur in markers of presynaptic neuronal activity, synaptic levels of GABA, and postsynaptic receptors for chandelier neurons and wide arbor neurons, the two principal classes of PV-containing GABA neurons.

Third, the PV-, but not the calretinin-containing, subclass of GABA neurons demonstrate decreased gene expression in subjects with schizophrenia, with at least some of these alterations evident at the level of the cognate proteins. These alterations are accompanied by an increase in the α_2 subunit of the $GABA_A$ receptor localized to pyramidal neuron axon initial segments, the synaptic target of PV-containing chandelier neurons. In addition, the altered gene expression in PV-containing neurons is strongly associated with reductions in BDNF-trkB signaling, suggesting a possible mechanism for the GABA changes.

Thus, these data suggest that a subpopulation of GABA neurons are altered in the DLPFC of subjects with schizophrenia, that these alterations are associated with specific

circuitry changes that arise during development, and that these abnormalities may contribute to the types of working memory disturbances that are central to the cognitive deficits observed in schizophrenia. Understanding these abnormalities in the context of the affected neural circuits may reveal novel therapeutic strategies for ameliorating the cognitive dysfunction of schizophrenia.

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Factor analysis classification and outlier detection of cortical neurons

Hamzei-Sichani, F., Kozloski, J., Yuste, R.

Classification of neurons is of central importance to Neuroscience, yet this task has traditionally been done using qualitative criteria, which are not universally accepted. We have explored the use of quantitative methods to classify neocortical interneurons, using a morphological and electrophysiological database from 30 randomly selected interneurons from layer 5 of mouse primary visual cortex. The data was examined using a combination of multivariate techniques including factor analysis (FA) and principal component & classification analysis (PCCA). These techniques allowed us to study the underlying structure of the data (FA) and the characterization of the resultant clusters (PCCA). For the 30 neurons, 34 measured morphological and physiological variables produced 3 principal orthogonal factors in descending order of their contribution to the total variance of the data. The first factor detected variables related to action potential characteristics and spike train. The second factor selected morphological variables, whereas the third factor identified differences in after-hyperpolarizations. Using these three factors, application of PCCA resulted in the neurons being grouped in 3 unique clusters, composed of 26 of the 30 neurons. The three clusters were characterized by a combination of electrophysiological and morphological characteristics. One cluster was composed of fast spiking cells, another by low threshold spiking cells and the third was a mix. These techniques allowed us to use a quantitative unbiased analysis to define neuronal classes as an important first step in understanding neuronal circuits.

Session 2: Neuromodulators and their alterations in schizophrenia
Chair: David A. Lewis

Prefrontal cortex: Structural and functional aspects of cognitive dysfunction in schizophrenia

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The stimulus-independent sustained activation of prefrontal neurons and their content-specific coding of information constitute the fundamental cellular basis of the brain's working memory functions. We have proposed that a breakdown in the circuitry mediating these functions may be responsible for the cognitive deficits which are increasingly considered core problems in schizophrenia (Goldman-Rakic, 1987; 1994). The two properties – persistent neuronal firing – that outlasts a stimulus by seconds, and preferential coding of stimulus parameters derive both from biophysical properties and network dynamics. My laboratory is focused on elucidating these mechanisms as they pertain to and underpin human mental capacity and its dissolution in numerous disorders.

A first order hypothesis for understanding persistent activity in prefrontal cortex is that it is mediated, in part, by recurrent excitation among pyramidal neurons. We have used two methods to examine these interactions. Simultaneous recording of multiple neurons in monkeys trained on working memory tasks to address questions of functional interactions between pairs of pyramidal cells. Our findings from diverse studies have established that microcircuits exist for memory phenomena and that they are constrained both by local circuit organization and by content (Constantinidis et al. , 2001). We have also studied the modulation of elemental neuronal interactions by dopamine both in cortical slices (Gao et al., 2001) and in the behaving animal (Williams et al., in preparation). Our findings indicate that dopamine depresses excitatory transmission between pairs of pyramidal neurons by actions at presynaptic D1 receptors both *in vivo* and *in vitro*. A presynaptic mechanism is supported by electronmicroscopic evidence that D1 receptors are located on axon terminals of excitatory afferents. These findings indicate, therefore, that a functional state of hypoglutamatergia can result from the actions of dopamine D1 receptors. Other equally important actions of dopamine in the prefrontal cortex arise from its modulation of inhibitory circuits that are engaged both in the spatial and temporal properties of prefrontal cognitive operations. (Rao et al., 1999; 2000; Constantinidis et al., 2002; Gao and Goldman-Rakic, in press).

The importance of the dopamine hypothesis of schizophrenia has been well known for half a century and will be further discussed in the context of recent studies on the existence of both D1 and D2 dopamine-receptor interacting proteins. As an example, the functional significance of D1 signaling mechanisms in schizophrenia has been highlighted by the recent finding that calcyon, an intracellular protein that interacts with D1 family receptors, is abnormally high in schizophrenic patients (Koh et al., in press). Further evidence from a recent genetic analysis has found that the region of chromosome 10 where the calcyon gene is located is at risk for schizophrenia or bipolar disorder (Jorgenson et al., 2002). Abnormalities

in the prefrontal cortex of patients have also been observed in two recent PET studies of D1 receptor binding potential (Okubo et al.,1997; Abi-Dargham et al., 2002). Both studies found significant correlations between D1 binding potential and cognitive performance.

The studies to be reported are building a highly differentiated portrait of prefrontal functional circuitry and its dopamine-mediated signaling mechanisms which constitute the brain's information processing system.

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Imaging dopamine transmission with Positron Emission Tomography in functional subdivision of the human striatum

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This presentation will illustrate how PET imaging, with state-of-the-art resolution and analytical techniques, provides a unique tool to study neurochemical pathways in the living human brain. The human striatum can be organized into limbic, associative and sensorimotor subdivisions, which process information related to emotional, cognitive and motor function. Dopamine (DA) projections ascending from the midbrain provide important modulatory input to these striatal subregions. The aim of this study was to compare dopamine transmission and stimulation of dopamine D_2 receptors following the administration of amphetamine in these functional subdivisions of the human striatum. D_2 receptor availability (V_3") was measured with Positron Emission Tomography (PET) and [^{11}C]raclopride in fourteen healthy volunteers under control conditions and following the intravenous administration of amphetamine (0.3 mg/kg). Amphetamine induced a significantly larger reduction in D_2 receptor availability (ΔV_3") in limbic (ventral striatum, -15.3± 11.8%) and motor (postcommisural putamen, -16.2 ± 9.6%) regions compared to associative regions (caudate and precommisural putamen, -8.1 ± 7.2%). This region of interest analysis was then confirmed by a voxel-based analysis. Subjects were also asked to rate their subjective experience following amphetamine, and reported an increase in euphoria which was associated with a greater ΔV_3" in limbic and motor regions, but not in the associative regions.

These results demonstrate significant differences in the DA response to amphetamine between the functional subdivisions of the human striatum. The mechanism behind these regional differences in amphetamine-induced DA release remain unclear, but could be related to the pattern of asymmetrical feed forward mediating the integration of limbic, cognitive, and motor function via the DA cell bodies in the ventral midbrain. The implication of these findings for the development of addictions and psychoses will be discussed.

Keeping the balance of 5-HT in the developing brain : a tightly controlled process

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The developmental role of serotonin (5-HT) has been stressed since the early studies of Buznikov on sea urchin embryos. Subsequent studies in the mammalian central nervous reported that 5-HT had a wide variety of effects, on proliferation, migration, neurite outgrowth and synaptogenesis. These studies led however to a somewhat blurred picture of what 5-HT is actually doing because of the wide variety of 5HT receptors and the difficulty in obtaining complete and selective inactivation or stimulation of these receptors with classical pharmacological compounds. Molecular genetic approaches in the mouse have recently contributed to sharpen the picture, and have allowed to clarify some of the targets of 5-HT during development. These studies also demonstrated the functional consequences of disrupting normal 5-HT metabolism *in vivo* during critical periods of development, both as concerns the wiring of the brain and the behaviour of adult animals.

One important concept that emerged from these studies is that 5-HT levels are tightly controlled, during development, by 3 major genes of the clearance pathway of 5-HT : the serotonin transporter (SERT), the vesicular monoamine transporter (VMAT2) and the monoamine oxidase A (MAOA). All 3 genes are broadly expressed at early time points in embryonic life and during late postnatal life. These genes are expressed in a wide population of non-monoaminergic neurones, particularly in the major sensory pathways. This transient developmental expression is shut down fairly sharply as the neurones mature in a cell autonomous manner. Disrupting the function of any of these 3 genes alters the level of brain 5-HT, either augmenting it, in the MAOA and SERT-knockout mice or decreasing it, in the VMAT2 knock outs. The consequences of these genetic changes on brain development are generally subtle : the basic structure of the brain is not modified but a number of alterations are found in the late process of maturation of the brain, such as the refinement of topographic axonal maps, the trophic-dependent cell survival, and the dendritic differenciation of several neuronal populations. These changes are important for normal adult brain function, since a number of behavioural changes are observed in these mouse strains such as altered aggressive behaviour, learning deficits, vulnerability to drug intake, or increased anxiety-related behaviour.

We will discuss in greater detail the effects of 5-HT excess or lack of 5-HT in the maturation of the barrel cortex in the primary somatosensory area and in the cingulate cortex. Different 5-HT receptor subtypes appear to be implicated in the maturation process of the various cellular components of this cortical circuitry - the 5-HT1B receptor are important for the maturation of the thalamocortical neurones and the 5-HT2 receptors in the maturation of the cortico-cortical neurones. This shows that only a thorough genetic molecular analysis will allow to completely decipher and tease out the once global "developmental effects" of 5-HT.

Antipsychotic treatment effects on the clinical and pathophysiological course of brain pathomorphology in schizophrenia

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Evidence suggests that morphologic abnormalities reflecting cellular and circuit based disturbances which underlie major mental illnesses like schizophrenia are either inborn and developmental,or progressive and possibly degenerative in nature or both. Despite the substantial evidence and intellectual appeal of the "Neurodevelopmental Theory" of schizophrenia, the progressive and deteriorating course of the illness is undeniable. Moreover, an increasing number of methodologically rigorous longitudinal neuroimaging studies which examined patients prospectively from their early stages of illness have demonstrated that regionally specific morphologic changes occur over, and in relation to, the course of their disorder. Among the questions that these findings have raised is what are the effects of treatment on brain morphology and in the progressive changes that occur.

Preclinical studies in rodents and subhuman primates have reported psychotropic drug induced neuroplastic alterations occurring in cell function and ultrastructural development that can produce changes that are morphometrically detectable. Human studies have to an extent replicated these findings. One problem in studying treatment effects on brain structure and function in animals is that they do not simulate the potential interaction between pharmacologic effects and disease pathophysiology. Even when animal models are used one can't be certain that the model (be it pharmacolgic, lesion-based or targeted gene modification) fully simulates in-vivo disease pathophyisology.

This presentation will present findings on treatment effects on brain morphology and examine them in terms of whether they are drug effects on normative function and structure (iatrogenic) or on disease pathophysiology (therapeutic), and then consider the mechanisms by which these occur (i.e. palliative, neuroprotective, neurogenetic).

NAA levels in the dorsolateral prefrontal region in the first years of schizophrenia are inversely related to disease duration

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Background: Magnetic resonance spectroscopy studies of schizophrenia have revealed consistently diminished N-acetyl aspartate (NAA) levels in chronic schizophrenic patients, but not in cases of recent onset. Studies on the relationship between this marker and disease duration have generally been negative, although it is also true that they have been performed on patients with long-standing illness.

Method: We have compared NAA levels in the dorsolateral prefrontal area in 16 recent-onset patients (RO; duration, 1.8 ±0.6 years), 19 chronic (CR; duration, 9.7 ± 6.1 years), and 20 healthy controls (CO). We have studied the NAA/ creatine and choline/ creatine ratios in the dorsolateral prefrontal region in both hemispheres, controlling for the effect of age.

Results: The chronic patients had significantly lower NAA/ Cr ratios in the left hemisphere compared to the recent-onset patients and healthy controls, with no difference in the Cho/ Cr ratio. There were no differences observed between controls and RO. There was a significant, inverse relationship between left side NAA/ Cr and disease duration.

Conclusions: These data support the hypothesis that prefrontal NAA levels may progressively decrease in schizophrenia. Taken within the context of the existing literature, these results indicate that this process may be limited to the first few years following onset of illness. Finding diminished prefrontal levels of NAA in schizophrenia may therefore be limited to chronic patients.

Key words: N-acetyl aspartate, schizophrenia, prefrontal, MRS, neurodegeneration, duration

Session 3: Synaptic function: Understanding the pathophysiology of schizophrenia
Chair: Patricia Gaspar

New approaches to synaptic vesicle exocytosis and endocytosis

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Kiss-and-run exocytosis is a rapid and efficient method of releasing secretory products to the extracellular medium through the transient formation of a fusion pore. During kiss and run, the vesicle membrane does not collapse into the plasma membrane, maintaining the secretory vesicle its position, identity and potentiality to perform a second round of exocytosis. In a previous report, we found that the incidence of kiss-and-run increases as the extracellular calcium concentration rises in the mM range (Ales et al. 1999, Tabares et al. 2001). These kiss-and-run events lasted only for milliseconds (average of 50 ms, called "Fast kiss-and-run") and released the same amount of transmitter than a conventional exocytosis. However, we did not demonstrate whether calcium mediated this effect extracellularly or by an indirect cytosolic calcium increase. To answer this question, we have done whole cell membrane capacitance measurements in peritoneal rat mast cells in which we could experimentally control both the extracellular and the intracellular calcium concentrations. This preparation also allows resolving individual fusion events. To monitor release, we have done simultaneous measurements of serotonin release with amperometry by positioning a carbon fiber electrode over the cell surface.

We analyzed well-defined single exocytotic fusion events from capacitance and amperometric measurements. The incidence of kiss-and-run increased when the rate of degranulation was decreased either by lowering the amount of secretagogue (GTPγS) or by dialyzing the cells with lower calcium concentrations. At similar amounts of GTPγS , the number of kiss-and-run events decreases as the cytosolic calcium concentration was raised from 100 nM to 1 μM. Simultaneous amperometric measurements revealed that these kiss-and-run events were slow (lasting on average 500 ms), and release only a small fraction of the vesicle contents. By contrast, when the extracellular calcium concentration was raised from 2 mM up to 90 mM, the incidence of kiss-and-run increased and the amount of serotonin was similar to the quantal contents of a rat mast cell granule, indicating the occurrence of fast kiss-and-run events.

These data reveal that fast kiss-and-run events are evoked by calcium acting from the extracellular medium and that these events represent a different kinetic process from the slow kiss-and-run events. We propose a kinetic model of exocytosis that account for slow and fast kiss-and-run exocytosis.

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Glutamate signalling and synaptic transmission

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Alterations of glutamatergic neurotransmission have been related to the neuronal damage observed after episodes of ischemia and hypoglycemia, as well as to the etiology of a series of neurological conditions including epilepsy, Alzheimer's disease, Huntington's chorea and amyotrophic lateral sclerosis. The cloning of a large number of glutamate receptor proteins and the discovery of their structural relationships have paved the way to most of our current understanding of the biophysical properties and the physiological role of each subtype in the mammalian brain. Of the glutamate receptor subtypes, NMDA, AMPA and kainate receptors, the latter is by far the less well understood, despite of their wide distribution in the brain.

Whilst the physiological role of these receptors is still not absolutely clear, recent advances have highlighted different aspects of their behavior (see ref 2 for a review). Progress in this field has been hampered by the lack of specific pharmacological tools. However, the discovery of a specific AMPA receptor antagonist, GYKI53655 (3,5), has made functional studies feasible. As a result, it has been proposed that kainate receptors play a role in both synaptic transmission and synaptic plasticity. In addition, it has been shown that the activation of kainate receptors modulates neurotransmitter release from a number of synapses. Indeed, in keeping with their presumptive role in epilepsy (e.g. ref. 1), kainate has been found to depress inhibitory GABA transmission in the rat hippocampus.

Kainate receptors share the membrane topology of ionotropic glutamate receptors (i.e. AMPA and NMDA receptors), they contain 3 transmembrane domains as well as a membrane domain that contributes to the ion channel wall. As a result, it was somewhat surprising to find, that inhibition of GABA release by kainate receptors in the hippocampus involved a second messenger cascade mediated by a Pertussis toxin sensitive G-protein and activation of Protein Kinase C (i.e. a rather classical metabotropic cascade; ref. 4). Since this discovery, further examples of such metabotropic activity have been reported, as well as other mechanisms through which kainate can act. However, in none of these cases has the receptor subunit involved in this activity been specified.

Hypothetically, the ionotropic and metabotropic activities of kainate receptors could be mediated through the same receptor complex. Alternatively, since the membrane topology of kainate receptors is in principle incongruous with the structure of G protein-coupled receptors, they may involve distinct or unrelated receptors. We have explored these possibilities in a simple cell model, that of cultured dorsal root ganglion (DRG) neurons.

These neurons express a rather homogeneous population of kainate receptors, predominantly containing GluR5 subunits. In a population of DRG cells, we found that kainate was able to elicit a G-protein dependent increase in $[Ca^{2+}]_{int}$. By using confocal microscopy and after application of kainate in the absence of extracellular Ca^{2+}, the levels of $[Ca^{2+}]_{int}$ were seen to increase in localized areas of the cell body, as well as in discrete spots along the neurites and close to bifurcation points. Measurements from these points indicated that the larger increases in $[Ca^{2+}]_{int}$ were in the neurites rather than in the soma. Furthermore, a brief exposure to kainate inhibited voltage-dependent Ca^{2+}-channels, a process that was sensitive to inhibitors of G-proteins and Protein Kinase C (PKC). This metabotropic effect of kainate does not seem to require ion channel receptor activity. However, it was not observed in neurons prepared from mice deficient for the ion channel forming subunit GluR5. Therefore, our results not only provide evidence for the existence of a metabotropic kainate receptor that inhibits Ca^{2+} channels, but also indicate that an ion channel forming subunit is involved independently in both ionotropic and metabotropic activities. Given that the nature of the signal transduction pathway triggered by the receptor determines the cellular response, our results indicate that kainate receptor activation may serve a number of novel functions.

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Basic developmental rules for the construction of cortical networks

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In the course of studies initiated in the late nineties, we have shown three basic developmental rules for the maturation of synaptic activity in a cortical circuit:

I) GABAergic synapses are initially excitatory because of a higher intracellular concentration of chloride in immature neurons. Consequently, the activation of GABAergic receptors and synapses lead to the generation of sodium and calcium potentials and an increase of (Ca)I by the removal of the voltage dependent Mg++ block from NMDA channels.

II) GABAergic synapses are formed before glutamatergic ones in both principal cells and interneurons and this has a morphological substrate: neurons are silent (no synapses) when they have no dendrites, they have only GABAergic synapses when they have a small apical dendrite (less than 50 uM) and they have also glutamate synapses when they have a large dendritic arbor that reaches the superficial layer. This sequence is observed earlier in interneurones (in utero in rodents at a time when pyramidal neurons are all silent); therefore, GABAergic interneurons that divide and arborize prior to the principal cells are the source and the target of the first synapses formed in the circuit and GABA is the principal excitatory agent at a time when glutamatergic synapses are absent.

III) There is in the developing network a unique pattern of network driven activity- generated by the excitatory actions of GABA in association with developing glutamatergic synapses- that provides most of the synaptic activity at an early developmental stage. We have now shown this dominating rhytm in vivo (patch recordings in the neonatal rat in situ) and in our intact hippocampi in vitro in which it is possible to study the propagation of these activities within the hippocampus and between hippocampi.

These rules appear to have been kept throughout evolution, as excit atory actions of GABA in immature neurons have been described in a large number of species and brain structures. We have suggested elsewhere (Ben-Ari, 2002) that this sequence enables to solve a dilemma that developing structures have, namely how to construct a structure without having a potentially toxic imbalance between excitation and inhibition. It is suggested that these patterns play an important role in the strategy followed by the brain to shift from a silent structure with no electrical activity and no synapses to an active one that possesses a highly diversified range of electrical signals and billion of selective synapses. Furthermore, the earliest formed GABAergic synapses are liable to alterations by the Hebbian principles and activity can also shift the type of neurons from one to the other (silent to GABA only for instance). We have recently extended this model to the primate by making the first recording of central neurons in utero in primates. Thus, these rules apply also to non-human primates and most likely to humans as well (Khazipov et al, 2001).

In the presentation, I shall also briefly review data on the role of activity in the formation of the first synapses and report that prior to synapses formation, very immature neurons have already a paracrine mode of communication by means of a non-vesicular non Calcium and non SNARE dependent release of GABA and glutamate that generates in target neurons, a slow current present only at early developmental stages. It is suggested that all these early mechanisms interfere in certain conditions with the normal construction of the brain leading to permanent deleterious conditions. This is exemplified by migration disorders that can lead to the formation of heterotopias and other disorders.

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Schizophrenia as a disease of the synapse: Gene microarray studies

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DNA microarray technologies have enabled researchers to analyze complex gene expression changes for tens of thousands of genes. This method is ideally suited for studying complex brain disorders like schizophrenia, where interactions of genetic and environmental factors hold the key to the disease. The revealed transcriptome changes often define expression signatures disease states, identifying patterns that suggest previously unknown molecular interactions.

Simultaneous assessment of the transcriptome for the majority of the human genome will lead to the individualization of diagnosis and will eventually link molecular phenotypes to the precise clinical profile of the disorder. In our recent DNA microarray studies we uncovered novel and consistent gene expression patterns in the prefrontal cortex of individuals with schizophrenia when compared to matched controls. We observed robust changes in the expression of genes related to presynaptic secretory release. Transcripts for Synapsin 2, N-ethylmaleimide-sensitive factor, SNAP, vesicular proton pumps and 14-3-3 levels were decreased in the majority of comparisons, albeit in a subject-specific manner. Furthermore, we also uncovered consistent transcripts related to GABAergic and glutamatergic transmission, which may be secondary to altered presynaptic drive. In addition, subjects with schizophrenia also reported consistent transcript decreases of multiple genes participating in various energy metabolism pathways and ubiquination, which may be also related to the presynaptic gene expression alterations.

Importantly, the presynaptic transcriptome alterations observed in schizophrenia showed a subject-specific pattern. That is, although all subjects showed decreased expression of multiple presynaptic gene products, the specific combination of affected transcripts varied from subject to subject. This finding strongly argues for a continuity of molecular phenotypes in schizophrenia that is likely to be related to the variability in the clinical manifestations of the disease.

The same microarray data set also revealed multiple changes that occur at the postsynaptic cell. These, amongst others, expression of AMPA2, regulator of G-protein signaling 4 (RGS4) and multiple genes involved in GPCR signaling were altered. Our microarray findings also seem to implicate multiple genes involved in both phosphatidylinositol and cAMP signaling pathways, which are likely consequences of more specific upstream changes in GPCR signaling, possibly involving dopamine signaling alterations.

In contrast, in a study of chronic haloperidol-treated monkeys we did not detect transcript alterations for the same presynaptic and postsynaptic genes. This suggests that the uncovered expression changes in schizophrenia may not be result of chronic antipsychotic treatment.

Undoubtedly, microarray studies and other complex transcriptome analysis methods will guide future attempts for deciphering complex molecular phenotypes associated with mental disorders. Defining disease-specific transcriptome changes will also facilitate development of animal models that closely mimic common molecular events associated with psychiatric conditions and holds a great promise for individualization of diagnosis and treatment.

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Molecular profiles of anatomically and electrophysiologycally characterized neocortical neurons

Toledo-Rodriguez M. , Blumenfeld B. ,Wu C.Z. & Markram, H

Multiple neurobilogical pathologies are result of neuronal malfunction. The understanding of these pathologies is based on the full knowledge of neuronal behavior in non -pathological conditions. Neurons influence the activity of other neurons and the microcircuit in general via neuromodulators. Up till now studies investigating neuromodulator expression and function have been limited due to methodological constrains. In order to study this diversity, we have undertaken a large-scale multiplex single cell RT-PCR study in which we examined the expression of 3 calcium-binding proteins, 10 neuropeptides, 5 synthesizing enzymes in anatomically, physiologically and molecularly characterized neurons at the single cell level. The study of more than 300 neurons using this technique lead us to the conclusion that the biochemical coexpression profiles are more complex that though till now, but nevertheless they follow three rules.

* SELECTIVITY->There is selectivity in the coexpression patterns, as only few of all the possible gene expression combinations are found.

* SPECIFICITY->Each co-expression pattern is specific for one or few morphological and electrophysiological groups.

* HOMOGENEITY->In some exceptional cases one neuropeptide is expressed homogeneously by all the members of a single anatomical group, as in the case of MCs that all express Somatostatin or SBCs that all express VIP.

Session 4: Development and plasticity: Role in the pathogenesis of schizophrenia
Chair: Pat Levitt

Synaptogenesis in the mammalian neocortex

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After neurogenesis and hodogenesis, synaptogenesis represents a crucial final step in cortical development. The cerebral cortex of the rhesus monkey provides an excellent animal model for the study of the development and plasticity of the human cerebral cortex. Description of the kinetics of synaptogenesis in several cortical areas of the macaque monkey from conception to death reveals several properties:

1) Onset of synaptogenesis is a very early developmental event, occuring in the first half of gestation, i.e. long before interactions with the environment.

2) The kinetics of synaptogenesis is complex rather than linear. Five distinct phases were identified in the whole cortical mantle of the macaque monkey. They were also described in rodent (deFelipe et al., Cerebral Cortex, 1997; 7: 619-634) and human brains, suggesting a highly conserved process through neocortical evolution. For the human cerebral cortex, due to sampling difficulties, the data are scarce and diverse (Larroche, Anat. Embryol., 1981; 162: 301-312; Huttenlocher and Dabholkar, J. Comp. Neurol. 1997; 387: 167-178; Zecevic, Cerebral Cortex 1998; 8: 245-252; Chugani, Neuroscientist 1999; 5: 29-40). We can tentatively propose an approximate timeline for each of these phases of synaptogenesis. Phase 1 (slow synaptogenesis in cortical preplate) would start near the 7th week of gestation. Phase 2 (slow synaptogenesis in cortical plate) would end near 17-22 weeks. Phase 3 (very rapid synaptogenesis in cortical plate) would start near the 26th week of gestation and end by 8-12 postnatal months in the primary visual cortex, and 2-3years in the prefrontal cortex. Phase 4 («en plateau» characterized by very high densities of synapses) lasts for 2-3 years in the visual cortex and a whole decade in the prefrontal cortex, until puberty. In phase 5, following puberty, the mean density of synapses drops to the density observed at birth. This density is maintained troughout adulthood, and then decreases significantly during senescence, until death.

3) Synaptogenesis comes into successive waves for diverse classes of synapses, progressively building the synaptoarchitectonic organization of the cerebral cortex. The developmental pattern of this organization is similar in the whole cortical mantle of the macaque and begins in the second half of gestation. Many anatomical (synaptoarchitectony, ocular dominance columns in primary visual cortex, columns in prefrontal cortex, etc..), physiological, and functional properties of these neocortical circuits are apparent prenatally and perinatally, and are thought to depend on innate mechanisms.

4) The early phases 1,2, and onset of phase 3 of synaptogenesis, are very robust developmental events. They take place even in challenging experimental (preterms, early bilateral enucleation, null mutations...) or pathological (double cortex, schizophrenia...) conditions.

5) Paradoxically, recent results suggest that «milder» affective perturbations such as maternal care (Liu D et al. Nature Neuroscience, 2000; 3(8): 799-806), or maternal deprivation (Helmeke C. et al. Cerebral Cortex, 2001; 11: 717-727) may cause discrete modifications of the synaptoarchitectony in specific cortical territories.

The early phases 1,2, and onset of phase 3 of synaptogenesis, appear controlled by genetic and epigenetic mechanisms intrinsic to the cerebral cortex. Later on, at the end of phase 3, and during phase 4 "en plateau", these intrinsic mechanisms are also modulated by the environment. This period of synaptic plasticity allows the fine tuning of the neurono-synaptic circuits involved in the progressive and coordinated maturations of all the sensory, motor, and associational cortical functions, until puberty.

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Determinants of pre- and postsynaptic interactions during development

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Regulation of the development of neuronal structures, such as axons and dendrites, affect the function of brain circuits. It is important to understand the mechanisms controlling the development of neuronal circuits in order to assess how these mechanisms are affected under pathological conditions. We used *in vivo* time-lapse imaging to determine the role of visual stimulation and glutamatergic synaptic transmission on the development of presynaptic retinal axon arbor structure in the visual system of Xenopus tadpoles. We imaged labeled retinal axons in Xenopus tadpoles in which surgery was used to induce binocular innervation of the optic tectum. Under these conditions, retinal inputs from the two eyes were initially overlapping and gradually segregated into eye-specific zones over time. This system permitted us to correlate structural dynamics of the arbor axon branches with degree of local innervation from axons from either the same eye or the opposite eye and thereby test whether patterned activity instructs axon arbor development. Axons eliminated branches preferentially from territory dominated by the eye opposite to their eye of origin, but added branches with approximately equal probability in both eye territories. NMDA receptor blockade prevented the selective stabilization of branches. In addition, the rate of branch additions was increased in sparsely innervated territory regardless of the eye of origin. These results 1) suggest that an activity-independent mechanism promotes branch additions in sparsely innervated brain regions, and 2) provide evidence for a correlation-based mechanism for the fine-tuning of axon arbor morphology in which the locations of branch stabilizations are governed by postsynaptic NMDAR-dependent signaling.

Imaging the structure and function of dendritic spines

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Dendritic spines are sites of most excitatory contacts in the cortex and have been practically inaccessible to functional measurements until the introduction of two-photon microscopy (1). Using a custom-built two-photon microscope (2) we have studied two questions:

A- Calcium compartmentalization of spines: Mechanisms of calcium influx and decay kinetics.

We have shown that dendritic spines can compartmentalize calcium (1) and explored the mechanisms of calcium influx into spines (3). Decay kinetics are controlled by (i) active calcium extrusion and (ii) diffusion of calcium from spine to dendrite (4). Although extrusion is likely to dominate in physiological conditions, the contribution of calcium pumps and diffusion varies from spine to spine, depending on the location of the spine along the dendrite, producing different sensitivity to synaptic depression (5). Decay kinetics change during spine motility (6).

B- Rapid motility of spines: Developmental regulation, mechanisms and possible functions.

We have characterized spine motility in acute and cultured slices (7). In all cells, dendritic protrusions (filopodia and spines) are highly dynamic, exhibiting a diversity of morphological rearrangements over short (<1 min) times (8). Spine motility declines during postnatal maturation, but changes are apparent in most spines in late postnatal neurons. Spine motility could be related to synaptogenesis. These morphological reorganizations are actin-based and can be regulated by Rho family GTPases (9). Finally, spines can move even with synaptic contacts (10).

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Hippocampal structure and function in schizophrenia

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Subtle yet significant volume reduction of the hippocampus is one of the most replicated brain abnormalities in schizophrenia. Neuroimaging studies have shown that smaller hippocampal volume predicts the onset of psychosis in high-risk subjects and is fully developed at the first presentation of psychotic symptoms, but may progress throughout the disease process. Postmortem studies have demonstrated that total hippocampal neuron number is not different in schizophrenia, but that subsets of neurons are functionally abnormal. Etiology and mechanism of the volumetric and cellular abnormality of the hippocampus in schizophrenia remain unclear, but appear to involve a disturbance of synaptic organization.

Recent functional neuroimaging experiments have supported the hypothesis that these structural abnormalities lead to functional deficits of the hippocampus in schizophrenia. Hippocampal recruitment during conscious recollection is impaired in schizophrenia, but simple recognition remains intact, even in the context of significant hippocampal volume reduction. Taken together, studies of hippocampal structure point to selective abnormalities of hippocampal architecture, resulting in impairments of some but not all hippocampal functions in schizophrenia.

Session 5: Models systems to investigate the underlying of etiology of schizophrenia
Chair: Guillermo Álvarez de Toledo

Molecular determinants of neurotransmitter release

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Our laboratory is interested in understanding how a presynaptic terminal contacts a postsynaptic neuron, and how the terminal secretes neurotransmitters in a tightly regulated and topologically restricted manner. Neurotransmitter release occurs by synaptic vesicle exocytosis that is triggered by influx of Ca^{2+} into the presynaptic neuron. Ca^{2+}-evoked synaptic vesicle exocytosis is one of the fastest and most tightly controlled reactions in biology, and can be modulated during synaptic plasticity. The speed and plasticity of neurotransmitter release are one of the major factors that shape the the exquisite speed and precision with which synaptic networks function.

In a longstanding program, we are investigating the molecular cascade that orchestrates neurotransmitter release. We are trying, in conjunction with other laboratories, to achieve a systematic description of all of the molecular components that mediate neurotransmitter release, and to analyse these components structurally and functionally. Although far from complete, these studies have provided an initial description of the molecular machinery that mediates neurotransmitter release, and provide clues to how the speed, precision, and plasticity of a synapse are achieved. For example, candidate Ca^{2+}-sensors for exocytosis have been identified in synaptotagmins, and integrators of presynaptic signalling at the active zone were described in RIM and Munc13 proteins. In my contribution I will discuss the current state of the project and its implications for a wider understanding of brain function.

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Increased GLT-1 expression in the prefrontal cortex of schizophrenics

F. Conti, C. Matute, M. Melone, A. Vallejo-Illarramendi and Fiorenzo Conti1, M.D.

High-affinity glutamate transporters (GluTs) are important regulators of glutamatergic transmission (Conti and Weinberg, 1999); increased Glu uptake may thus reduce glutamatergic transmission. The possibility that increased Glu uptake plays a role in the pathophysiology of schizophrenia is suggested by the demonstration that in rat frontal cortex clozapine reduces the expression and function of GLT-1 – the astrocytic GluT responsible for the greatest proportion of Glu transport - in a strong and selective manner, thereby raising Glu levels (Melone et al., 2001, 2002).

We thus studied the prefrontal cortex (PFC) of 2 drug-free schizophrenic subjects to verify whether the reduced efficacy of glutamatergic transmission reported in schizophrenia depends on the increased expression of GLT-1, the transporter responsible for most glutamate (Glu) transport.

Samples from areas 9/46 were used to study GLT-1 mRNA with DNA microarray and GLT-1 protein by immunocytochemistry and immunoblotting, and for measuring L-3H-Glu uptake. Gene expression and immunocytochemical studies showed that both GLT-1 mRNAand protein was robustly upregulated compared with controls. Functional studies revealed that Glu uptake was nearly 4-fold higher than in controls.

The hypothesis that GLT-1 is abnormally high in the PFC of schizophrenics is tenable and merits large-scale studies.

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A mouse model of mental illness based on a known risk factor

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There is considerable evidence that exposure of pregnant women to viral infection during a critical period can increase the incidence of schizophrenia and possibly autism in their offspring (reviewed in 1). We find that inducing an influenza virus respiratory infection in pregnant mice cause highly abnormal behavioral responses in their offspring, tested as adults (2). These tests include open field, novel object, social interaction and prepulse inhibition (PPI) of the acoustic startle response, and the results are consistent with abnormalities observed in subjects with schizophrenia and autism. Moreover, the deficit in PPI is corrected by anti-psychotic drug treatment, and exacerbated by psychomimetic drug treatment.

A variety of abnormalities have been observed in the cortex and hippocampus of mice born to infected mothers, including the distribution of SNAP-25 and reelin, and shrinkage of pyramidal neurons (3-5). Many of these findings are consistent with those reported for schizophrenic brain. We now find that these offspring also display a loss of Purkinje cells, specifically in lobules VI and VII of the cerebellar vermis (6). We also occasionally see what appear to be misplaced Purkinje cells in the white matter of lobule VII. In addition, the molecular layer of lobule VII appears to be thinner in these mice. This highly specific neuropathology is of particular interest because it coincides with that reported in both imaging and postmortem studies of autistic brains. This finding is also consistent with histological and behavioral abnormalities that have been linked to the cerebellum in schizophrenia.

We hypothesize that the abnormal brain development seen in the mice born to infected mothers is due to the maternal immune response, rather than to influenza infection of the fetal brain. In support of this idea, we find no evidence of viral RNA in the fetal brains of exposed mice. Moreover, a PPI deficit is found in adult mice born to mothers that were treated with poly(I:C), a synthetic, double stranded RNA that is known to evoke an anti-viral like immune response (2).

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Determinants of developmental trajectory: Models of early insult leading to delayed or long-lasting psychopathology

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A primary pathogenetic event of relatively short duration causing pre- and/or postnatal disruptions of brain development has been proposed to underlie schizophrenia. If this neurodevelopmental hypothesis is correct, then the resultant behavioral and cognitive signs and symptoms remain dormant for some time after the primary insult. This hypothesis of schizophrenia is attractive for a variety of reasons, and there are several converging lines of evidence to support such a model. However, few examples in which delayed-onset disruption of structure-function relationships have been documented. We will discuss two models, one genetic and one environmentally-induced, that result in different developmental trajectories and adaptive changes in brain structure and function.

In our investigations of a mouse (*waved-1; wa-1*) carrying a single gene mutation that causes gradual, postnatal hypomorphic expression of transforming growth factor-alpha (TGFα, peripubertal changes in brain structure and in learned fear conditioning occur, and are expressed with a gender bias[1]. It is noteworthy that in the full TGFα null mouse line in which expression of this growth factor is completely removed throughout development does not exhibit any functional or structural brain abnormalities. In contrast, we have used high-resolution structural magnetic resonance imaging of *wa-1* mice to document selective enlargement of ventricles in the absence of major loss of tissue parenchyma, changes that are reminiscent of schizophrenia. Moreover, alterations in learning appear to be at least partially due to adolescent onset of abnormal stress responsiveness in wa-1 male mice. We have assessed the levels of transcript encoding TGFα and EGFR, the receptor for TGFα, and have observed a gender dependent mRNA expression pattern in select HPA structures. In contrast, gender specificity was not observed in transcript expression patterns in learning associated brain regions such as the amygdala and hippocampus. This structure-dependent pattern of mRNA expression supports the importance of the HPA axis in assigning the gender specific phenotype in *wa-1* that develops during adolescence.

In a second group of studies, we have used psychostimulant exposure to alter monoamine neurotransmitter function during prenatal brain development. In this model of gestational exposure to cocaine, administration of the drug for a very limited period of time during a "sensitive period", results in disrupted dopamine (DA) D_1 receptor signaling that

remains permanently altered for the life of the animal[2,3]. The disrupted signaling also causes abnormal development of certain neurons in regions of the cerebral cortex that receive dense DA innervation, including the prefrontal cortex and anterior cingulate. Strikingly, the offspring are permanently desensitized to the locomotor effects of psychostimulants, whereas adults exposed to the same level of cocaine instead exhibit intense sensitization, suggesting that the biological response of the CNS to this pharmacological insult varies not only in magnitude, but even in direction, depending on the developmental maturity of the nervous system when the insult is received.

Although the particular insults to the CNS used in these studies are not intended to model any specific causes of schizophrenia, these findings reflect the importance of timing on the disruption of neurodevelopment, and illustrate the vast diversity of resultant neuroadaptations. The study of such alterations in developmental trajectories are vital steps in understanding the complex additive and interacting effects that can lead to the eventual expression of the hallmark features of schizophrenia. Such developmental events can result in highly selective outcomes that reflect the culmination of adaptive (and sometimes maladaptive) changes that may underlie the pathophysiology of schizophrenia.[4]

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POSTERS

Alantamine induces neurotransmitter release likely by blocking SK channels

Eva Alés, Francesca Gullo, Esperanza Arias, Antonio G. García and Manuela G. López

Galantamine has shown increased clinical efficacy and safety for the treatment of Alzheimer´s disease. This drug has been described as a selective acetylcholinesterase inhibitor that allosterically modulates nicotinic receptors. However, here we present evidence of a new mechanism for galantamine, i.e. blockade of SK channels (Ca^{2+}-activated K^+ channel).

These channels are activated by an increase of intracellular calcium and their activation causes membrane hyperpolarization, which inhibits cell firing and limits the firing frequency of repetitive action potentials.

This process termed spike-frequency adaptation is essential for normal neurotransmission.

We have observed that galantamine can inhibit SK channel currents in a concentration-dependent manner and, as apamin (a selective blocker of SK channels), can potentiate the secretory responses induced by brief acetylcholine and K^+ pulses (10 s) applied to fast-superfused bovine adrenal chromaffin cell populations. At the single cell level, galantamine caused a mild increase of the cytosolic calcium and catecholamine release, measured simultaneously using a fura-2 fluorescence dye and an amperometric carbon fiber microelectrode. This effect could not be reproduced in all tested cells. However, this action when observed, was clearly dependent of calcium and galantamine concentrations. Furthermore, a potentiation of exocytosis by galantamine could be observed when acetylcholine and potassium were applied.

Our hypothesis is that galantamine can block SK channels and inhibit the hyperpolarization induced by acetylcholine or depolarizing stimuli.

Therefore, galantamine could potentiate the rate of action potential firing and consequently, neurotransmitter release; this is a process that has been described to be depressed in Alzheimer´s patients and other phycopathological diseases.

Catecholamine innervation of identified pyramidal neurons in the human temporal cortex

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Catecholamines have been shown to be involved in a variety of processes and pathologies such as Alzheimer's disease and schizophrenia. Previous studies have established that catecholaminergic innervation of both pyramidal and non-pyramidal cells occurs, and that this is selective for area, laminar and neuronal cell types. In the present study we have quantified catecholamine inputs to identified pyramidal cells in the human temporal cortex. To this purpose, we filled individual pyramidal neurons with Lucifer Yellow in fixed cortical tissue and immunostained with tyrosine hydroxylase (the rate limiting enzyme in catecholamine synthesis). Pyramidal cells (n=8) were reconstructed from serial confocal microscope images, and putative contacts between tyrosine hydroxylase immunoreactive (TH-ir) axon terminals and dendritic spines and shafts of labeled pyramidal cells were quantified at high magnification. After dividing the basal and apical dendritic fields of pyramidal cells in three portions (proximal, intermediate and distal), catecholamine contacts were located mainly in the intermediate portion. Moreover, pyramidal neurons received a remarkable narrow variation of 15 to 31 (average of 23) putative contacts from TH-ir axons per neuron. These results suggest that pyramidal neurons recieve an optimal number of cathecolaminergic synapses and that these synapses are stereotypically organized in the human temporal cortex.

Cortical AMPA and kainate receptor subunit expression in schizophrenia, bipolar disorder, and major depressive disorder

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Several lines of evidence suggest abnormal expression of ionotropic glutamate receptors in schizophrenia. Recently, converging studies have found that disturbances in glutamatergic neurotransmission may also play a role in the pathophysiology of other psychiatric illnesses. Although alterations of expression of ionotropic glutamate receptors have been reported in limbic cortex and medial temporal lobe structures in schizophrenia, possible changes in the expression of these receptors in mood disorders have not been well studied. In the present study, we have determined AMPA and kainate receptor subunit expression in the dorsolateral prefrontal (dlPFC) and anterior cingulate (aCg) cortex of brains from subjects with schizophrenia, bipolar disorder, major depression, and a comparison group, using samples from the Stanley Foundation Neuropathology Consortium. We have studied these receptors by using in situ hybridization for subunit transcript expression and by receptor autoradiography. We found differences between the alterations that occur in the dlPFC versus ACg. There were no differences between the subjects with any mental illness and the control group in the Acg.

Interestingly, in dlPFC, a significant decrease of GluR2 and KA2 transcripts expression was observed, but only in major depression. These results suggest abnormal glutamatergic neurotransmission in the cerebral cortex in major depressive disorder, specific to dlPFC and not seen in aCg. Interestingly, these two receptor families are spared in these cortical areas in schizophrenia and bipolar disorder in this particular cohort of subjects.

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Neurobiological and behavioural abnormalities induced by prenatal stimulation of dopamine D1 receptors

Sajnani, G., Meyer, G., Abreu, P. and Castro, R.

The fact that dopamine receptors are found early in brain development, prior to the formation of subcortical and cortical synaptic connections, suggests that dopamine, acting through dopamine receptors, may play an important function in neural development (1,2,3). We studied the long-term effects of prenatal SKF-38393 (a D1 receptor selective agonist) on monoamines metabolism in mesolimbic and mesostriatal systems of puber male rats. We also studied the behavioural consequences of such a manipulation.

Our results showed that low doses of SKF-38393 (0.05 mg/Kg s.c.) induced a selective reduction of either serotonin innervation in dorsal hippocampus.

Moreover, there was either a significant reduction of serotonin and 5-HIAA content, and GAP-43 expression levels, in hippocampus. These changes were followed by a selective decrease in dopamine and Dopac levels in mesolimbic system regions. Finally, a diminished vertical-locomotor activity and an altered stress-response was also found in puber rats.

These results suggest that early abnormal occupation of dopamine D1 receptors may have long-term consequences on development and function of brain limbic regions. Neuropathological and neuroimaging abnormalities have been reported in the same limbic regions in schizophrenia.

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Altered gene expression of a cell adhesion molecule subfamily in schizophrenia

Philip Ebert, Frank Middleton, Karoly Mirnics, David Lewis, and Pat Levitt

Schizophrenia is a complex disorder affecting ~ 1% of the population. Current evidence suggests that aberrant synaptic function may mediate in part the onset and progression of this disease; the functional deficits associated with schizophrenia, in addition to neuroanatomical studies, suggest that the defects underlying synaptic dysfunction are not global in nature, instead originating due to aberrant expression or function of molecules expressed in restricted regions and circuits. Members of the IgLON(K) family of genes (LSamp, OBCAM, Neurotrimin and Kilon), expressed in restricted regions of the brain (including the limbic system), encode GPI-anchored cell adhesion molecules of the Ig superfamily. Recent reports have revealed that Ig superfamily cell adhesion molecules such as SynCAM1 and neuroplastin2 are involved in synaptic assembly and maintenance, respectively, and therefore could play a role in the synaptic dysfunction associated with schizophrenia. We investigated by gene microarrays and in situ hybridization the expression of the IgLON(K) family in subjects with schizophrenia, focusing on the dorsal prefrontal cortex. The analysis revealed that the expression of the IgLON(K) family is consistently and significantly down-regulated in schizophrenia, with Kilon and OBCAM being the most robustly changed. The analysis further revealed distinct patterns of expression for each molecule in layers 5 and 6 of the human cortex. We suggest that alterations in expression of the IgLON(K) molecules reflect more global problems with synaptic function in schizophrenia.

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The effect of antipsychotic medication on the occupancy of the D2 receptor by endogenous dopamine

W. Gordon Frankle, Mark Slifstein, Anissa Abi-Dargham and Marc Laruelle

All pharmacological treatments which improve the psychotic symptoms of schizophrenia act as antagonists at the dopamine D2 receptor. The exact mechanism by which D2 antagonism improves psychotic symptoms is unclear. One hypothesis is that blockade of the D2 receptors by antipsychotic medications decreases the occupancy of these receptors by endogenous dopamine. Imaging studies have determined that the risk of extrapyramidal side effects increases when the striatal D2 receptor occupancy by an antagonist is greater than 80% [1]. However, the level of occupancy at which antipsychotic medications are effective is less well established. Some medications, notably clozapine, achieve efficacy at lower D2 occupancy levels than other medications [2]. The goal of the present study was to better characterize the degree of receptor occupancy by antipsychotic medications required to reduce occupancy of the D2 receptor by endogenous dopamine to control levels.

Our group, by examining the effect of dopamine depletion on [^{123}I]IBZM binding with SPECT, has previously demonstrated that baseline striatal dopamine levels are elevated in individuals with schizophrenia [3]. The present study utilized data of the18 healthy controls and 18 medication-free schizophrenic subjects from this prior study. The average free dopamine in both controls and schizophrenic subjects was calculated using the flowing formula [4]: (1) Free dopamine = K_D * (BP_2/BP_1 – 1), where K_D is the dissociation rate constant of dopamine at the D2 receptor and BP_1 and BP_2 are the binding potentials pre- and post- dopamine depletion, respectively. The calculated average free dopamine is 30 nM in individuals with schizophrenia and 15.5 nM in healthy individuals. The occupancy of the D2 receptor by dopamine can be calculated using this free concentration and the equation: (2) Occupancy = $F/(K_D + F)$, where F is concentration (nM) of free dopamine and K_D is the dissociation rate constant of dopamine at the D2 receptor (160 nM). Using this formula the calculated occupancy by dopamine of the D2 receptors is 15.8% in medication-free schizophrenic individuals and 8.8% in healthy individuals.

Equation (2) can be expanded to allow for the calculation of receptor occupancy by one species (e.g. dopamine) in the presence of another (e.g. antipsychotic). The expanded equation is: (3) Occupancy = $(F/K_D)/(1 + F_{ANT}/K_{DANT} + F/K_D)$, where F, F_{ANT}, K_D and K_{DANT} are the free concentrations and the dissociation rate constants of dopamine and the antipsychotic, respectively. With equation (3) we can calculate the striatal D2 occupancy by an antipsychotic necessary to reduce the 15.8% D2 occupancy by dopamine in schizophrenics to control levels of 8.8%. Assuming a starting level of free dopamine of 30 nM in schizophrenic subjects (15.8% occupancy) the level of D2 occupancy by an antagonist that would reduce the dopamine occupancy to that of healthy controls (8.8%) is 47%. Based on

these results it is unlikely that the sole mechanism of action of antipsychotic medications is to reduce D2 occupancy by dopamine, since, with the exception of clozapine, the D2 occupancy level at which antipsychotic medication is effective is generally greater than 50%.

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Lateral association of Neuroligin mediate synaptic adhesion with Neurexin and induce the formation of presynaptic terminals

Francisco G. Scholl, Camin Dean, and Peter Scheiffele

The precision of synaptic connectivity is essential for normal brain function and the formation of wrong connections leads to specific disease states such as schizophrenia where synaptic activity is upregulated. The understanding of the mechanisms that rule synaptogenesis is essential to overcome defects in synaptic activity.

Previous work has demonstrated that neuroligins, post-synaptic transmembrane proteins, can trigger the assembly of presynaptic specializations in axons contacting neuroligin transfected non-neuronal cells (1). Now, we have analyzed the mechanisms of neuroligin induced synaptogenesis in the CNS. Beta-Neurexins are plasma membrane proteins that associate *in vitro* with the extracellular domain of Neuroligins (2). However direct evidence of beta-neurexins as functional neuroligin receptors during synapse formation is still missing. In fact, the subcellular localization of neurexins has remained controversial. Here we demonstrate that neurexins localize to synaptic puncta in mouse cerebellar neurons. Overexpression of neuroligin-1 in neuronal cells results in a 5 fold increase in the number of presynaptic terminals contacting the transfected cells. Neurexins are recruited to the cell-cell contact site of such neuroligin-induced synapses. Neuroligin mutants lacking synaptogenic activity fail to aggregate beta-neurexin-expressing cells, however the ability of these mutants to bind soluble neurexins is not altered. The inactivating mutations map to a loop in the neuroligin extracellular domain that is required to form oligomers. These and additional results suggest that lateral association of neuroligin molecules in the postsynaptic membrane induces strong adhesion to neurexins in the presynaptic cells which trigger the formation of presynaptic terminals.

We are now studying the pathway downstream of neurexin in the presynaptic terminal. The cytoplasmic domain of neurexins link to the actin/spectrin cytoskeleton *in vitro*(3). To analyze the function neurexins might play in the assembly of the actin cytoskeleton, we microinjected beta-neurexin cDNA into NIH 3T3 cells. Neurexin expression induces a dramatic reorganization of the actin filaments. Microinjected cells form actin patches underneath plasma membrane domains containing beta-neurexin proteins. We speculate that neurexins may have a functional role on the regulation of the actin cytoskeleton at the synapse.

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Altered gene expression for BDNF signaling in the prefrontal cortex of subjects with schizophrenia

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Gene expression for GAD67 and GABA transporter-1, markers of inhibitory neurotransmission, appears to be selectively altered in the parvalbumin (PV)-containing subclass of GABA neurons in the prefrontal cortex (PFC) of subjects with schizophrenia. Brain-derived neurotrophic factor (BDNF) has been shown to regulate the expression of these markers of GABA neurotransmission. To test the involvement of BDNF signaling in altered GABA-related gene expression in schizophrenia, we analyzed mRNA expression for BDNF and its receptor tyrosine kinase, TrkB, in PFC area 9 from 15 pairs of schizophrenic and control subjects, individually matched for age, sex and postmortem interval, using *in situ* hybridization. Expression of both BDNF and TrkB mRNAs, as assessed by film autoradiography, was significantly decreased in subjects with schizophrenia, whereas no change in mRNA expression was detected for TrkC, a receptor tyrosine kinase for NT-3. The magnitudes of the decreases in BDNF and TrkB mRNA expression were similar and significantly correlated across subject pairs.

Furthermore, these changes were correlated with the changes in mRNA expression for GAD67 and PV. TrkB mRNA was expressed abundantly in PV mRNA-positive neurons but not in calretinin mRNA-positive neurons in control subjects, suggesting that PV-containing neurons are most affected, compared to other PFC GABA neurons, by altered BDNF signaling in schizophrenia. The absence of the altered BDNF or TrkB mRNA expression in monkeys treated with haloperidol suggests that the gene expression changes are related to the disease process, but not to the treatment of schizophrenia. Altered BDNF signaling might affect the maintenance of inhibitory synaptic functions in the PFC of subjects with schizophrenia.

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Evidence for an interaction between N-terminal redox sensitive cysteines and a polyamine modulatory site of the NR1/NR2A NMDA receptor

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The NMDA subtype of glutamate receptor is sensitive to reducing and oxidizing agents in a subtype specific-manner. Cysteines 744 and 798 on NR1a are responsible for most of the redox sensitivity of NR1a/NR2B and NR1a/NR2C receptors. However, NR1a(c744a/c798a)/NR2A receptors remain sensitive to redox modulation. This has been attributed to cysteines in the amino terminal domain (ATD) of NR1 and NR2A (Choi et al., J. Neurosci. 21:392;2001). We have previously shown that 30uM spermine blocks the effects of DTT in NR1a(c744a,c798a)/NR2A receptors (Herin et al., SFN Abstracts 703.12; 2001), in a tricine-insensitive manner. This suggests a zinc-independent effect of spermine on redox modulation at the N-terminal redox-sensitive cysteines. Evidence exists that spermine alone modulates NR1a/NR2B (but not NR1a/NR2A) receptors via the N-terminal regulatory domain of NR1a. We hypothesized that spermine block of DTT potentiation in NR1a(c744a/c798a)/NR2A could be modified by alterations of the ATD of NR1a. Mutation of cys79 in NR1a(c744a/c798a)/NR2A receptors resulted in a decrease of DTT potentiation of NMDA-induced currents, as reported earlier (Choi et al., ibid.). In support of our hypothesis, the inhibitory actions of spermine on DTT potentiation were markedly attenuated in this construct. These results suggest an interaction between cys79 of NR1a and a spermine modulatory site in NR1a/NR2A receptors.

Neurotransmitters action before synaptogenesis: GABA and glutamate modulate the migration of hippocampal pyramidal cells

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Previous studies suggested that neuronal migration in the neocortex is modulated by neurotransmitters, mainly GABA and glutamate. We recently report that a calcium- and SNARE-independent release of transmitters underlies a paracrine mode of communication before synapse formation (Demarque et al., 2002). This results in the presence of tonic, spontaneous and evoked currents in embryonic and neonatal CA1 neurons mediated primarily by the activation of GABA-A receptors. These currents persist in the presence of calcium channel blockers, botulinium toxin and are observed in Munc18-1 deficient mice in which vesicular release is abolished (Verhage et al., 2000). We postulate that such a communication may be involved in the regulation of developmental processes. In the present report we analysed if this paracrine action of transmitters contributes to modulate the migration of hippocampal neurons. Cell migration was evaluated on organotypic explants from E18 rats or mice (wild type or KO for Munc 18-1, kindly provided by Dr. TC Südhof). To follow migration two procedures were used: 1) co-culture of explants from green mice (EGFP; a gift from Dr. M. Okabe) and non-fluorescent mice, what allowed the visualisation of fluorescent migrating cells in a non-fluorescent environment. Migrating cells were recorded using the patch-clamp technique in the whole-cell configuration. 2) BrdU labelling of pregnant animals 2 hours before dissection. Slices were treated with GABA(30 μM) or GABA-A receptor antagonist (50 μM Bicuculline) or glutamate (300 μM) or NMDA receptor antagonist (10 μM MK801). We observed that bath application of GABA and glutamate evoked currents in migrating cells, indicating that these cells already express functionnal ionotropic receptors to GABA and glutamate. Furthermore, the application of GABA-A and NMDA receptor antagonists during 1 day in culture dramatically reduces pyramidal cells migration. Since transmitters are thought to be mainly released from axonal growth cones through a vesicular mechanism, pyramidal cells migration was also investigated in co-cultures of green mice and Munc-18-1 KO mice explants. As reported before (Verhage et al., 2000) CA1 pyramidal cells migration persists in Munc 18-1 KO mice, suggesting that a synaptic release of transmitters is not required for neuronal migration. Our results suggest that GABA and glutamate released through a non-synaptic, non-conventional pathway modulate pyramidal cells migration.

Effects of the antipsychotic drug clozapine on the expression and function of the glutamate transporters GLT-1, GLAST and EAAC1 in the frontal cortex of rats

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To evaluate the potential role of glutamate transporters in the pathophysiology of schizophrenia, we studied the effects of the antipsychotic clozapine on GLT-1, GLAST and EAAC1 in the neocortex of normal rats which were given clozapine in drinking water (0,5 mg/ml; 25/35 mg/Kg/day; estimated clozapine plasma levels 20-60 ng/ml) for 9 weeks.

Immunocytochemical and immunoblotting studies showed that clozapine induced a strong reduction of GLT-1 expression in the frontal cortex (42,5%-70%) whereas GLAST and EAAC1 expression in the same cortical region were unchanged compared to controls. Glu transport in *Xenopus* oocytes injected with mRNA from the frontal cortex of treated animals was decreased by 60%. In the frontal cortex of treated animals, basal and KCl-evoked Glu output was significantly higher than in controls (about 4-fold increase for basal Glu output and up to 4-fold increase for KCl-evoked Glu output).

Our data show that clozapine administration strongly reduces GLT-1 but not GLAST and EAAC1 expression in the frontal cortex of normal rats. Thus, the robust decrease of Glu uptake and the subsequent increase in Glu levels can be attributed to a specific effect of clozapine on GLT-1. These findings suggest that clozapine exerts at least partly its therapeutic effect by acting on GLT-1 expression and function thereby potentiating glutamatergic transmission. Furthermore, the present data suggest that glutamatergic hypofunction in schizophrenia may partly result from changes in GLT-1 expression and/or activity.

C-fos expression after cocaine or morphine sensitization in CB1 knockout mice

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Repeated intermittent treatment with psychostimulants or opioids sensitizes animals to the locomotor activating effects of these drugs. Such sensitization processes may have important implications for the understanding of addiction, mental illness, and the cellular basis of memory, but the neurobiological mechanisms involved remain only partially understood [5].

Sensitization to the locomotor responses after a chronic treatment with morphine or cocaine, and the rewarding effects of these compounds have been evaluated in CB1 knockout mice [2]. While the acute locomotor effects of morphine were preserved in these mice, in agreement with previous studies [1], some adaptive responses to chronic morphine treatment seemed to be suppressed in CB1 knockout mice. Thus, the sensitization to hyperlocomotor activity produced by chronic morphine in wild-type animals was abolished in CB1 knockout mice. Furthermore, repeated morphine administration did not exhibit rewarding responses in the conditioned place preference paradigm in CB1 knockout mice, whereas wild-type animals showed a clear preference for the morphine-associated compartment. These data indicated that stimulation of the CB1 receptors are necessary for expressing morphine-induced motivational responses, such as the sensitization to locomotor effects and the rewarding properties. In the case of cocaine administration, acute locomotor effects of this compound and the adaptive responses to repeated cocaine administration were preserved in CB1 knockout mice. Thus, chronic cocaine responses was similar in both genotypes.

On the other hand, the protooncogene c-fos produces a phosphoprotein, Fos, which regulates gene transcription processes. In neuronal systems, Fos has been proposed to couple synaptic transmission to changes in gene expression by acting in the cell nucleus in concert with other proteins to form complexes in the promoter regions of target genes in response to extrinsic signals [3,4]. Therefore, mice were treated with either morphine or cocaine by using the same schedule than for the locomotor sensitisation procedure, and were then tested for induction of c-fos. We reasoned that expression of Fos could serve as a sensitive indicator of differential gene activation by drugs of abuse in a region of the brain that is directly implicated in the effects of these drugs.

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Reduced flow through NMDA-receptor channels as a basis for cognitive disorganisation in schizophrenia

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Cognitive disorganisation involves impairment of dynamic organizational processes such as contextual modulation and dynamic grouping. There is both neurophysiological and psychological evidence for these processes, and they are at the centre of the theory of cortical computation known as Coherent Infomax (Phillips & Singer, *Behavioral and Brain Sciences* 1997, 20: 657-722). This theory has been developed both analytically and by computer simulations. Dynamic organisation could be implemented by those long-range connections within and between cortical regions that operate via NMDA-receptor channels, and which control gain as a result of their voltage-dependence. An impairment of these mechanisms is central to PCP-psychosis, and cognitive capabilities that they could provide are impaired in schizophrenic patients. Evidence for the hypothesis that schizophrenia involves impaired cognitive coordination due to NMDA-receptor hypofunction will be summarized (Phillips & Silverstein, *Behavioral and Brain Sciences*, in press).

Dopamine D1 receptors in the macaque monkey thalamus: selectivity for thalamic projection neurons

Margarita Rodríguez-Moral and Carmen Cavada

The monkey thalamus is notably innervated by dopamine (DA), mostly nuclei connected with associative and limbic cortical areas, neostriatum, and amygdala. This suggests that thalamic DA is involved in the modulation of cognitive, motor and emotional functions. With the purpose of searching for the receptors mediating DA neurotransmission in the thalamus, we studied the localization of dopamine D1 receptor in the brains of adult *Macaca nemestrina* monkeys using a subtype specific antibody.

Surprisingly, we found that D1 is present in large neuronal somata widespread throughout all thalamic nuclei. Their large size and the lack of expression of GABA or GAD indicated that the D1-immunoreactive neurons are projection neurons, not interneurons. This was confirmed by retrograde labeling from the cerebral cortex together with D1 immunodetection.

Finally, the D1 immunoreaction exhibits a perikaryal localization and does not appear associated to the plasmatic membrane of neuronal bodies, suggesting that D1 may be associated to the plasmalemma away from the neuronal body.

We propose that D1 is present in thalamocortical axon terminals based on: 1) the localization of the receptor protein in the perikaryon of thalamocortical projection neurons, and 2) the presence, previously described by others, of presynaptic D1 in axon terminals of the macaque cerebral cortex forming asymmetric synapses. Because D1 receptors have been proposed to modulate the release of neurotransmitters, including glutamate, and because thalamocortical neurons are considered glutamatergic, D1 may function as a presynaptic modulator of glutamate neurotransmission in thalamocortical systems.

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Electrophysiological study of motor function in a-Synuclein null-mice

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a-Synuclein (a-Syn) is a 14 kDa protein of the mature synapses which has been implicated in the physiopathology of Parkinson's disease. However, the physiological functions of a-Syn are not yet well understood. In hippocampal neurons in culture, downregulation of a-Syn by antisense has been shown to reduce the number of synaptic vesicles, indicating that this protein may regulate the size of the presynaptic vesicular pool (1).

Expression of mutant human a-Syn forms in mice generates animals with brainstem neuronal degeneration and motor neuron pathology, this last finding suggesting that a-Syn has also a role on the synapses maintenance of the neuromuscular junction (2).

We have used mice carrying a knock-out of the Snca gene (3) to study the effect of the lack of a-Syn on motor function. The amplitude of the compound motor action potentials (CMAP) on the EDL and GN muscles in response to prolonged repetitive stimulation of the sciatic nerve were compared in knock-out and wild-type mice. In both groups, the initial amplitudes of CMAPs were similar and the decline on CMAPs amplitudes during trains of either short or prolonged high-frequency stimulations (20-100 Hz) presented similar time course. The rates of recovery of the amplitude of the CMAPs were also very similar in wild-type and knock-out muscles. Estimation of the number and size of motor units on the GN and EDL shows not differences between null- and wild-types. We also examined motor performance in rotating rod experiments (progressive speed acceleration up to 36 rpm, 5 minutes test). The motor performance of the two groups revealed no differences.

In summary, our study shows no gross sings of impair performance of the motor system on a-Syn null mice, suggesting that, at least, at this level other synapses proteins may compensate for that deficit.

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Antigliosis: Evidence for glial effects on synaptic plasticity in schizophrenia

Keith A. Young, Paul B. Hicks and Leigh A. Holcomb

Most investigations of glial pathology have failed to find evidence for activation or proliferation of glia (gliosis) in schizophrenia. We have used stereological techniques to estimate total glial cell populations in the anterior thalamus in subjects from the Stanley Foundation Neuropathology Consortium. There is a significant reduction in the normal glia/neuron ratio in this nucleus in schizophrenic, but not bipolar or depressed subjects. This pattern of antigliosis has also been reported in frontal cortical regions in schizophrenia (Pakkenberg, 2001). Because the schizophrenic subjects in the Stanley Foundation collection were younger than many other post-mortem studies, our data provide evidence for early glial insults in schizophrenia. We are currently in the process of studying specific glial cell subtypes to determine whether glial cell number reductions are specific for microglia, astrocytes and/or oligodendrocytes.

During the past decade, there has been a reformation in our understanding of the role of glia in CNS neurotransmission. It is well established that glia perform important developmental and trophic actions for neurons. But in addition to these functions, recent findings implicate glia as partners in local signaling activity through glial calcium gradients, and have established microglia and astrocytes as active participants in neurochemical modulation. Most recently, it has been demonstrated that normal synaptic plasticity is dependent on glial cell activity. For instance, glial-derived TNF-alpha is necessary for maintenance of normal synaptic strength of excitatory synapses (Beattie et al., 2002).

Antigliosis in schizophrenics is therefore a prime candidate in the impairment of synaptic plasticity in this mental illness. Genetic findings from our VA Cooperative Studies cohort (Skol et al., 2002) and other recent studies (Steffansson et al., 2002) have provided further support for glial involvement in schizophrenia by identifying candidate genes that participate in the determination of glial cell fates and glial activity. In combination with microarray identification of abnormal glial gene products in schizophrenic brain tissue, these recent findings suggest that antigliosis may be a intimately involved in the pathophysiology of schizophrenia.

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