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Abstract Book

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Title

Frequency-dependent transformation of GABAergic inhibition into glutamatergic potentiation in astrocyte-neuron networks

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Objectives: Accumulating evidence indicate the existence of bidirectional communication between astrocytes and neurons. We have investigated the response of astrocytes to activity of GABAergic interneurons and the consequent effects on CA3-CA1 glutamatergic synaptic transmission. Material and Methods: Using electrophysiological and Ca2+ imaging techniques in murine hippocampal slices (P12-P18), we performed paired recordings from GABAergic interneurons and pyramidal neurons in the CA1 area, while simultaneously monitoring intracellular astrocyte Ca2+ levels. We stimulated Schaffer collateral single synapses using the minimal stimulation method to quantify the synaptic transmission properties. Results: Depolarization-evoked single action potentials in interneurons induced a transient inhibition of synaptic efficacy and probability of release at single CA3-CA1 synapses. This transient inhibition was abolished by picrotoxin, indicating that it is mediated by activation of presynaptic GABAa receptors. In contrast, trains of action potentials evoked by long depolarizations (500-700 ms) transiently increased the synaptic efficacy and the probability of release at the same single synapses. This phenomenon was abolished by GABAb and group I mGlu receptor antagonists (CGP-55845 and MPEP+LY-367385, respectively), and was associated with elevations in the intracellular Ca2+ levels of the surrounding astrocytes. Both the GABA-induced astrocyte calcium elevations and the synaptic potentiation were absent in the IP3-R2 knockout mice (in which G-protein-mediated astrocyte Ca2+ signal is impaired), indicating that high frequency interneuron activity activates GABAb receptors in astrocytes, elevating their Ca2+ levels and stimulating the subsequent release of glutamate, which potentiates synaptic transmission by the activation of presynaptic group I metabotropic glutamate receptors at Schaffer collaterals. Conclusions: Astrocytes decode interneuron activity to induce frequency-dependent differential modulation of synaptic transmission. As a consequence of this property, astrocytes transform in a frequency-dependent manner GABAergic inhibition into potentiation of glutamatergic excitatory synapses.

Title

The auditory contralateral advantage as a model system for studying connectivity

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Previous M/EEG studies have successfully used dynamic causal modeling (DCM) for evoked responses to model connectivity networks, but there are few models of already-established connectivity differences in the auditory system. We studied whether DCM for M/EEG can effectively model the well-established asymmetrical response to left/right auditory stimulation. Participants heard monaural pure tones on either the right or the left ear, and responses were recorded using MEG. To analyze these data, DCM models were constructed with single equivalent current dipole (ECD) sources in left and right auditory cortex, using a single-shell sphere model. We compared models with separate input to each source, versus models with a unitary input to both sources, and also models with and without lateral connections between the sources. Model comparison showed that the DCM without lateral connections, but with separate inputs to left and right cortex was the best account. This was true in both the planar gradiometer data, as well as the magnetometer data. The results suggest that DCM for M/EEG is an effective model for connectivity for the auditory evoked field.

Title

Glutamate release through the cystine/glutamate antiporter contributes to ischemic neuronal damage

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Excessive release of glutamate during brain ischemia triggers neuronal death by overactivating N-methyl-D-aspartate (NMDA) receptors. However, the mechanisms of alteration of glutamate homeostasis, and whether this occurs in the synaptic or extrasynaptic space is still a matter of debate. In this study, we monitored glutamate release by measuring ischemia-gated currents in pyramidal cortical neurons and determined neuronal damage after ischemia-like conditions (oxygen and glucose deprivation) in acute slices and organotypic cultures of cerebral cortex. We were unable to detect inhibition of such currents or any reduction in neuronal damage after oxygen and glucose deprivation by blocking excitatory amino acid transporters (EAATs) or vesicular glutamate release. In contrast, pharmacological inhibition of the extrasynaptic glutamate regulator, the cystine/glutamate exchanger (known as system xc-), significantly attenuated ischemic-gated currents and neuronal cell death after oxygen and glucose deprivation. We provide evidence that extrasynaptic but not synaptic NMDA receptor antagonists reduced ischemia-gated currents and cell damage after oxygen and glucose deprivation. Accordingly, cystine/glutamate antiporter is located outside of the synaptic cleft. Finally, we provide evidence that ischemia induced an increase in system xc- function. Altogether, these data suggest that ischemia up-regulates cystine/glutamate antiporter expression and function, which contributes to increase extracellular glutamate concentration, overactivation of extrasynaptic NMDA and ischemic neuronal death.

Title

Towards objective neuroimaging markers in clinical neuroscience: insights from Information Theory and Complex Networks Analysis

Author(s)

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I will discuss recent results in clinical neuroscience in patients with deficit of consciousness after traumatic brain injury. In concrete, I will report on recent results which are based on the functional magnetic resonance imaging (fMRI) at the resting state (rs) of those patients. We have analyzed the rs-fMRI networks by using different methods based on Information Theory and Complex Networks Analysis. We are proposing possible candidate markers that can account for the level of deficit of consciousness found on those patients. The main motivation of this research is the need for new markers that based on the rs-fMRI dynamics might improve not only the diagnosis but the prognosis of those patients.

Title

Can blood samples be used as a surrogate of brain samples in methylation studies?

Author(s)

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INTRODUCTION A growing body of evidence suggests that epigenetic mechanism play a central role in the regulation of gene expression in the nervous system during development and adulthood. Thus, epigenetic profiles of an organism significantly influence the ageing process of the central nervous system, hence being able to determine the onset and progress neurodegenerative disease. As matter of fact, it has been found that many neurological disorder display aberrant methylation patterns in the nervous system, and thus it has become now a high priority for neuroepigenomic studies to find non invasive biomarkers that could be use as a surrogate of brain samples in methylation analysis. MATERIAL AND METHODS We have performed an Array-based DNA methylation profiling of two groups of paired samples using the the Illumina Infinium HumanMethylation27 BeadChip. The first group of samples included cortex, hippocampus and blood samples from 10 individuals while the second comprised blood and lymphocyte samples from 8 individuals. Comprehensive bioinformatic analysis was conducted in order to identify those epigenetic biomarkers that display same methylation patterns in brain when compared to blood, and therefore could be used to test the methylation status of those genes in brain. We also compared the methylation patterns of those matched blood and lymphocytes, and matched cortex and hippocampus. We finally measured the interindividual variability of the methylation levels of each of the genes contained in the array, by comparing the methylation of 112 cortex from public data, so that we could identify those genes that display the largest interindividual differences in brain, and thus could be considered more interesting. Single markers were further validated by means of pyrosequencing. RESULTS We found a list of 525 genes for which all individuals shown the same methylation status in brain and blood samples and were also significantly variable in cortex (>50% of the maximum variability). 3 of those 525 genes were successfully biologically validated in an independent set of 50 matched blood and brain pairs by pyrosequencing. When we compared methylation between lymphocytes and blood, and cortex and hippocampus, we found that that none of the genes included in the array have consistent different methylation status. CONCLUSION Blood samples can be used as a surrogate of brain samples for the methylation analyses of certain genes. In the current work we have provided a list of genes for which all individuals shown the same methylation status in brain and blood samples and displayed a high variability in cortex.

Title

LRRK2 is involved in the inflammatory response in Parkinson disease

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Mutations in the leucine-rich repeat kinase 2 (LRRK2) gene contribute to both familial and idiopathic forms of Parkinson disease (PD), but the normal function of the protein and its role in the disease are still unknown. In this work, we have studied the involvement of LRRK2 in inflammation in a cellular model with endogenous expression of normal and two mutated forms of LRRK2 associated to PD. We determined COX-2 expression in primary dermal fibroblasts from patients with the G2019S and R1441G mutations in LRRK2, idiopathic PD and healthy individuals. We found that basal levels of COX-2 RNA were remarkably elevated in the fibroblasts of the patients. Lentiviral-mediated silencing of LRRK2 demonstrated that LRRK2 regulates basal COX-2 levels and COX-2 induction after a pro-inflammatory stimulus. Additionally, in samples from patients with LRRK2R1441G and with idiopathic PD, we found a re-distribution of the COX-2 RNA stabilizing protein HuR to the cytoplasm. Furthermore, the LPS-induced inflammatory response was attenuated in these two groups, which showed weak induction of pro-inflammatory cytokines, as well as reduced NFκB pathway activation. We confirmed these results in post-mortem brain samples, where COX-2 was high in the putaminal samples of most PD patients. Finally, we have taken advantage of cellular reprogramming and have generated induced pluripotent stem (iPS) cell lines with LRRK2G2019S and LRRK2R1441G. Preliminary results indicate that iPS-derived LRRK2R1441G neurons show impaired NFκB activation after an IL-1β stimulus. In summary, PD patients display multiple defects in inflammatory pathways in which LRRK2 appears to be critically involved. These defects are more prominent in LRRK2R1441G and idiopathic PD than in those carrying LRRK2G2019S. Further studies are required to establish the relevance and therapeutic implications of inflammatory dysregulation in the pathophysiology of the disease.

Title

The efficacy of a new generation Cognitive Rehabilitation Program for Psychosis: clinical symptoms and functional outcome improvement in chronic schizophrenia

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Background and aims: Cognitive remediation improves cognition in schizophrenia, but its effectiveness on other relevant factors such as clinical symptoms and functional outcome has not been studied to the same degree. The aim of this study was to analyze the influence of the cognitive rehabilitation program REHACOP Method: Eighty-four inpatients with chronic schizophrenia were recruited. All subjects underwent pre and post-treatment assessment including neurocognition, clinical symptoms, insight, and functional outcome. Patients were randomly assigned to either neuropsychological rehabilitation (REHACOP) or control group for 3 months in addition to treatment as usual. REHACOP is an integrative Spanish program that taps all basic cognitive functions. Results: REHACOP group showed significantly greater improvements at 3 months in neurocognition, negative symptoms, disorganization and emotional distress measures compared to the control group (Cohen´s effect size for these changes ranged from d = 0.47 for emotional distress to d = 0.58 for disorganization symptoms). REHACOP group also improved significantly in both GAF (d = 0.61) and DAS-WHO total score (d = 0.57). More specifically, they showed significant improvement in vocational outcome (d = 0.47), family contact (d = 0.50) and social competence (d = 0.56). Conclusions: Neuropsychological rehabilitation may be useful for the reduction of some clinical symptoms and functional outcome. These findings support the feasibility of integrating neuropsychological rehabilitation into treatment as usual programs for patients with chronic schizophrenia.

Title

Metabotropic glutamate mGlu2/3 receptor coupling to G proteins in postmortem frontal cortex of schizophrenic subjects

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Glutamate neurotransmission is emerging as a new target for the pharmacological treatment of psychosis and schizophrenia. Different preclinical and clinical studies suggest metabotropic glutamate 2 and 3 receptor (mGlu2/3R) agonists as potential antipsychotic drugs. Previous findings have suggested that the density of these receptors is decreased in postmortem human brain cortex of schizophrenic subjects. Moreover, mGlu2 receptors downregulation seems to be secondary to repressive histone modifications induced by the treatment with atypical antipsychotic drugs in its promoter. Although in heterologous systems and rodent models mGlu2/3R have been shown to couple and activate Gi/o proteins, the G protein coupling pattern of these receptors in postmortem human brain remains unknown. Similarly, there is no data about the possible coupling abnormalities associated with schizophrenia and antipsychotic treatment. In this context, the aim of the present study was to evaluate the functional coupling of mGlu2/3 receptors to the different subtypes of Ga proteins (Gai1, Gai2, Gai3, Gas, Gaz, Gao and Gaq/11) in postmortem human frontal cortex of 26 schizophrenic subjects and 26 matched controls. The selectivity of the findings for mGlu2R and not for other mGlu and non-glutamate receptors was confirmed by experiments in mGlu2R knock-out mice and control littermates. Stimulation of the [35S]GTPgS binding by the mGlu2/3 receptor agonist LY379268 (3x10-8 M and 3x10-6 M) was determined by Scintillation Proximity Assays using specific antibodies against each Gα subunit. The relative level of Gα proteins was assessed by Western Blotting. In human frontal cortex, LY379268 induced a concentration-dependent stimulation of the [35S]GTPgS binding to all the different Gα proteins (range Emax 106±2% to 122±3%). Activation of the Gα subunits was abolished by the mGlu2/3R antagonist LY341495 (10-5 M). No differences in the activation of Gαi1, Gαi2, Gαi3, Gαs, and Gαo by LY379268 were observed in schizophrenic subjects as compared to controls (Student´s t-test; p>0.05). In contrast, Gαz and Gαq/11 stimulation induced by LY379268 was significantly decreased in schizophrenic subjects (Gαz: D≈-11% at 3x10-8 M, p<0.01; and D≈-9% at 3x10-6 M, p><0.05 / Gaq/11: D≈-4% at 3x10-8 M, p>0.05; and D≈-8% at 3x10-6 M, p<0.01). Additionally, Gαi1 stimulation induced by LY379268 was decreased in antipsychotic-treated schizophrenic subjects as compared to matched controls (D≈-10%, n=8, p><0.05). The immunoreactive density of the different Gα proteins did not significantly differ between schizophrenic and control subjects. The stimulation of [35S]GTPgS binding to all the different Gα proteins induced by LY379268 (10-5 M) in brain cortex of wild-type mice (range 113±0.2% to 125±4%, n=3-5) was absent in brain cortex of mGlu2R knockout mice. Together, these findings suggest a functional coupling of the mGlu2 receptor to pertussis toxin (PTX)-sensitive as well as to PTX-insensitive G proteins. A reduced mGlu2 receptor-mediated Gαz and Gαq/11 coupling was observed in schizophrenia. Additionally, mGlu2 receptor-dependent Gαi1 coupling was decreased in antipsychotic-treated schizophrenic subjects. Further work is needed to determine the functional role of these findings in schizophrenia brain and on mechanisms of antipsychotic drug

Title

Dual effects of the Cannabinoid agonist WIN55,212-2 in Cholinergic basal forebrain cells

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The cholinergic basal forebrain cells (CBFC) are involved in the acquisition of memory and in learning processes that are consolidated in cortical areas (Cx) and hippocampus. The loss of CBFC in the nucleus basalis of Meynert (nbM) and in the medial septum (MS) is associated with the cognitive impairment in Alzheimer´s disease (AD). The endocannabinoid system that modulates the cholinergic signaling in these brain areas is altered in postmortem brains of AD patients, although the reported data are unclear. Thus, some authors have described a decrease in CB1 receptor density in hippocampus and Cx, while others have reported an increase in CB1 and CB2 densities surrounding the senile plaques. The aim of the present study was to analyze the effects of the CB1/CB2 agonist WIN55,212-2 in organotypic cultures of hemibrain slices containing CBFC from neonatal rats (P7). The effects of the treatment with the specific CBFC immunotoxin 192IgG-saporin, were evaluated as an ex vivo model of cholinergic lesion. Cell death was analyzed by propidium iodide uptake and immunohistochemistry was used to label CBFC (p75NTR) and CB1 receptors. The toxicity induced by 192IgG-saporin was reduced after pre-treating the brain slices with WIN55,212-2 (1 nM - 10 nM). However, higher concentrations of the agonist (1 μM - 10 μM) increased the cytotoxicity. Moreover, a modulation of CB1 immunoreactivity was observed in Cx and MS in cultures treated with 192IgG-saporin when compared with those treated with the vehicle. The CB1 mediated specificity and the phenotype of the affected cells are being analyzed. The organotypic hemibrain culture model of CBFC has been developed as a versatile method with which identify pharmacological strategies to palliate their specific neurodegeneration.

Title

Cannabinoids modulate information transmission of sensorimotor circuit of basal ganglia

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The CB1 cannabinoid receptor which is densely located in the basal ganglia is known to participate in the regulation of movement activity. The aim of this study was to determine the effect of cannabinoids (Δ9-tetrahidrocannabinol (Δ9-THC), and WIN 55,212-2) on spontaneous and cortically evoked activity in the substantia nigra pars reticulata (SNpr) by extracellular recording techniques in anaesthetized animals. Administration of Δ9-THC (0.5 mg/kg, i.v.) stimulated (by 127 ± 14%) 6 of 11 SNpr recorded neurons, whereas it inhibited (by 73 ± 14%) the remaining 5 neurons. After Δ9-THC the regularity of neuron activity was increased in all recorded neurons and the firing pattern changed toward a more bursting discharge. On the other hand, administration of WIN 55,212-2 (125-250 µg/kg, i,.v.) increased the firing rate of SNpr neurons (by 125 ± 7%, n=6) and the regularity of neuron activity, whereas did not modify the firing pattern. Previous administration of the cannabinoid receptor antagonist AM 251 (1 mg/kg, i.v.) completely blocked the effects induced by both agonists. Moreover, when AM 251 (1 mg/kg, i.v.) was administered alone also induced an increase (13 of 21 SNpr recorded neurons) or a decrease (remaining 8 neurons) in firing rate. After Δ9-THC or WIN 55,212-2 administration, the inhibitory component of the cortically evoked response (activation of the direct striatonigral circuit) and the late excitatory response (activation of the indirect striato-pallido-subthalamo-nigral circuit) were decreased or completely lost. However, the early excitatory response (activation of the hyperdirect cortico-subthalamic circuit) was not modified by cannabinoids administration. Previous administration of AM 251 (1 mg/kg, i.v.) completely blocked these effects without any modification of the cortically evoked responses. These results suggest that CB1 receptor activation modulates the sensorimotor transmission through the trans-striatal pathways. This modulation may be relevant in the understanding of involvement of the cannabinoid system in motor control.

Title

Control of M-Current Density

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In contrast to the ubiquitously expressed SGK1, the neuronal isoform SGK1.1 interacts with phosphoinositide-phosphatidylinositol 4,5-bisphosphate (PIP2) and is distinctly localized to the plasma membrane (Arteaga et al., 2008). To evaluate the effect of the kinase in neuronal excitability, we generated a transgenic mouse (Tg.sgk) expressing a constitutively active form of SGK1.1 (S515D). Transgenic mice with increased SGK1.1 activity showed diminished sensitivity to kainic acid-induced seizures. While 50% of the animals died after this treatment, no tg.sgk mice die as a consequence of this insult. Superior cervical ganglion (SCG) neurons isolated from Tg.sgk mice showed a significant increase in M-current levels, paralleled by reduced excitability and more negative resting potentials. The M-current formed by tetramerization of Kv7.2 and Kv7.3 subunits is a neuronal voltage-gated K conductance that controls resting membrane potential and cell excitability. SGK1.1 upregulates the Kv7.2/3 current in Xenopus oocytes and in mammalian human embryonic kidney HEK293 cells. A mutant with disrupted PIP2 binding sites produced no effect on Kv7.2/3 current amplitude. These results suggest that the kinase increases M-channel membrane abundance, and thereby protects mice from kainic evoked overexcitability.

Title

Higher sensitivity of Wistar Kyoto compared to Wistar rats to the stimulatory effect of 5HT7 receptors agonist, AS19 on locus coeruleus noradrenergic neurons

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One of the main drawbacks in major depression treatment is the onset of antidepressants to produce their therapeutic effect. Recently, it has been proposed the 5HT7 autorreceptor as a new target for depression treatment, since blocking this receptor accelerates the therapeutic response of some antidepressants. Studies of the noradrenergic nucleus, locus coeruleus (LC) have highlighted mechanisms underlying the ethiopathology of depression and cellular effects of antidepressants. The goal of this study was to characterize the effect induced by the activation of 5HT7 receptors onto LC activity of Wistar Kyoto (WKY) rat, a proposed animal model of depression, and its outbred control, the Wistar (Wis) rat. For that purpose, we studied the effect induced by a selective 5HT7 receptors agonist AS19 on the LC neuron activity in vivo, using single unit extracellular recordings, and in vitro, using whole-cell voltage-clamp recordings. In vivo, AS19 (2.5-10mg/kg i.v.) had a dose-dependent stimulatory effect on LC noradrenergic neurons, and the selective antagonist of 5HT7 receptor SB 269077 (1mg/kg i.v.) did not block neither reverse this effect. Conversely, intracerebroventricular administration of kynurenic acid, an unspecific glutamatergic receptors antagonist (1µmol) partially blocked the effect induced by AS19. When LC slices were perfused with AS19 (100 µM), neither the frequency nor the amplitude of spontaneous excitatory postsynaptic currents were altered. Similarly, AS19 did not change the basal current neither the inward current induced by glutamate (100 µM). Interestingly, the in vivo sensitivity to AS19 was greater in LC neurons from WKY compared to Wis rats. Overall, these results suggested that WKY rats are more sensitive to AS19 which stimulatory effect seems to be indirect and partially mediated by the excitatory amino acid system.

Title

Ultrastructural localization of the CB1 receptor in the muscle mitochondria

Author(s)

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CB1 receptor is the main cannabinoid receptor expressed in the brain, but it is also present in peripheral organs where it participates in energy metabolism. Our recent data have shown that CB1 receptors are localized in mouse neuronal mitochondrial membranes where they regulate cellular respiration and energy production (Benard et al., 2012). However, nothing is known concerning the presence of CB1 receptors on peripheral mitochondria. The goal of this investigation was to determine whether CB1 cannabinoid receptors are localized in striate muscle mitochondria, an organ where the endocannabinoid system is present (Crespillo et al., 2011). Highly specific CB1 antibodies were used in combination with a pre-embedding silver-intensified immunogold method for high resolution electron microscopy. Gastrocnemius muscles were removed from transcardially perfusion-fixed wild-type (CB1-WT) mice and mutant littermate mice constitutively lacking the CB1 receptor gene (CB1-KO). CB1 immunoparticles were distributed on mitochondrial membranes of CB1-WT but not of CB1-KO mutants. The statistical analysis revealed that about 26% of mitochondria were immunopositive in CB1-WT, whereas, these values dropped to 0.37% in CB1-KO mice. The CB1 immunolabelling density was significantly different in WT and KO animals (0.231 versus 0.003 particles/µm, respectively). Interestingly, CB1-KO muscles had significantly fewer mitochondria than WT (0.36 versus 1.06 mitochondria/µm2). Mitochondria of CB1-KO mice displayed also a trend to be smaller than CB1-WT (mitochondrial area: 0.091 and 0.068 µm2 in WT and KO, respectively; p=0.7935). In conclusion, these anatomical data show that CB1 receptors, like in the brain, are also located in muscle mitochondrial membranes, where they might exert an intracellular regulation of metabolism through control of mitochondrial functions.

Title

Immunoelectron localization of the transient receptor potential vanilloid type 1 at inhibitory synapses in the mouse dentate gyrus

Author(s)

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The transient receptor potential vanilloid type 1 (TRPV1) is a non-selective cation channel that acts primarily as pain sensor in the periphery but also modulates neurotransmitter release and synaptic plasticity in the brain. TRPV1 function must lay on its anatomical distribution in the peripheral and central nervous system regions involved in the physiological roles of the channel. However, the anatomical localization of TRPV1 is well established in the periphery, but in the brain it is a matter of debate. We have recently shown that TRPV1 is highly concentrated in postsynaptic dendritic spines to asymmetric perforant path synapses in the outer 2/3 of the ML, being poorly expressed at the excitatory hilar mossy cell synapses in the inner 1/3 of this layer. However, the TRPV1 distribution at inhibitory synapses in the dentate molecular layer is still an open question. To investigate this, we have used TRPV1 antibodies combined with a highly sensitive pre-embedding immunogold method for high resolution electron microscopy. TRPV1 immunoparticles were observed in dentate granule cell dendrites receiving symmetric inhibitory synapses. The silver-intensified gold particles were mostly confined to postsynaptic membranes and distributed at a relative short distance from the inhibitory synaptic contacts. Importantly, the TRPV1 pattern distribution at inhibitory synapses disappeared in the molecular layer of TRPV1-knockout mice. These findings give additional knowledge on the fine TRPV1 localization in the rodent hippocampus by means of high resolution electron microscopy.

Title

Ultrastructural localization of DAGL-α and NAPE-PLD in the mouse ventromedial nucleus of the Hypothalamus

Author(s)

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It is well established that the endocannabinoid system and the hypothalamus, including the ventromedial nucleus (VMH), are associated with homeostatic and behavioral functions, such as regulation of food intake and energy balance. In a previous study, we showed that CB1 receptors in the VMH are mainly localized in inhibitory GABAergic and also in cortical and subcortical excitatory glutamatergic synaptic terminals. However, the distribution of the enzymes responsible for the synthesis of the main endocannabinoids (2-AG and anandamide) in the VMH is not known. Therefore, the aim of this study was to investigate in the VMH the precise ultrastructural distribution of the enzymes DAGL-α (for the synthesis of 2-AG) and NAPE-PLD (for the synthesis of anandamide). For this purpose, a high resolution pre-embedding silver-intensified immunogold method for electron microscopy was used. As a summary, DAGL-α was distributed in postsynaptic membranes of dendritic elements, as well as in dendritic spine heads and necks, while NAPE-PLD was localized in both postsynaptic dendrites and presynaptic terminals. Current extensive statistical analysis will define the contribution of these enzymes to the presynaptic and postsynaptic compartments of synapses demonstrated to localize CB1 in the VMH. This anatomical knowledge shown here will contribute understanding the complex regulation of appetite by the endocannabinoid system. Key words: immuno-electron microscopy, endocannabinoid synthesizing enzymes.

Title

Distribution of phospholipids in the brain of CB1 knockout mice by imaging mass spectrometry

Author(s)

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Much progress has recently been made in studies of brain lipids. Neural lipids play a role in synaptic vesicle fusion, regulation of receptors and ion channels, and formation of raft microdomains (where cannabinoid receptors are present) for neuronal cellular communication. Phospholipids (PL), are precursors of lipid species that participate in signalling processes and neurotransmission. Some of these neural lipids, such as the endocannabinoids, activate G protein coupled receptors (GPCR). The CB1 cannabinoid receptor is the main subtype involved in CNS synapses. Therefore, the localization of the different species of phospholipids in CB1 knockout mice would contribute to the understanding of the metabolic involvement of this class of lipids with the endocannabinoid signalling.

Title

Length and diameter of axon initial segment of pyramidal neurons in layer II and layer III of anterior cingulate cortex of the rat besides number and distribution of incoming axo-axonic synaptic boutons

Author(s)

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This paper presents parametric data of the axon initial segment (AIS) of principal cells in layers II-III of Vogt's area 24b of rats. Three classic spiny pyramidal neurons were studied by means of the rapid Golgi impregnation under correlated light- and electron microscopy. One cell sat in layer II and the remaining in layer III. In pooled AIS of layer III cells, the mean length was 35.04 ± 11.62 µm, whereas the mean diameter was 0.69 ± 0.11 µm (the individual diameters had been averaged from estimates of the proximal, intermediate and distal segments of each AIS). The number of axo-axonic synaptic boutons apposed to the pooled AIS was 13 ± 5.86; density (boutons divided per AIS-length) was 0.37 ± 0.04. In turn, the AIS of layer II cell measured 53.52 µm in length and 0.72 µm in diameter. It received 22 incoming synaptic boutons, with a density of 0.41. Thus, the AIS of the classical spiny pyramidal neuron was longer and more innervated in the cell of layer II. Results were compared to others of occipital cortex of rats [1]. The outcome was that the AIS of classic pyramidal neurons of layers II-III was longer, thinner and less innervated —both in the absolute numbers and density— in area 24b of anterior cingulate cortex than in occipital cortex. Patterns of innervation along AIS also differed in both regions.

Title

Deep interstitial periventricular white matter cells of frontoparietal have axon projection to anterior cingulate and dorsomedial prefrontal cortex in adult rabbits.

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In this study we observed that interstitial deep white matter cells (IdWM cells) have long-range axonal projection in particular to anterior cingulate (24b) and dorsomedial prefrontal cortex (PrcM). These cells were retrograde labelled following injections of Ctb in areas 24b and PrcM of adult rabbits. IdWM cells were retrograde labelled after injections in the anterior dorsal claustrum too. Ctb-labelled IdWM cells lay between the lateral subventricular zone (SVZ) and claustrum in the frontoparietal levels. Some of these Ctb-labelled IdWM cells were immunopositive for GABA as well. In addition, we observed Ctb-labelled cells in the antero-lateral SVZ; however, the morphology of these Ctb-labelled SVZ cells cells was different from Ctb-labelled IdWM cells. In prenatal and postnatal development, retrograde labelled cells after injections of DiI in the claustrum, and immunopositive GABA, Calbindin and Calrretinin immunolabelled cells stand as a continuum from the lateral SVZ into the ventro-lateral migratory stream to the external capsule; some of them occur in the claustrum too [1,2]. We consider that the Ctb-labelled IdWM cells observed in adult animals in the present study belong to the same cell class than those cells previously seen in the ventro-lateral migratory stream during development. Neurons in the white matter seem to be actively involved in coordinating interareal and subcortical connectivity and regulation of blood flow. They have been associated with a variety of neurological and psychiatric disorders [3]. Our present results add knowledge on neurons of deep white matter by showing that IcdWM cells, some of them GABAergic, have long-range axon projection to areas 24b and PmcM. The function of long-range projection cells in the adult SVZ remains to be elucidated. Work funded by UPV/EHU Grants GIU07/14 and UFI11/41. [1] Reblet C et al. (2002). Brain Res. Bull. 57, 495. [2] Reblet C et al. (2005). Brain Res. Bull. 66, 461. [3] Judas M et al. (2010). J. Anat. 217, 381.

Title

Acute impairment of microglial phagocytosis in the hippocampus of epileptic mice

Author(s)

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Apoptotic debris must be rapidly removed to prevent the spillover of cellular contents and the initiation of and inflammatory response. The professional phagocytes in the brain are microglia, which belong to the monocyte-macrophage lineage and orchestrate the brain inflammatory response. We have recently shown that in physiological conditions, apoptotic newborn cells in the adult hippocampal neurogenic niche are phagocytosed by unchallenged microglia. This microglial phagocytosis is fast and efficient, as measured by the phagocytic index (percentage of apoptotic cells being phagocytosed), phagocytic capacity (number of apoptotic cells being phagocytosed by each microglial cell) and clearance time (time taken by microglia to fully degrade an apoptotic cell). When apoptosis is triggered in the hippocampus by acute neuroinflammation using bacterial lipopolysaccharides, the microglial phagocytic capacity increases and thus the phagocytic efficiency remains constant. In contrast, when apoptosis is increased by kainic acid-induced excitotoxicity and seizures, the microglial phagocytic capacity and efficiency result drastically impaired. The mechanisms leading to this dysfunctional state of microglia as well as its consequences are currently under investigation and will be discussed.

Title

S-adenosylmethionine levels regulate the Schwann cell DNA methylome

Author(s)

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Axonal myelination is essential for rapid saltatory impulse conduction in the nervous system, and malformation or destruction of myelin sheaths leads to motor and sensory disabilities. DNA methylation is an essential epigenetic modification during mammalian development and in diverse pathological conditions, yet its role in myelination remains obscure. Here, using high-resolution in vivo methylome maps, we show that DNA methylation plays a key gene regulatory role in peripheral nerve myelination and in the pathogenesis of diabetic neuropathy, the most common and debilitating complication of diabetes. Importantly, we find that S-adenosylmethionine (SAMe), the principal methyl donor in cytosine methylation, regulates the methylome dynamics in these mice models. These critical observations establish a link between SAMe and DNA methylation status in a defined biological system, and provides a novel mechanism that could direct methylation changes during cellular differentiation and in diverse pathological situations.

Title

P2X4 receptors control the fate and survival of activated microglia

Author(s)

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Microglia, the resident immune cells of the central nervous system, responds to brain disarrangements by becoming activated to contend with the brain damage. Here we show that P2X4 receptor mRNA is upregulated in inflammatory foci and in activated microglia in the spinal cord of rats with experimental autoimmune encephalomyelitis (EAE) as well as in the optic nerve of multiple sclerosis patients. To study role of P2X4 receptors in microgliosis, we activated microglia with LPS in vitro and in vivo. We observed that P2X4 receptor activity in vitro was increased in LPS-activated microglia as assessed by patch recordings. In addition, P2X4 receptor blockade significantly reduced microglia membrane ruffling, TNFα secretion and morphological changes, as well as LPS-induced microglial cell death. Accordingly, neuroinflammation provoked by LPS injection in vivo induced a rapid microglia loss in the spinal cord that was totally prevented or potentiated by P2X4 receptor blockade or facilitation, respectively. Within the brain, microglia of the hippocampal dentate gyrus showed particular vulnerability to LPS-induced neuroinflammation. Thus, microglia processes in this region retract as early as 2h after injection of LPS and die around 24h later, two features which are prevented by blocking P2X4 receptors. Together, these data suggest that P2X4 receptors contribute to controlling the fate of activated microglia and its survival.

Title

Blood-Brain Barrier Modeling with Immortalized Endothelial and Primary Glial Cells Co-cultures.

Author(s)

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Many therapeutic and diagnostic drug candidates for the central nervous system (CNS) cannot be applied to the treatment of neurologic diseases due to their low concentration in the brain even when acceptable doses are administered. This is due to the fact that the delivery of many compounds to specific areas of the brain is restricted by the blood-brain barrier (BBB), a dynamic and complex interface between the blood and the CNS. The development of a standard in vitro BBB model is an imperative tool to study the structure and function of the BBB at the cellular level as well as to pursue the considerations of the "3 Rs" principles of animal research. To this end, epithelial and endothelial cells are being used to build paracellular barriers which protect the tissue from the external and internal environment. The aim of the present study was to evaluate the role of astrocytes on the modulation of paracellular permeability and morphogenesis in different BBB cell culture-based models. Four immortalized cell lines: the human intestinal cell line (Caco-2), the Madin-Darby Canine Kidney Cells (MDCKII), the brain endothelial cells (bEnd.3) and the human brain microvascular endothelial cells (hcmec/D3), were cultured alone, on conditioned medium or co-cultured with rat primary astrocytes. The development of the different in vitro BBB models was monitored by the measurement of the transendothelial electrical resistance as well as enzymatic activities as γ-glutamyl transpeptidase activity and alkaline phosphatase activity. Lucifer Yellow, Dextrans 10, 40 and 70 permeability assays and tight junction citometry studies were carried out to detect functional differences on the BBB models. The results showed that the presence of glial cells in the co-cultures and the use of conditioned medium enhance the properties of the BBB models. In the presence of astrocytes, co-cultures developed elevated trans-endothelial electrical resistance respect to cell cultures without astrocytes. Likewise, paracellular transport studies indicated lower markers absorption values and therefore, an increase in selective permeability. In co-cultures was also observed higher tight junctions expression compared to cells grown as monolayer. However, the different culture conditions work at different ways. Adhesion, enzyme activity and permeability properties of the BBB models change depending on the type of the endothelial cell and culture form, noticing a poor correlation between the four in vitro models. Future efforts should be directed towards improving existing models.

Title

Glial-Neuronal Interactions in Adult Retina Cell Cultures

Author(s)

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Introduction: Following retinal disease or injury, for example in glaucoma or retinal ischemia, axonal degeneration and death of retinal ganglion cells (RGC) results in irreversible blindness. The retinal glial cells, astrocytes and Müller glia, provide structural and trophic support to RGCs in the healthy retina and may also have a function in promoting cell survival after injury. Objective: We are interested in understanding the mechanisms of regeneration in adult RGCs and how glial plasticity may be influencing this process. In rat models of glaucoma and ischemia, retinal glia were found to adopt a branched morphology, suggesting that there exists a degree of cell plasticity, which may have functional significance. Methods: In this study, we have investigated the relationship between adult rat glia and RGCs in the healthy and damaged retina in vivo and also how these cells interact in vitro. Using a cell culture system, we have investigated the interactions between retinal glia and RGCs using cells isolated from adult rat. Results: The organisation of astrocytes in the retina of a rat model of glaucoma was found to be significantly different than in a healthy retina. These alterations in glial cell morphology may reflect changes in their relationship with RGCs. We found that cultured adult RGCs in close contact with adult Müller cells exhibit improved cell viability and significant neurite elongation. Müller cell conditioned media was also found to have a positive effect on RGC growth, however to a lesser extent. Conclusion: These results suggest that Müller glia support RGC regeneration not only by direct interaction, but also releasing soluble trophic factors. Further understanding of the relationship between retinal glia and RGCs is important in order to identify potential therapeutic targets to encourage retinal neuroregeneration.

Title

Amyloid β oligomers upregulate myelin proteins in oligodendrocytes

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Alzheimer´s disease (AD) is a neurodegenerative disorder that is characterized by the presence of senile plaques formed by amyloid beta (Aβ) peptide aggregates. Although AD is primarily a neuronal disease, oligodendrocytes in gray and white matter are also affected and their damage may contribute to cognitive decline in AD. However, the mechanisms underlying oligodendrocyte demise in AD and the contribution to AD pathophysiology remain poorly understood. In this study, we have investigated the signaling cascades triggered by Aβ1-42 oligomers in primary oligodendrocyte cultures derived from the rat optic nerve and in cerebellar organotypic slices. First, we found by RT-qPCR that incubation of organotypic slices with Aβ oligomers (200 nM; 48 h) upregulate the expression of myelin proteins (MBP, CNPase and PLP) and of PDGF receptors. These findings were confirmed by western blot and immunocytochemistry assays of Aβ-treated oligodendrocytes and cerebellar slice samples. To understand the signaling pathways underlying Aβ-mediated myelin increase, we analyzed the regulation of AKT and ERK phosphorylation, two key proteins involved in oligodendrocyte differentiation. We found that Aβ oligomers triggered a sustained ERK phosphorylation and AKT dephosphorylation in primary oligodendrocyte cultures. These effects were diminished by UO126 and SF1670, inhibitors of MEK 1 kinase and PTEN phosphatase, respectively. Importantly, inhibition of both Aβ-activated pathways reduced the MBP expression in oligodendrocytes. Our data demonstrate that Aβ oligomers signal directly to oligodendrocytes and induce the expression of myelin proteins through MAPK/ERK and PI3K/AKT signaling pathways. These mechanisms may be relevant to AD pathophysiology.

Title

Acute effects on learning and memory mediated by cannabinoid receptors activation

Author(s)

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The cholinergic system plays an important role in learning and memory processes that are affected in Alzheimer's disease (AD). The muscarinic receptor (MR) antagonist, scopolamine (Scop), causes memory impairment in the rat. Controversial data have been reported in relation to cognitive functions induced by cannabinoids in AD. In addition, cannabinoids exert different effects in learning and memory processes depending on the behavioural test, dose or via of administration used in the studies. The aim of the present study was to evaluate the effect of WIN55,212-2 (1 mg/kg; i.p.) in the learning and memory impairment model in rodent induced by the administration of Scop (2 mg/kg; i.p.) previously to the passive avoidance test. The acquisition trial was done 30 min after drug administration and the retention trial was performed 24 h after the training. The latency to enter the dark compartment was recorded. The animals were anesthetised and dissected 24h later after the last administration. [35S]GTPgS autoradiography was used to evaluate the functional activity of cholinergic MR and CB1 receptors in diverse brain regions related with learning and memory. The administration of WIN55,212-2 or the vehicle did not modify the learning and memory trials. But, when the cannabinoid agonist was administered 90 min before Scop, an slight protection from the memory impairment induced by Scop was recorded in the latency time (WIN55,212-2+Scop: 223 ± 34 sec vs vehicle+Scop: >300 sec). The in vitro experiments showed that the activation of [35S]GTPgS induced by WIN55,212-2 (CB1 activity) was higher in the ventral hippocampal CA1 area in the group treated with both drugs when compared to that of the animals which received only Scop previously to the test (Scop: 187 ± 44 % over basal value vs Scop+WIN: 337 ± 42 % of stimulation; Scop: 211 ± 35 % vs Scop+WIN: 499 ± 117%, respectively in stratum lacunosum and radiatum; $p<0.05$, n=8). On the contrary, the in vitro stimulation by carbachol of the [35S]GTPgS binding (MR activity) was lower in the dentate gyrus of the group which received both drugs (Scop: 70 ± 10 % vs Scop+WIN: 35 ± 10 %; $p><0.01$, n=8). These preliminary results support the existence of an interaction between cholinergic and cannabinoid systems in brain areas related with learning and memory. Further behavioural and neurochemical studies are necessary to analyse the modulation of MR-mediated activity by cannabinoid

Title

The study of the microRNAs expression pattern and their targets in the rat visual cortex under experimental conditions directed to neuroplasticity

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Currently, microRNAs are key post-transcriptional regulators of gene expression, given their ability to degrade mRNA targets or inhibit the translation of the mRNA targets into the corresponding proteins. Recent studies have documented the involvement of the microRNAs in neuronal plasticity processes like neurite outgrowth, axonal pathfinding and regulation of synapses. However the mechanisms underlying this phenomenon still remain unlargely unexplored (1). Previous experiments in our laboratory have demonstrated that magnetic field application and also visual deprivation caused in the visual cortex of rodents changes in the levels of expression of different proteins implicated in cellular mechanisms of neuroplasticity (2, 3). For this reason, we focused our attention on the study of the microRNAs involved in the neuronal plasticity of the visual system. In order to achieve this aim, we first identified by microarray the microRNAs whose expression pattern is altered in the primary visual cortex under different experimental conditions (dark rearing, magnetic stimulation and dark rearing with magnetic stimulation) with respect to control cases. Then, a qPCR assay was performed in order to determine further statistical details related to the expression level of the microRNAs. Afterwards, another qPCR assay was conducted to validate the corresponding targets of each microRNA which had shown significant differences in the previous analysis. Based on the microarray data, the expression pattern of forty-seven microRNAs resulted to be altered in comparison with control cases under the experimental conditions. Five of them presented its expression significantly down-regulated in animals subjected to dark rearing or eyelid suture and magnetic stimulation: let-7b*, miR-330, miR-338*, miR-376c and miR-542-5p. Consequently, the deregulated expression of these microRNAs in the experimental conditions above mentioned caused significant alterations in the level of expression of their corresponding predicted targets (BDNF, Gjb2, Tnr, Cntn4, Sncb), with the exception of miR-542-5p. Therfore, this study has further deepened the role of microRNAs in the regulation of gene expression of molecules involved in the synaptic efficacy, axonal and dendritic remodeling in the visual system. (1) Kosik KS. Neuroscience 2006 (7): 911-920. (2) Rienda B. Tesis doctoral 2011. (3) Bozzi Y et al. Neuroscience 1995 69(4): 1133-1144.

Title

The effects on the cortex of specific anti-miRNAs against BDNF and β-synuclein

Author(s)

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MicroRNAs (miRNAs) are a class of small non coding regulatory RNAs that have key functions in cell processes such as development, apoptosis and regulation of synaptic plasticity(1). Using anti-miRNAs, molecules designed to specifically bind and inhibit endogenous miRNA, this study pretends to inhibit rno-let-7b* and rno-miR-376c which are responsible for downregulating BDNF and β-synuclein respectively. BDNF is a neurotrophin linked to neuronal plasticity and is involved in learning and memory (2, 3); and a reduction of this molecule levels has been reported in several diseases such as depression or Parkinson diseases among others (4). Moreover, β-synuclein modulates α-synuclein neurotoxicity by reducing α-synuclein protein expression (5), which could be relevant because abnormal aggregations of α-synuclein have been described in some neurodegenerative diseases such as Parkinson and Alzheimer (6). This study aims to inhibit specific miRNA (rno-let-7b* and rno-miR-376c) by injecting the corresponding anti-miRNA directly in the cortex and analyze the changes in the target proteins. For this study, the anti-miRNA was injected in the anteroposterior axis of the left hemisphere and the effect of this administration was analyzed in two different conditions: in the first one, the animal was not exposed to any experimental changes; whereas in the second one, the animal underwent a right eyelid suture in P9 and was exposed to a magnetic field for a week in the adulthood. Afterwards, the brains were processed with ELISA for BDNF and immunohistochemistry for α and β-synucleins. Preliminary results showed that rats overexpressed BDNF in the cortex after the injection of anti-let-7b*. Moreover, β-synuclein was highly overexpressed in the area surrounding the anti-miR-376c injection, although the reduction of α-synuclein was not so robust. Additionally, qRT-PCR showed that both, rno-let-7b* and rno-miR-376c were downregulated under the condition of eyelid suture and magnetism. Based on these results, the anti-miRNAs have been demonstrated to be a powerful tool in the regulation of miRNAs expression in a specific way. So, the administration of anti-miRNAs may eventually find application in a new therapeutic mechanism to treat neurodegenerative diseases which manifests a decrease in the expression of BDNF or an increase in the level of α-synuclein. (1) Lim LP et al. Nature 2005 (433): 769–773. (2) Alsina B et al. Nat Neurosci 2001 (4): 1093–1101. (3) Heldt SA et al. Mol Psychiatry 2007 (12): 656–670. (4) Chauhan NB et al. J Chem Neuroanat 2001 (21): 277–288. (5) Fan Y et al. Human Molecular Genetics 2006 Vol. 15, No. 20: 3002-3011. (6) Shibayama-Imazu T et al. Mol. Reprod. Dev. 1998 (50): 163-169.

Title

Neurochemical and cellular mechanisms underlying opiate tolerance and dependence

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The prescription of opioids as pharmacotherapy is limited by the development of dependence and tolerance to their effects. The locus coeruleus (LC), which is the major noradrenergic nucleus in the brain, has been widely used as a model to study the mechanisms of opioid tolerance and dependence. Thus, previous work has suggested the involvement of glutamatergic and nitric oxide (NO) signalling pathways in opioid withdrawal and tolerance, and in the desensitization of μ opioid receptors (MOR) in the LC. The main aim of our studies is to reveal the neurochemical and cellular mechanisms underlying opiate tolerance and dependence. The specific aims were: (1) to study the effect of modulating glutamatergic system on opioid withdrawal syndrome in behavioural models and neuron activity recordings in the LC; (2) to explore the involvement of NO-dependent signalling pathways (i.e., soluble guanylate cyclase and reactive oxygen species generation) in MOR desensitization and behavioural and electrophysiological tolerance to opioids; and (3) to explore the intracellular mechanisms underlying the functional turnover and trafficking of MOR, and its modulation by opioid agonists with different pharmacological profiles. For these purposes, we used behavioural tests and electrophysiological recordings in the LC from rat brain slices. Our results can be summarized as follows: (i) presynaptic processes regulating the glutamate neurotransmission (such as the release or reuptake) can be targeted by drugs that affect both the behavioural and the cellular responses induced by morphine withdrawal in the rat LC; (ii) signalling pathways linked to NO, such as the guanylate cyclase or the reactive oxygen species generation, are involved in the development of cellular and analgesic tolerance to opioids and in MOR desensitization induced by specific opioid agonists, and (iii) MOR in the LC undergoes a rapid and constitutive turnover that depends on calcium- and activity-regulated trafficking from a cytoplasmatic pool of receptors; prolonged treatments with different opioid agonists induce marked changes in the kinetics of MOR turnover that prevent LC neurons from recovery to normal functionality. In conclusion, our data suggest different neurochemical and molecular events in brain nuclei such as the LC that account for the neuroplasticity accompanying prolonged administration of opioids: presynaptic glutamate release and uptake during opioid withdrawal, NO-dependent signalling cascades in the induction of MOR desensitization and opioid tolerance, and functional turnover and trafficking of MOR as an efficient process that is regulated by opioids. This better understanding of the neuroadaptive changes during chronic use of opiates may be relevant for improving the therapeutic benefits of opiates in the patient.

Title

Neither environmental enrichment nor physical exercise alone is enough to recover astrocytic population from dark-rearing. Synergy is required.

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Elimination of sensory inputs (deprivation) modifies the properties of the sensory cortex and serves as a model for studying plasticity during the postnatal development. Many studies on the effects of deprivation have been performed in the visual cortex using dark-rearing as visual deprivation model, inducing changes in all cellular and molecular components, including astrocytes which play an important role in the development, maintenance and plasticity of the cortex mediated by cytokines that have been termed angioglioneurins. When one sense is deprived, a compensatory mechanism called cross-modal plasticity increases performance in the remaining senses. Environmental enrichment is so far the best-known method to compensate sensorial deprivation. The aim of this work is to study the effects on astroglial population of exercise and enriched environment, separately or the potential synergistic effect of their combination during the rat visual system development, in order to determine an hypothetical capacity of these components to compensate the negative effects produced by visual deprivation over visual cortex astrocyte density. Rats were raised in one of the following rearing conditions: control rats with standard housing (12-h light/dark cycle); in total darkness for the dark-rearing experiments; dark-rearing in conditions of enriched environment without and with physical exercise; and dark-rearing with physical exercise. The astrocytic density was estimated by immunohistochemistry for S-100β protein. Quantifications were performed in layer IV. The somatosensorial cortex barrel field was also studied as control. The volume of layer IV was stereologically calculated for each region, age and experimental condition. Our main result shows that rats raised in enriched environment in combination with exercise, get reverse the negative effects that the darkness has on astroglial population. This effect is from the beginning of the critical period, and remains beyond it, so that rats with a double combination of enrichment and exercise have higher astrocytic population that maids in a control and much higher than those reared in darkness without any stimulation.

Title

Anxiety-like responses and LTP induction changes in mice overexpressing hippocampal 5-HT1A receptors

Author(s)

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Several studies have investigated the role of the postsynaptic 5-HT1A receptors by pharmacological approaches in specific projection areas of the serotonergic system since it is well known the 5-HT1A receptors plays an important role in the neurobiology of depression/anxiety disorders. In this study, we used mice overexpressing postsynaptic 5-HT1A receptors in serotonergic projection areas in which we have evaluated: (a) their behavioral performance in the anxiety-like paradigm of light/dark box (LDB), (b) LTP induction by electrophysiological techniques and (c) BNDF and Arc mRNA expression levels by qPCR. 5-HT1A TG mice exhibited a decreased anxiety-like response under basal circumstances in the LDB test (light/dark: 1.44±0.12 vs 1.00±0.08 in WT; $p<0.05$). Interestingly, the pre-exposure to a single mild stressing factor tended to increase the anxiety response in the TG mice (1.27±0.08 vs 1.45±0.12 in TG naïve) but not in the WT group, whereas the addition of a second more severe stressing factor, resulted to be clearly anxiogenic for both WT (0.62±0.12; $p><0.05$ vs naïve and injection), and TG mice (0.51±0.09; $p><0.001$ vs naïve and injection). Electrophysiological experiments demonstrated that the hippocampal induced LTP did not differ between both genotypes under basal conditions. After pre-exposure to acute stress (forced swimming session), LTP was decreased in WT mice (116.8±2.5 vs 139.1±2.7 in non stressed WT; $p><0.001$) but not in TG mice (145.21±3.4%). Interestingly, this stress-resistance in TG mice was suppressed after acute administration of the 5-HT1A antagonist WAY-100635. We further analyzed the expression of genes related to the synaptic consolidation, as brain-derived neurotrophic factor (BDNF) that induces the immediate early gene activity-regulated cytoskeleton-associated protein (Arc). BDNF expression was similar for naïve WT and TG mice (1.00±0.06 vs 0.90±0.11, respectively), and was decreased in both groups after double stressing protocol (0.43±0.02 vs WT, $p><0.001$; 0.42±0.04 vs TG, $p><0.001$). In contrast, a different pattern of expression was observed for Arc. Under basal conditions it is decreased in TG mice compared to WT counterparts (0.52±0.07 vs 1.00±0.03 for WT; $p><0.01$), while an increase was observed in TG but not WT mice following the exposition to double stressor (1.06±0.06 vs 0.42±0.04 for TG naïve; $p><0.05$). Our results demonstrate that 5-HT1A TG mice show a differential anxiety-like response depending on the intensity of the stressor and exhibit a resistance to acute stress-induced impairment of LTP. This appears to be related to changes in hippocampal functionality and level of BDNF and Arc

Title

Environmental enrichment reverts cognitive and neurovascular deficits produced by the kinase inhibitor of VEGFR-2 Vandetanib in the rat visual cortex

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An enriched environment has been shown to significantly facilitate recovery from brain injury due to important cortical changes that occur mainly during the critical period. Vandetanib, is a small-molecule tyrosine kinase inhibitor (TKI) that targets VEGF receptor (VEGFR2-3) signalling which apart from angiogenesis processes is involved in neuroprotective, neurotrophic and neurogenic pathways. Our aim is to investigate neurovascular and cognitive effects of Vandetanib oral-administration and the role of enriched environment to counteract Vandetanib effects during the critical period of the rat visual cortex development.

Title

Effect of Geomagnetic field changes on the expression of an activity related gene by Encephalic Neurons

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It has been previously shown that changes of the horizontal or vertical component of the natural geomagnetic field elicits the expression of the activity related c-fos gene in the mesencephalon of the rat`s brain [1]. A geomagnetic field stimulation platform that allows a shift of 120º in the horizontal or vertical component was built in our laboratory. After 1 h of application, this modified geomagnetic field triggered a high expression of c-fos in all cortical areas and hippocampus as well. We explored the participation of signaling pathways activated by membrane receptors that could trigger the expression of c-fos. Injection of Raf derivative peptides or pharmacological inhibitors of MAP kinases dramatically decreased the c-fos expression. Similar geomagnetic fields were applied 3 times a day during I week to visually deprived rats as a model of experimental amblyopia. A microarray study of the expression changes of c-fos promoted microRNAs in control visual cortex in comparison with amblyopic cortex revealed a lower expression of microRNAs like Rno-let-7b, Rno-miR-330 and Rno-miR-376c and consequently an increased expression of BDNF, Neurotrophin 3 and Synuclein beta, which are the main targets of these microRNAs. These changes were accompanied by an increase of histone acetylation in supragranular visual layers which is considered a parameter of visual recovery. In the future we will explore the effects described here to study the effects of geomagnetic modified fields on memory mechanisms, aversive effects or circadian rhythms changes involved in nervous diseases. [1]P. Nemec, et al. 2001. Neuroanatomy of magnetoreception: The superior colliculus involved in magnetic orientation in a mammal. Science, 294: 366-368.

Title

GFAP Knock-Out mice with blunted adult neurogenesis show anxiety-like behavior, altered plasticity pathways and modified endocannabinoid signaling

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It has been certainly reported that endocannabinoid system is involved in depression/anxiety disorders. Additionally, anxiolytic effects of cannabinoid drugs appear to be dependent on hippocampal neurogenesis. In order to further investigate this linkage, we have studied different aspects of GFAP (Glial Fibrillary Acidic Protein) conditional knock-out mice, in which adult hippocampal neurogenesis was blunted. For this purpose, ganciclovir was administered to induce the hippocampal neurogenesis impairment and subsequently: a) hippocampal proliferation by inmunohistochemistry; b) anxiety-like responses in the open field test; c) functionality of CB1-receptors by [35S]GTPgS binding; and d) BDNF/TrkB expression levels by in situ hybridization were assessed. Inmunohistochemistry studies confirmed impaired hippocampal neurogenesis in TG mice as evidenced by a significant decrease in BrdU+ expression in the dentate gyrus of hippocampus (% of reduction: 37±7%; $p< 0.01$). In the open field, TG mice showed an anxiogenic phenotype reflected by increased central ambulation (central entries= 12±5 vs 21±8 for WT; $p< 0.01$). In addition, the [35S]GTPgS binding stimulated by WIN 55,212-2 (10-5 M) was significantly decreased in both the dentate gyrus of hippocampus (% of reduction: 34±5; $p< 0.01$) and medial prefrontal cortex (% of reduction: 56±2; $p< 0.01$) of TG mice. Finally, lower levels of BDNF associated to up-regulation of TrkB mRNA expression in some brain areas (medial prefrontal and frontal cortices) were also detected. In conclusion, we demonstrate that GFAP knockout mice with impaired adult neurogenesis exhibit an anxious phenotype, CB1-receptor desensitization in hippocampus and medial prefrontal cortex, and an alteration in BDNF-TrkB plasticity markers signalling.

Title

Environmental enrichment reverts the cognitive effects of altitude hypoxia

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Microvascular environment plays a fundamental role in the adaptive response to increases in synaptic activity. The increased demand in blood supply leads to local brain hypoxia and subsequent angiogenesis. VEGF is a the archetypal angioglioneurin which can be induced by hypoxia. Interestingly, we have previously reported that environmental enrichment (EE) leads to an increase in VEGF in the brain promoting active angiogenesis. Altitude hypoxia mimics the increase in energy demand and can be used to study the interplay between the vascular and neuronal networks in cognitive functions and neurodegeneration. In this work, we addressed whether altitude hypoxia can interfere with spatial memory in Long Evans rats and we investigated whether EE can rescue this effect. Our results indicate that rats born and raised (P49) at moderate altitude (600m, Fribourg, CH) show a diminished exploratory activity, and perform similarly in spatial learning tasks as compared with rats of the same age raised in an EE. In contrast, rats, exposed from P49 to P56 to high altitude (3450m, JungfrauJoch High Altitude Station, CH), display increased exploratory behavior as compared to the rats raised at 600m, but their spatial memory performance is reduced when compared to cage controls at moderate altitude. This indicates a detrimental effect of altitude hypoxia on neuronal networks function. Interestingly, rats that were kept in an EE displayed an increased angiogenic response and had rescued the memory deficit at high altitude. This indicates that the EE paradigm can be neuroprotective to the effects of altitude hypoxia. To further understand whether altitude hypoxia has a direct effect on gene expression in the hippocampus, we investigate the expression of Notch1, which is important signaling factor implicated in learning and memory. We observed that the levels of Notch1 were strongly induced in hippocampal CA1 in the rats exposed to EE as compared to cage controls at moderate altitude. This indicates that the enriched paradigm leads to an increase in network activity however the increase in Notch1 expression does not correlate with the spatial memory performance of the rats. Moreover, the levels of Notch1 were strongly induced in rats exposed to altitude hypoxia both in cage control and EE conditions. This would indicate that altitude hypoxia “per se” is enough to induce Notch1 expression, and that other mechanisms are responsible for the rescue in memory performance. Indeed, Notch1 has been shown to be induced by cerebral hypoxia ischemia and may contribute to inflammation and neurodegeneration. On the whole, moderate altitude has effects on spatial memory, which is compensated by rearing in enriched environment including physical exercise

Title

A neural extracellular matrix-based method for in vitro hippocampal neuron culture and dopaminergic differentiation of neural stem cells

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Background The ability to recreate an optimal cellular microenvironment is critical to understand neuronal behavior and functionality in vitro. An organized neural extracellular matrix (nECM) promotes neural cell adhesion, proliferation and differentiation. Here, we expanded previous observations on the ability of nECM to support in vitro neuronal differentiation, with the following goals: (i) to recreate complex neuronal networks of embryonic rat hippocampal cells, and (ii) to achieve improved levels of dopaminergic differentiation of subventricular zone (SVZ) neural progenitor cells. Methods Once isolated and expanded, both rat hippocampal neurons and SVZ progenitor cells were differentiated on PLL- and ECM-coated coverslips. The progression of hippocampal and SVZ cultures was observed on both substrates by neuronal and glial immunostainings. Results When seeded on nECM-coated substrates, both hippocampal cells and SVZ progenitor cells showed neural expression patterns that were similar to their poly-L-lysine-seeded counterparts. However, nECM-based cultures of both hippocampal neurons and SVZ progenitor cells could be maintained for longer times as compared to poly-L-lysine-based cultures. As a result, nECM-based cultures gave rise to a more branched neurite arborization of hippocampal neurons. Interestingly, the prolonged differentiation time of SVZ progenitor cells in nECM allowed us to obtain a purer population of dopaminergic neurons. Conclusions We conclude that nECM-based coating is an efficient substrate to culture neural cells at different stages of differentiation. In addition, neural ECM-coated substrates increased neuronal survival and neuronal differentiation efficiency as compared to cationic polymers such as poly-L-lysine.

Title

A unified model for the neural competence of adult human connective tissue stem/precursor cells

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Resident neural precursor cells (NPCs) have been reported for a number of adult tissues. Understanding their physiological function or, alternatively, their activation after tissue damage or in vitro manipulation remains an unsolved issue. Here we investigated the source of human dermal NPCs in the adult. By following an unbiased, comprehensive approach of cell surface marker screening, cell separation, transcriptomic characterization and in vivo fate analyses, we found that p75+ Sox2+ dermal stem/precursor cells of human trunk skin can be traced back to the Schwann cell (CD56+) and pericyte lineages (CD56-), which seem to be developmentally interrelated. Moreover, neural differentiation of dermal stem/precursor cells was restricted to the Schwann-like cells. These cells were similarly obtained from human heart stromal tissue. We postulate a model by which neural competence of connective tissue stem/precursor cells is dependent on Sox gene expression levels. We further hypothesize that Sox2+ resident precursors arise by dedifferentiation of Schwann cells at peripheral nerve endings of multiple organs.

Title

Foxa2 for the selection of midbrain dopamine neurons derived from pluripotent cells.

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Parkinson disease (PD) is a neurodegenerative disorder characterized by a selective degeneration of dopamine (DA) neurons in the substantia nigra. Cell replacement therapy using human embryonic mesencephalic tissue has been shown to restore dopamine transmission upon engraftment in the striatum. Remarkably, this has been achieved in different PD animal models and also in PD patients. Human embryonic stem cells (hESC) and induced pluripotent stem cells (iPSC) are potential cell sources for regenerative medicine and in vitro modeling. Despite optimized differentiation protocols to generate midbrain DA neurons (mDANs) from pluripotent cells, there is still a need for selection strategies to obtain a homogeneous cell population, required for their application to cell replacement or drug screening. Here we developed a selection approach to enrich mDANs derived from mouse and human pluripotent sources using the transcription factor (TF) Foxa2 that is expressed in mDANs early in development. We designed a lentiviral vector driving the expression of GFP controlled by the promoter of Foxa2. We validated the specificity of the vector in mouse and human cell lines and in human DA progenitors (hDAPs) against a promoterless construct. DAPs derived from mouse embryonic stem cells were transduced with the lentiviral vector and selected by FACS. Analysis was carried out immediately after sorting by quantitative real time PCR (qRT-PCR) and showed enrichment in several DA markers with respect to the unsorted population. This fraction was enriched in neural progenitors expressing nestin rather than post-mitotic neurons positive for β-III tubulin. Similar to mouse cells, we transduced hESC-derived neural progenitors to select DAPs expressing Foxa2. In the GFP positive fraction there was an increase in TF transcripts expressed in the ventral domain of the midbrain (Nkx2.2, Lmx1a, Foxa1) and floor-plate markers (Six6, Foxa2). Dorsal TFs such as Pax6 were more abundant in the GFP negative fraction. Next we compared the transcriptional profiles of human neural progenitors selected with the Foxa2 promoter with those transduced with a retroviral vector to over-express Foxa2 during the differentiation. Both populations showed increased mRNA levels of TFs expressed in the ventral midline (Foxa2, Netrin, Lmx1a) but not in mature DA markers. These results suggest that additional(s) DA determinant(s)/surface marker(s) will have to be combined with Foxa2 towards an efficient selection of mDANs. A dynamic expression pattern of TFs in mDANs derived from hESC could result in a narrow window and further complicate optimal selection of human midbrain DAPs using this strategy. Additionally, differences in Foxa2 expression by mDANs might exist between rodent and human, especially in the mature stage. Our work contributes to better understand the role of Foxa2 in mDAN development and to improve the biotechnology of pluripotent cells toward the relevant cell type lost in PD.

Title

Purinergic and serotoninergic signalling in adult neurogenesis

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Background. Stem cells possess high therapeutic relevance owing to their potential to differentiate into specific cell types as well as their capacity to be expanded in vitro. The adult mammalian brain still holds the capacity to generate neural cells. Mainly, there are two germinal zones in the adult mammalian brain that continue to generate new neurons and glia: the subventricular zone (SVZ) and the hippocampal subgranular zone (SGZ). These regions contain multipotent precursor cells that can be isolated and grown in vitro as floating neurospheres, maintaining self renewal capacity and multipotentiality in culture. Differentiation of neurospheres can be modulated by growth factors, hormones or neuromodulators like extracellular ATP and serotonin. ATP signals are mediated by fast ionotropic (P2X1-7) or metabotropic (P2Y1,2,4,5,6,8-14) receptors. Serotonin is reported to be present right from the early embryonic stages and plays an important role in early patterning. In addition, it has been suggested that serotonin plays an important role in neurogenesis through a large number of receptors (5HT family). Moreover, serotonin and ATP can be actively co-released from neurons and other cell types. Objectives. Given that serotonin and extracellular ATP modulate the differentiation of neuronal progenitor cells, the aim of this work was to characterize the effect of serotonin and purinergic P2X receptors on neuronal differentiation. Results. The effect of serotonin and extracellular ATP on neurogenesis was studied by evaluating proliferation and neuronal differentiation of cultured rat neurospheres from the subventricular zone. Proliferation in the presence of either serotonin or ATP-gS was decreased, an effect that was further intensified by simultaneous administration of both agonists. In turn, activation of serotonergic or purinergic receptors led to neuronal or oligodendrocyte differentiation, respectively. Thus, serotonin acting at 5HT1A receptors, stimulated neuronal differentiation since their specific agonist/antagonist (clozapine and WAY 100135) mimicked the effect of that neurotransmitter. Finally, in order to discriminate which P2X receptor was involved in the modulation of neurosphere differentiation we performed qRT-PCR of all P2X receptor mRNAs at various stages of differentiation and found that among them, the expression of P2X1, P2X3 and P2X5 mRNA was increased, P2X4 mRNA was decreased and P2X2, P2X6 and P2X7 mRNA was not significantly changed. These findings suggest that both serotonin and purinergic receptors modulate the differentiation of neural cells in the SVZ.

Title

Genetic and Functional Characterization of the Neurogenic Differentiation Potential of Human Dental Pulp Stem Cells (DPSC)

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Introduction The dental pulp of adult teeth harbors a very active stem cell population with a neural crest cell phenotype: the so-called dental pulp stem cells (DPSC). These are potentially a very attractive option for its use in neuroregenerative cell therapies. As compared to many other stem cell types that can be found in the adult human body, DPSC are unique in that they basally express a wide variety of neural cell markers. DPSC present other substantial advantages, such as a much higher accessibility than endogenous neural stem cells, together with a big capacity of ex-vivo expansion and subsequent neural differentiation, which may leave the door open to autologous cell therapy for neuroregeneration. Objective The aim of our research is to characterize the neurogenic differentiation potential of DPSC, with the final goal to determine which are the factors that influence the conversion of non-differentiated ectomesenchymal DPSC to neural cells, capable to integrate within a host nerve tissue. Methodology We analyzed, by immunocytochemistry and RT-PCR, the expression profile of pluripotency markers, neural markers, neurotrophins and neurotransmitter receptor in DPSC, grown in serum-containing or serum-absence culture conditions. We assessed the functionality of the neurotransmitter receptor machinery present in DPSC, by Ca2+ microfluorimetry. Results Freshly-plated DPSCs form adherent proliferative colonies. In the presence of 10% Fetal Bovine Serum (FBS) in the culture medium, cell proliferation is sustained to eventually create a uniform collagen I-containing monolayer. However, DPSC grown in media formulations devoid of FBS cease early to proliferate, these changes correlating with a cell morphology elongation, including the appeareance of long and thin processes closely resembling neurites. Despite this, marker expression profiles for pluripotency (Oct4, Sox2), mesenchymal (Vimentin, Collagen), and neural (Nestin, β3-Tubulin) phenotypes were retained in both experimental conditions. The same was observed for neurotrophin expression, where NGF, BDNF and NT3 were all found to be expressed by DPSC. However, subtle differences were appreciated in neurotransmitter receptor expression profiles, where ionotropic glutamate AMPA and Kainate receptors where found in both conditions, together with GABA-B and muscarinic Acetylcholine receptors. However, only DPSC grown in the absence of serum were found to express ionotropic ATP P2X7 receptors. In order to confirm these differences, and assess the functionality of neurotransmitter receptors present in DPSC, we performed live Ca2+ microfluorimetry assays, using DPSC grown in both serum presence/absence conditions. Discussion and Conclusions DPSC retain many of the characteristics of neural cells, including expression of neural markers, neurotransmitter receptors and neurotrophins. However, they additionally present other features that can be hardly attributed to genuine nerve-tissue cells, such as collagen production, and pluripotency. Serum deprivation may be one of the factors that influence the differentiation of DPSC toward a neural phenotype.

Title

Genetic Inducible Fate Mapping to Trace the Differentiation of Adult Hippocampal Neural Stem Cells in a Rodent Model of Temporal Lobe Epilepsy

Author(s)

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A population of radial neural stem cells (rNSCs) that persists throughout adulthood in the dentate gyrus (DG) of most mammals, including humans, is able to generate new neurons that integrate into the hippocampal circuitry. This process referred to as adult hippocampal neurogenesis is important form memory formation, spatial learning, pattern separation, fear conditioning, and anxiety. We have found that in a model of temporal lobe epilepsy (MTLE), the most common form of epilepsy, adult hippocampal neurogenesis results chronically impaired, which in turn implies the loss of the cognitive functions associated to neurogenesis and the loss of the potential to regenerate the neurons that die by excitoxicity in MTLE. Based on our previous results we have hypothesized that the main reason to explain the loss of neurogenesis in MTLE might be the massive activation and conversion into reactive astrocytes of rNSCs. We have confirmed our hypothesis by resorting to inducible transgenic Nestin-Cre-ERT2-Rosa26-YFP mice to trace the differentiation of rNSCs in a well-established model of MTLE: the intrahippocampal injection of the glutamatergic agonist kainic acid.

Title

Radial Neural Stem Cells Contribute to Hippocampal Sclerosis in a Rodent Model of Temporal Lobe Epilepsy

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New neurons are generated in the hippocampus throughout the life span of mammals, including humans, due to the persistence in the dentate gyrus (DG) of a population of radial glia cells that act as neural stem cells (rNSCs). The integration of newborn neurons into the hippocampal circuitry has been shown to participate in memory formation, spatial learning, pattern separation, fear conditioning, and anxiety. Adult hippocampal neurogenesis declines with age and is impaired in neurological disorders such as temporal lobe epilepsy. Using intra-hippocampal injection of the glutamatergic agonist kainic acid, a well-established model of mesial temporal lobe epilepsy (MTLE) we have observed that seizure-inducing neuronal hyper-excitation induces a massive activation of rNSCs and their differentiation into reactive astrocytes. As a result neurogenesis becomes result chronically impaired in the DG which in turn implies the loss of the cognitive functions associated with hippocampal neurogenesis and the loss of the potential to regenerate the neurons dead by excitoxicity. Furthermore, these results describe a new property of adult hippocampal rNSCs: their conversion into reactive astrocytes and their contribution to hippocampal sclerosis the anatomical distortion with neuronal death and dispersion and glial activation that constitutes a pathological hallmark of MTLE in the human brain.

Title

Hair follicle is a highly efficient source of MSC-derived neurons

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Introduction. Epithelial stem cells constitute a contemporary focus in skin stem cell research. However, previous studies indicate that hair follicle may bear other progenitor. Stem cells in hair follicle express mesenchymal stem cell (MSC) markers, suggesting the presence multipotent stem cells. Several reports have shown that rodent hair follicle stem cells can differentiate into neurogenic lineage in vitro. These results added to our own research in human skin cell research (immunophenotype and differentiation to fat, bone and cartilage, hair follicle stem cells) propose the possibility that human hair follicle holds a neurogenic potential. The objective of this study was the determination of the neurogenic differentiation potential of stem cells from hair follicle culture using immunostain with anti-neurofilament 200kDa. Neurofilaments are present in neurons and neuronal processes comprising the axoskeleton, bearing functions in maintaining neuronal caliber, and also playing a role in intracellular transport to axons and dendrites. Materials and Methods. Human samples were obtained from postsurgery materials after approval by a research ethics committee. Specimens were washed with PBS buffer followed by plating in 25 cm2 tissue culture flasks, and maintain in a humidified incubator at 37 °C and 5 % CO2. Cells were culture until 80% confluence in Dulbecco's Modified Eagle Medium (DMEM-Sigma Aldrich) supplemented with enriched human serum growth factors and then transferred to a neural differentiation assay. For all experiments control and specimen cells were harvested from different passages, seeded at a density of 2500 cells/cm2 in 12-well plates and cultured for 24h. A neural differentiation media (AdvanceSTEM Neural Differentiation Kit- Thermo Scientific HyClone) was used after rinsing cells with PBS. The medium was changed every 48h until neuron-like cells were observed (72h). Cells were plated on polylysine coated coverslips for microscopic visualization. After 1 week of culture in neurogenic medium, cells were fixed and incubated in blocking solution followed by staining with a primary antibody (Anti-Neurofilament200, Sigma Aldrich) and a secondary antibody (IgG anti-rabbit conjugated with Alexa647, Cell Signaling Technology). Cultures incubated only with secondary antibody were used as control. The cultures were visualized on a fluorescence microscope. Results and Conclusions. Human follicular cells exposed to 7 days of in vitro neurogenic differentiation show neural morphology, and immunostaining revealed neurofilament 200kDa expression in differentiated cells but not in control cells. Cells undergo reshaping after 48h and begin to develop dendritic processes. Along with our previous results, the present findings indicate that human follicular cells express surface markers that characterize MSCs, such as CD90 and CD105. Additionally, they also undergo differentiation toward fat, bone, cartilage, and neurons. These results suggest that hair follicle stem cells may be highly similar to MSCs derived from bone marrow. The ease of accessibility to collect specimens along with their high neurogenic potential makes the hair follicle an ideal source of adult stem cells for future therapeutic applications in personalized medicine.

Title

Glioma stem cells in ENU-induced gliomas are grouped in niches inmunophenotypically similar to neurospheres

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Introduction: The ENU (Ethyl-nitrosourea) glioma model has been widely used as a suitable model to study glioma progression, angiogenesis and also formation of neurospheres. Neurospheres are structures developed in vitro under non-adherent conditions by neural stem cells. Glioma stem cells (GSC) are a small subpopulation of the tumoral cells that have self-renewing, multipotency and tumour-induction capacities. Furthermore they share some inmunomarkers with the neural stem cells. Objective: The aim of this work is to identify the glioma stem cells subpopulation and try to elucidate their distribution related to the tumour growth. Methods: For this study we have used 96 tumours from 83 Sprague-Dawley rats with gliomas induced by prenatal exposure to N-Ethylnitrosourea on the 15th day of pregnancy. Nestin and CD133 have been used as glioma stem cell markers for inmunohistochemistry and inmunofluorescences assays, as well as other markers such as GFAP, VEGF, Glut-1 and LEA. Results: Nestin and CD133 positive cells are found inside the gliomas in different locations related to their morphology. Some of them appear as dense aggregations of cells (niches) located close to the vessels ("perivascular niche") or isolated throughout the tumour ("spheroid niche"). "Perivascular niches" show cells with big cytoplasm and large prolongations, whereas "spheroid niches" show round and dense cytoplasms around the nuclei, without cellular prolongations. A small amount of cells inside the "spheroid niches" are positive for GFAP and VEGF, but most of the VEGF/GFAP positive cells are located around the niches, like "encapsulating" them. Data show a link between the increase in the number of "spheroid niches" and the glioma progression, with a statistically significant increase correlated to glioma growth. The blood-brain barrier around or inside the "spheroid niches" seems to be conserved, as we have observed Glut-1 positivity in the vessels that are located around and sometimes inside these niches. Both "perivascular and spheroid niches" keep similar inmunophenotype to the reported neurospheres, but only "spheroid niches" resemble morphologically to the neurospheres. Conclusion: Cellular niches in vivo seem to display similar features to in vitro neurospheres, allowing the study of their interaction with the tumour microenvironment.

Title

In vivo therapeutic effects and oligodendrocyte protection from excitotoxicity by the MAGL inhibitor JZL184

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Multiple sclerosis (MS) is a chronic disease of the human central nervous system that is characterized by focal lesions with inflammation, infiltration of immune cells, demyelination and axonal damage. Activation of cannabinoid CB1/CB2 receptors is considered a potential therapeutic strategy for the treatment of MS, based on the evidence that exogenous cannabinoid agonists exert neuroprotective and immunosuppressive effects in experimental models of the disease. Nevertheless, the therapeutic use of synthetic and/or plant-derived agonists acting on brain cannabinoid receptors is limited by the possible adverse responses related to memory and learning impairment. An alternative approach that could avoid this limitation consists of enhancing the concentration of the main endocannabinoids anandamide (AEA) and/or 2-arachidonoylglycerol (2-AG), by increasing their synthesis or decreasing their degradation. JZL184 is a selective inhibitor of 2-AG hydrolyzing enzyme monoacylglycerol lipase (MAGL) that induces anxiolytic effects without cognitive alterations when administered at low doses. The main objective of this study was to analyze the effects of JZL184 in the chronic experimental autoimmune encephalomyelitis (EAE) model of MS. Mice were treated daily with a low and a high dose of JZL184 (8 and 32 mg/kg) or vehicle from the onset of the motor symptoms. Comparison of the motor score curves indicated that both doses of JZL184 ameliorated the deficits observed in vehicle-treated mice during the disease course. Nevertheless, the beneficial effect of the 32 mg/kg dose was no longer evident in mice scored at 40 dpi. Treatment with 8 mg/kg JZL184 reduced the conduction latency of the corticospinal tract measured at the end of the experiment, whereas administration of the 32 mg/kg dose had no effect on this parameter. Immunohistochemical analysis indicated reduced demyelination in the spinal cord of mice treated with 8 mg/kg JZL184, together with a decrease in the number of inflammatory lesions and associated microglial activation. Importantly, chronic treatment with JZL184 elicited a dose-dependent reduction in the coupling ability of brain CB1 receptors to Gi/o proteins, measured by [35S]GTPγS autoradiography, suggesting that inadecuate funcionality of CB1 protein may underlie the reduced therapeutic efficacy of the MAGL inhibitor when administered at a high dose. Excitotoxic damage to oligodendrocytes is considered a key pathogenic mechanism in MS and EAE. Noteworthy, preincubation of oligodendrocyte precursors with JZL184 prevented AMPA-induced cytotoxicity in vitro, an effect that was blocked by the CB1 receptor antagonist AM281. Our findings suggest that chronic administration of MAGL inhibitors may be a promising strategy for the treatment demyelinating disorders.

Title

1–42 β -Amyloid peptide requires PDK1/nPKC/Rac 1 pathway to induce neuronal death

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Small GTPases of the Rho family, whose best characterized members include RhoA, Rac1 and Cdc42, are key players in complex signaling networks that control normal activity in most of cell types. Like other small GTPases, Rho GTPases function as molecular switches to control cellular signaling pathways. Since these monomeric G proteins are implicated in almost all aspects of cell biology, changes in their regulatory cycles can affect normal cell functionality in an irreversible manner. Signaling pathways originated from deregulated GTPase activities can be involved in cell transformation and metastasis, in the Wiskott-Aldrich syndrome, Faciogenital Dysplasia, Tangier disease and other pathologies. Recently, it has been shown that they may also be implicated in Alzheimer's Disease (AD). It is well established that RhoGTPases control cytoskeleton dynamics in neurons, thereby modulating synaptic plasticity. In fact, AD entails progressive dendritic spine loss, synaptic dysfunctions and morphological changes in dendrites. In normal brain, neuronal RhoA attaches to synapses and dendritic microtubules, however, in AD RhoA expression is decreased in synapses and increased in dystrophic neurites. The molecular mechanisms leading to development of AD are not fully characterized. This neuropathology is associated with massive accumulation of two types of protein aggregates; senile plaques that are constituted mostly by 1-42 β Amyloid (Aβ1-42) peptide and by neurofibrillary tangles containing hyperphosphorylated Tau protein. There is mounting evidence that the Aβ1-42 peptide mediates many aspects of the pathogenesis of AD. In vitro this peptide is toxic to endothelial cells, smooth muscle cells, astrocytes, neurons and oligodendrocytes. The mechanisms by which Aβ1-42 peptide exerts its cytotoxic action are not fully understood. Currently, there are ongoing efforts to discover the signaling pathways that are mediated by the Aβ1-42 peptide. Several signaling cascades may be involved in cell damage and they appear to be activated by the Aβ1-42 peptide, including oxidative stress generation, impaired Ca2+ homeostasis and mitochondrial dysfunction, generation of NO, and microglia activation. Here, we studied how the Aβ1-42 peptide uses the cellular machinery for signal transduction leading to neuronal cell death in the cell line SN4741, in primary embryonic cortical neurons from rats as well as in neuronal organotypic cultures of hippocampus and entorhinal cortex. This signaling cascade involves specifically the Rac1 GTPase, which is regulated upstream by the PI3Kinase/PDK1/nPKCs pathway. This novel molecular characterization identifies nPKCs and Rac1 as potential therapeutic targets to block neuronal death program induced by the β-amyloid peptide.

Title

Neuroprotection by Galanin in ex vivo models of Oxidative Stress

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Galanin is a neuropeptide that innervates cholinergic basal forebrain neurons (BFN) which are implicated in memory and learning processes. BFN project to cortical and hippocampal areas. Galanin also hyperinnervates the surviving BFN in Alzheimer's disease (AD) patients. In addition, the pathology of AD has been related to different biochemical observations such as the deposition of bA peptide, inflammatory responses, oxidative stress and mitochondrial dysfunction. Therefore, our aim was to test the role of galanin as a neuroprotective agent in different ex vivo models of oxidative stress: hemibrain organotypic cultures from neonatal rat (P7) and retinal pigmental cells (ARPE-19). The former, allow us to maintain the cell viability and the cholinergic pathways which are degenerated in AD and the latter, have been analysed previously as a model for the study of oxidative stress affecting neuronal tissue. Hydrogen peroxide (H2O2) was used as the oxidative agent in combination with TNFa. The results showed a dose-dependent vulnerability of the cells to the oxidative stress conditions in both models. The incubation with galanin (10 mM) before the treatment with H2O2 (1 mM) reduced cell death in diverse areas such as, caudate-putamen, nucleus basal of Meynert and cortex, in the organotypic cultures. Cell death was evaluated by propidium iodide assay. Also, when ARPE-19 cells were pre-treated with the neuropeptide for 24 h, there was a decrease (30%) of the apoptotic marker used. This effect was mediated by galanin receptors as was demonstrated by the fact that it was prevented by the galanin antagonist, M35. The observed neuroprotective and antiapoptotic effects induced by galanin could have important implications, which are currently been evaluated, for the treatment of neurodegenerative diseases in which the cholinergic system is especially vulnerable, such as AD.

Title

Prolonged L-DOPA treatment modifies the electrical activity of entopeduncular nucleus and substantia nigra pars reticulata neurons in 6-hydroxydopamine lesioned rats

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The prolonged L-DOPA treatment leads to disabling motor complications including dyskinesia in Parkinson´s disease (PD) patients and animal models. The mechanisms that underlie L-DOPA-induced dyskinesia (LID) are not clear, although the implication of the basal ganglia output nuclei, the substantia nigra pars reticulata (SNr) and the internal segment of the globus pallidus (GPi, entopeduncular nucleus in rat, EP nucleus), has been proposed. Here, we studied the involvement of the EP nucleus and the SNr in LID, using single-unit extracellular recordings and behavioural approaches in hemi-parkinsonian rats chronically treated with L-DOPA. Additionally, we also investigated the possible correlation between subthalamic nucleus (STN) neuron activity and its target nuclei (EP nucleus and SNr) neuron activity. Our results show that L-DOPA chronic treatment modified the electrophysiological parameters of EP nucleus and SNr neurons in 6-hydroxydopamine lesioned rats. We did not find any correlation between abnormal involuntary movement (AIM) scores and the electrophysiological parameters of EP nucleus neurons recorded 24 h or 20-120 min after the last L-DOPA administration. Whereas, we found positive correlation between the limb and orolingual AIMs and the electrophysiological parameters of SNr neurons recorded 24 h after the last L-DOPA administration. These correlations disappeared after the acute L-DOPA challenge. Moreover, we also found positive correlations between STN neuron activity and EP nucleus or SNr neuron activity in dyskinetics rats. Altogether, these results show that in dyskinetic animals the electrical activity of EP nucleus and SNr neurons is altered, revealing a correlation with STN neuron activity.

Title

Role of the RNA-binding protein HuR in Neurofibromas and Malignant Peripheral Nerve Sheath Tumour

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Malignant peripheral nerve sheath tumors (MPNST) are aggressive soft tissue sarcomas that arise within the peripheral nerve with very poor prognosis. MPNSTs are sporadic or arise in individuals with neurofibromatosis type 1 (NF1). NF1 patients develop benign dermal neurofibromas and/or benign plexiform neurofibromas, which can undergo malignant transformation to MPNST. Schwann cells are the crucial pathogenic cell type in NFs. Genome-wide gene expression and DNA methylation profiling have shown significant differences between normal Schwann cells, NF1-derived benign and malignant cells. During tumour development, cancer cells acquire novel physiologic changes that allow them to develop, adapt, survive, proliferate and metastasize by expressing a distinct catalogue of proteins. In addition to genetic and epigenetic mechanisms, post-transcriptional regulation of gene expression by RNA-binding proteins (RBP) also strongly influences the aberrant expression of many of these proteins. The RBP HuR is aberrantly expressed in several types of cancer and a strong correlation has been found between its expression levels and advancing stages of malignancy. We found that HuR expression was significantly increased in NF and MPNST samples compared to normal nerves, with a strong correlation between HuR expression and degree of malignancy, both by immunohistochemistry, and qPCR analysis and Western Blotting, of human tissue arrays and solid human tumors, respectively. RNA immunoprecipitation coupled with microarray analysis (RIP-ChIP) of solid human tumors and human MPNST cell lines showed that the number of mRNAs bound to HuR increased as malignancy progresses. Amongst them, several ones with well-defined roles in oncogenesis were identified. HuR silencing in vitro using MPNST cell lines significantly reduced the expression of these genes and proliferation, migration, colony formation and invasion and also made these cells more sensitive to apoptotic death by UV irradiation. In summary, we propose that HuR plays a key role in the control of expression of critical cancer-associated genes that regulate oncogenic functions such as proliferation, cell survival and metastasis.

Title

Unravelling the ubiquitin network during nervous system development, function and disease

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The post-translational modification of proteins with ubiquitin, a reaction that is carried out by the sequential action of an ubiquitin activating (E1), conjugating (E2) and ligating (E3) enzymes, plays very important roles in the regulation of neuronal development and function. In fact, its failure has been associated to a number of neurological disorders such as Parkinson's disease, Alzheimer's disease, Autism Spectrum Disorders (ASD) or the Angelman Syndrome (AS). Since the fraction of any protein being ubiquitinated is very small, the study of this post-translational modification is a difficult task to perform. For instance, in a disease like the AS, in which the function of UBE3A, one out of several hundred existing E3 ligases, is disturbed in the brain, the neuronal proteins that are no more targeted for ubiquitination remain undiscovered. This results on a poor understanding on the etiology of this disorder, therefore hindering any therapeutical approach to compensate the symptoms. We developed a novel approach to isolate ubiquitin conjugates from the Drosophila nervous system, which allowed us to identify dozens of ubiquitinated proteins during development of the embryonic brain (Franco et al., 2011) and now also from the fly adult brain. Seeing the efficiency that this strategy has, we now have combined it with available fly models of AS/ASD, with the aim of understanding better the molecular mechanisms of those disorders, both being associated to the activity of UBE3A. Preliminary results indicate a direct role in the regulation of protein homeostasis, and could explain many of the observations reported so far.

Title

Proliferative response after the administration of Endocannabinoids in Hypoxic-Ischemic brain injury rat model

Author(s)

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INTRODUCTION Perinatal hypoxic-ischemic (HI) encephalopathy is one of the main causes of disabilities in term-born infants. The immature brain is susceptible to HI damage and the intensity and timing of asphyxia determine the extension, degree and severity of the damage. The discovery of a new intercellular communication network called endocannabinoid system, which acts as neuromodulator in many physiological processes, has sparked special interest as neuroprotector after cerebral damage. The aim of the present study was to evaluate histologically in a neonatal rat model of HI damage, the neuroprotective effect of the administration of two endocannbinoids, 2AG or AEA, analyzing in hippocampus and parietal cortex the number of viable neurons, glial response and proliferation. METHODS 7-day-old postnatal Wistar pups were exposed to a HI insult by means of the Rice-Vanucci procedure. Left common carotid artery was permanently occluded and after recovering, pups were maintained under hypoxic conditions (8% oxygen balanced with nitrogen) for 2 hours. Four different groups were assigned: Sham, HI, HI+2AG and HI+AEA. In treated group, immediately after the HI insult, the animals were treated with a single dose of 2-AG (1mg/Kg) or AEA (5mg/Kg) and then sacrificed 7 days after the HI event. 5-Bromo-2-deoxyuridine (BrdU 50 mg/kg) was injected intraperitoneally twice daily from days 1 to 7 after HI. Brains fixed by perfusion were stained with Nissl (n=8) for morphological studies and other brains (n=5-8) stored in 30% sucrose for immunofluorescence staining with NeuN, GFAP and BrdU staining. All the experimental procedures met the National Institutes of Health guidelines for the care and use of laboratory animals, and the European Communities Directive 86/609/EEC regulating animal research. RESULTS After the hypoxic-ischemic damage we have observed different grades of damage ranging from moderate to severe in the ipsilateral side of the brain, mainly located on the areas supplied by the middle cerebral artery such as parietal cortex and hippocampus. However, when both endocannabinoids were administrated the infarct volume decrease showing few morphological differences with Sham group. The number of viable neurons in hippocampus and parietal cortex undergoes an important reduction following hypoxic-ischemic event. By contrast, endocannabinoid-treated animals showed a significative reduction in the number of damaged cells, reaching values very similar to those observed in Sham group. Our data also suggest a significant increase in the astrocyte response in the HI group, which is correlated with neuronal death, but after the administration of 2AG or AEA, the length of the processes of the astrocytes was similar to Sham group. Concerning to the proliferation study, HI group showed a high number of proliferative cells per mm2 in comparison to Sham group, meanwhile treated groups had different response after damage. Thus, 2AG endocannabinoid response was similar to Sham group while AEA endocannabinoid showed significative proliferative. CONCLUSION Our results show that post-HI treatment with endocannabinoids 2AG and AEA, not only attenuates brain injury at neuronal level but also promotes proliferative response, suggesting a neurogenic response.

Title

Cell membrane microarrays as a novel tool for screening drugs that act through acetylcholinesterase

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The cholinergic neurotransmission ends by the hydrolysis of acetylcholine, mainly due to the action of the acetylcholinesterase (AChE) enzyme. For this reason, AChE is the primary target of a wide spectrum of compounds used as pesticides, nerve agents or therapeutic drugs for neurodegenerative diseases such as Alzheimer´s disease. In this context, and taking into account the continuing increase of the aging population, and consequently of the neurodegenerative diseases prevalence, the development of time and cost saving high-throughput screening biotechnologies for drug discovery directed to inhibit AChE activity results of notably interest. Therefore, the aim of the present work is to develop a new biotechnology tool for drug discovery based on tissue cell membrane microarrays. This platform enables to detect the AChE activity, and to determine the potency of candidate therapeutic agents directed to inhibit AChE in a large-scale collection of membrane homogenates, which maintain the integral proteins functionality, isolated from different tissues and organs of the rat. To prove its usefulness, the AChE activity was evaluated in cell membrane microarrays and in brain slices, obtaining a good correlation between both techniques. Moreover, we determined the potency of the known AChE inhibitors in cerebral cortex and striatum: tacrine and physostigmine, first generation drugs for AD symptomatic treatment, and donepezil and galanthamine, second generation drugs, being the results congruent with those reported elsewhere by conventional methods. Furthermore, the IC50 of donepezil was evaluated simultaneously in 41 tissues and organs using this technology, demonstrating the great potential of cell membrane microarrays, which enable not only to analyze the potency of candidate compounds, but also to predict their unwanted effects. In summary, this single miniaturized assay provides a powerful tool for studying the effect of AChE inhibitors in several tissues and organs together, accelerating the drug discovery process and predicting the possible side effects with animals, time and cost saving.

Title

Generation of human recombinant prions. Model for understanding the Gerstmann–Sträussler–Scheinker syndrome

Author(s)

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Human transmissible spongiform encephalopathies (TSEs) or human prion diseases belong to a group of fatal neurodegenerative disorders that includes kuru, Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome (GSS), and fatal familial insomnia (FFI) among others. Human TSEs may occur sporadically or on a genetic or iatrogenic basis. Gerstmann-Sträussler-Scheinker disease (GSS) is a genetically determined TSE characterized by adult onset of memory loss, dementia, ataxia, and pathologic deposition of amyloid-plaques in the brain. It is caused by a range of mutations within the open reading frame of the prion protein (PrP) gene (PRNP). The P102L substitution was the first discovered and the most common mutation. The consequences of pathological mutations to the human PrP conformation and their effects on pathogenesis are still poorly understood. The aim of this study is to generate a useful model for understanding GSS. To achieve this, Protein Misfolding Amplification (PMCA) is being used, as it mimics PrPC to PrPSc conversion in vitro with accelerated kinetics. Specifically, PMCA based on recombinant PrP (recPMCA) is being used instead of brain-derived PrP. A substrate based on P102L mutated human recombinant PrP was subjected to serial rounds of recPMCA until a recombinant human GSS P102L PrPSc was generated spontaneously. The recombinant prion was unable to propagate the misfolding to a wild type human recombinant PrP based-substrate by recPMCA. These results are comparable to in vivo results using both non-human primates and rodents as recipients for different GSS isolates from human patients. We are currently performing different biochemical, biological and structural studies trying to characterize the new recombinant PrPSc as a model for understanding Gertsmann-Straussler-Scheinker syndrome.

Title

VEGF-releasing micro and nanotechnologies for the treatment of Alzheimer's disease

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Alzheimer's disease (AD), the most common adult-onset dementia, is a complex degenerative disorder clinically characterized by a progressive loss of memory and deterioration of other cognitive functions. Neuropathological hallmarks of AD involve neuronal degeneration, extracellular deposition of amyloid beta (Aβ) peptide in senile plaques and neurofibrillary tangles of phosphorylated tau protein, associated with neuronal loss, and vascular toxicity [1]. However, the exact mechanism of neuronal degeneration in AD has not been clearly identified. Accumulating evidence suggests that cerebrovascular dysfunction contributes to cognitive decline and neurodegeneration in AD. Therefore, vascular clearance can critically influence brain Aβ accumulation, and induce neuronal and vascular associated toxicity. Considering the above, an interesting and promising approach to treat AD is the use of vascular endothelial growth factor (VEGF). VEGF is an endothelial-specific growth factor principally implicated in the creation of new blood vessels, blood-brain barrier integrity, neuroprotection and axonal outgrowth [2]. Major problems for the clinical application of the rapid degradation rate and its short half-life in vivo, which derives in a need for continuous and direct administration of the factors into the brain. Such inconveniences make necessary the development of suitable technologies to allow a continuous and localized delivery of physiological amounts of VEGF. During the last years, our group has explored two different ways to achieve a suitable VEGF delivery in the treatment of AD. One interesting approach is the encapsulation of VEGF-secreting cells into alginate microcapsules. Here, engineered somatic cells are protected against immune cell-mediated and antibody-mediated rejection through immobilization in a polymer matrix surrounded by a semipermeable membrane. The latter regulates the bidirectional diffusion of nutrients, allowing the controlled and continuous delivery of therapeutic proteins in the absence of immunosuppression. On the other hand, the second strategy is based on biodegradable PLGA nanoparticles, which have been extensively investigated as drug delivery systems for the treatment of several diseases. These biodegradable nanoparticles can protect the encapsulated drug from degradation, release the drug in a controlled manner, improve its biodistribution and allow drug targeting. In order to assess the efficacy of these two technologies, in vivo studies were carried out in amyloid precursor protein/presenilin-1 (APP/Ps1) mice by administering VEGF-nanoparticles or VEGF-secreting microcapsules through minimally invasive craniotomy. Because excessive Aβ accumulation is associated with neuronal degeneration and disturbed cognitive function in AD mouse models, we investigated this pathological hallmark in our experimental model. Our results showed that VEGF release from both PLGA nanoparticles and cell-loaded alginate microcapsules promoted angiogenesis in vivo and consequently reduced brain Aβ plaques, enhancing its clearance along the walls of the microvasculature. Besides, VEGF further enhanced proliferation of neuronal progenitors in hippocampal region. Altogether, such physiologic recovery was translated into behavioral improvements. Indeed, mice treated with VEGF-nanoparticles and VEGF-secreting microcapsules presented higher exploratory activity levels measured by T-maze test, as well as improved short-term memory, usually studied with the object recognition test [3-5]. In conclusion, these results suggest that both technologies may represent promising tools to release morphogens for the treatment of AD, as they were able to improve behavioral deficits, decrease Aβ deposits and promote either angiogenesis or neurogenesis, while reducing neuronal loss and cerebrovascular abnormalities. References [1] A.K. Desai et al. Neurology. 64 (2005): S34-9; [2] M. Shibuya et al. FEBS J. 276 (2009): 4636-4643. [3] Spuch C, et al. Biomaterials. 31 (2010):5608-18. [4] Herrán E, et al. J Control Release. (2013). [5] Antequera D, et al. J Alzheimers Dis. 29 (2012):187-200.

Title

Phospholipase C-β1 is strongly downregulated in cortical synaptic fractions of chronically epileptic rats

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Epilepsy is a chronic disease characterized by sudden recurring neuronal discharges, usually short in time, known as epileptic seizures. It is one of the most common neurological disorders in humans, affecting about 1-3% of the population. About 50% of cases of chronic epilepsy are provoked by a previous brain insult, with temporal lobe epilepsy being the most common type of acquired chronic epilepsy. During the last years, several lines of evidence point at phospholipase C-β1 (PLCβ1) downregulation as a major responsible for the emergency of recurrent epilepsy. Thus, Plcb1 knock out mice develop epilepsy during postnatal maturation (Kim et al., 1997), and homozygous deletion of the human PLCB1 gene is associated with malignant migrating partial seizures in infancy (Poduri et al., 2012). Using the lithium-pilocarpine model of temporal lobe epilepsy, we have examined, by Western blot in subcellular fractions and immunohistochemistry, the expression of PLCβ1 protein in the brain cortex of chronically epileptic rats, 28 days after pilocarpine-induced status epilepticus. By sequential centrifugation of rat cortical homogenates and Triton X-100 (TX100) treatment of the plasma membrane fraction, we obtained [i] TX100-resistant (synaptic), [ii] TX100-soluble, [iii] microsomal and [iv] cytosolic fractions, which were enriched in [i] postsynaptic markers, [ii] Na+/K+ ATPase, [iii] the endoplasmic reticulum marker GRP78/BiP and [iv] β1-tubulin, respectively, as assessed by Western blotting. PLCβ1 was expressed in all fractions, but more intensely in the synaptic fraction. Moreover, of the two splice variants of PLCβ1 (PLCβ1a and PLCβ1b), PLCβ1b was expressed almost exclusively in the synaptic fraction. Western blot in whole homogenates of cortical tissue showed a slight decrease of PLCβ1-immunoreactivity in epileptic rats compared with controls. The analysis of subcellular fractions, revealed a drastic decrease of PLCβ1-expression (more than 50%) in the synaptic fraction of epileptic rats, whereas no changes were observed for the postsynaptic makers PSD95 and Homer1b/c, indicating that the observed effect in PLCβ1 expression was not the consequence of a loss of synapses in the epileptic cortex. Immunohistochemical analysis of rat brain sections showed a clear redistribution of the immunoreactivity in the cortex of chronically epileptic rats, leading to a drastic decrease of the staining in apical dendrites of layer II-III and layer V cortical pyramidal neurons, along with a general and marked decrease in the neuropil labelling. These changes observed in apical dendrites of pyramidal neurons and neuropil contrasted with a higher staining intensity in perikarya of pyramidal neurons, suggesting a redistribution of PLCβ1 from the dendritic to the somatic domain, which is in agreement with the biochemical observation that PLCβ1 is specifically downregulated in the synaptic fraction. The present results, together with the known role of PLCβ1 as a postsynaptic effector that regulates synaptic activity via production of second messengers and endocannabinoids, suggest that downregulation of this enzyme in the epileptic brain may underlie hyperexcitability in cortical neurons of the epileptic brain.

Title

Synergistic effects of VEGF- and GDNF-releasing biodegradable polymeric nanospheres in a partial lesion model of Parkinson's disease

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Parkinson's disease (PD) is the second most common neurodegenerative disorder, and is characterised by the progressive degeneration of the nigrostriatal dopaminergic pathway. The dopamimetic therapies presently available for PD are not effective in the long-term and cannot repair the already damaged area [1]. That is why, current research efforts are being focused in new neuroprotective and neuroregenerative strategies that will halt the neurodegenerative process. Therefore, an interesting approach to raise this challenge is the use of neurotrophic factors such as glial cell line-derived neurotrophic factor (GDNF) and vascular endothelial growth factor (VEGF). These proteins play critical roles in the induction, specification, survival, maturation and protection of developing neurones. However, the critical problems for the clinical application of GDNF and VEGF are their rapid degradation rate and their difficulty in crossing the blood-brain barrier [2]. To overcome these problems, in a recent study published by our research group, VEGF and GDNF were encapsulated in biodegradable and biocompatible poly-lactic-co-glicolic (PLGA) nanospheres. This strategy made possible the administration of these two factors in the brain, getting a continuous and simultaneous drug release. The results obtained demonstrated regenerative effects of the combined administration of VEGF and GDNF-NS in a severely lesioned rat model of PD [3]. Continuing with the study mentioned above, the aim of this novel work was to evaluate the combination of lower doses of VEGF and GDNF PLGA nanospheres (PLGA-NS) in a partially lesioned rat model (equivalent to an earlier stage of PD) and analyze the neuroregenerative potential of these factors individually and in combination. PLGA VEGF and GDNF loaded NS were prepared by double emulsion solvent evaporation technique. The NS particle size was about 200 nm, showing a biphasic release profile in vitro. The VEGF and GDNF bioactivity assays were conducted in endothelial and in pheochromocytoma cell lines respectively, and we found that cell viability was increased after treatment with factors released from NS. To carry out in vivo studies, 34 Female albino Sprague-Dawley rats were partially lesioned with 6-OHDA. 3 weeks after 6-OHDA-induced lesion, PLGA-NS were implanted into the right striatum divided in 5 different experimental groups: (1) only vehicle, (2) empty-NS, (3) VEGF-NS (2.5 µg), (4) GDNF-NS (2.5 µg), and (5) VEGF+GDNF-NS (1.25 µg + 1.25 µg). Once the PLGA-NS were implanted, in vivo efficacy of encapsulated VEGF and GDNF was assessed during 12 weeks using an amphetamine rotational behavior test. Our results showed that VEGF+GDNF-NS significantly reduce the number of rotations induced by amphetamine. In addition TH+ inmunohistochemical analysis in Substantia Nigra (SN) demonstrated a significantly enhanced of neurons in VEGF+GDNF-NS treatment group. Taking all results together it may be concluded that the synergistic effect of lower VEGF and GDNF-NS dose may be a neuroregeneration-neuroreparation strategy to treat Parkinson disease. References [1] L. Aron, et al. Trends Neurosci. 34 (2011) 88-100. [2] G.J. Siegel, et al. Brain Res. Rev. 33 (2000) 199-227 [3] Herrán E, et al. Eur. J. Pharm. Biopharm. (2013) In Press.

Title

Resveratrol protects neonatal brain after Hypoxia-Ischemia when administered before injury

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Introduction Hypoxia-ischemia (HI)-induced perinatal encephalopathy is a major cause of acute mortality and neurologic morbidities such as cerebral palsy, mental retardation and epilepsy. Resveratrol is a natural polyphenol present in red wine with anti-oxidant and anti-inflammatory properties that is thought to be neuroprotectant against experimental brain injury. The aim of the present work was to evaluate the effect of resveratrol administered 10 minutes before or immediately after hypoxic-ischemic (HI) brain injury in neonatal rats using the Rice-Vannucci model. Material and Methods Thirty two Sprague-Dawley seven-day-old (P7) rats were randomly separated into 4 groups: Sham (n=8), HI (n=8) and HI treated with resveratrol 10 minutes before (n=8) and immediately after (n=8) the injury. The three hypoxic-ischemic groups were subjected to permanent legation of left common carotid artery and then asphyxiated for 2 hours and a quarter with 8% O2.Treated animals received a single intraperitoneal injection of 20 mg/kg resveratrol 10 minutes before (RvtB) or immediately after HI event (RvtA). Resveratrol was diluted in DMSO and in saline solution. Pups without injury were used as controls (Sham). Seven days after surgery, brains were collected and coronal sections were Nissl-stained. Analysis of infarct area was done using a quantitative analysis Image J program and a semi-quantitative neuropathological scoring system. Results 7 days after the injury, brains of HI group usually showed an infarct area in the ipsilateral core with loss of brain tissue. Indeed, asphyctic animals presented swollen and deformed neurons in the CA1 and CA2/CA3 areas and in the dentate gyrus of the hippocampus and in the parietal cortex ipsilateral to the site of insult. Animals pretreated with resveratrol revealed a mild damage in the ipsilateral side of the brain, but this damage showed a remarkable reduction compared to HI group. Moreover, RvtB animals presented mild neuronal damage, specially having effect in the maintenance on the hippocampal cells. No infarcted areas were observed. By contrast, animals treated with resveratrol immediately after HI-injury in general term showed an intensity of tisular damage similar to those of HI group. Regarding the quantitative analysis of the infarct area by Image J program, a high percentage of damage is showed in HI group in comparison with Sham and RvtB. RvtA revealed also a severe percentage of tissue loss. The semi-quantitative neuropathological scoring system demonstrated damage located at the level of hippocampus and parietal cortex of ipsilateral hemisphere in HI group. Protection effect by resveratrol injected 10 minutes before the injury was evident when injury was assessed by neuropathological scoring, but resveratrol did not protect when is injected just after. Conclusion Our results suggest that pretreatment with resveratrol led to a neuroprotective effect, reducing infarct volume and maintaining the structural features of the hippocampus and cortex, but not when administered immediately after HI.

Title

Effects of hypoxia on retinal ganglion cells and astrocytes in the neonatal pig retina

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Introduction: Neurodegeneration in retinal pathologies, such as glaucoma, is often exacerbated by hypoxic conditions. The consequences of the decrease in oxygen on the behavior of retinal ganglion cells (RGC) and retinal glia are not fully understood. Objectives: The aim of the present study was to analyse the survival of RGCs and also assess the morphological changes in astrocytes in the neonatal pig retina under control and hypoxic conditions. Materials and Methods: We induced mild hypoxia to neonatal pigs by decreasing the oxygen concentration to 12-14% for 90-120 minutes, following by re-oxygenation at a concentration of 21-35% for 240 minutes. Subsequently, eyes were enucleated and the retina carefully extracted and fixed for immunochemistry analysis. RGCs nuclei were labeled with anti-Brn3a and astrocytes with anti-GFAP specific markers. The number of RGCs/mm2 was quantified in the central and peripheral retina and the morphology of astrocytes in these areas was analysed. Results: We observed that, following hypoxia, retinal astrocytes adopt a more disorganised distribution with an increase in lateral process extension. There were no significant changes in the number of RGCs at this time point. Conclusions: This study found that mild hypoxia induces rapid alterations in retinal glia morphology but had no immediate effect on the number of RGCs. The downstream consequences of these glial changes require further investigation. A deeper understanding of the relationship between RGCs and astrocytes in retinal pathologies may aid the effort to improve neuroregeneration.

Title

Oscillatory activity associated with the contralateral and ipsilateral auditory pathways

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In relation to the electrophysiological response to sensory stimulation, evoked (time- and phase-locked) and induced (not time- and phase-locked) activity are differentiated. In the time domain, auditory stimulation leads to an increase in the amplitude of signals such as the EEG, MEG or LFP at around 0.1 s post-stimulus onset (N100m) in temporal cortex, which occurs earlier and/or has a higher amplitude during contralateral stimulation as compared to ipsilateral stimulation. In the frequency domain, auditory stimulation leads to evoked low-frequency (around 10 Hz) and gamma-range (around 35 Hz) power increases. Some reports indicate that monaural auditory stimulation induces tau-band (6.5 - 9.5 Hz) suppression (relative to prestimulus baseline), which is stronger in contralateral stimulation than in ipsilateral stimulation over the right hemisphere. Induced gamma-band power increase was reported in auditory stimulation in ECoG studies. The current study aimed at investigating simultaneously evoked and induced activity and assessing contralateral advantage in each of the observed patterns using magnetoencephalography. Six neurologically healthy subjects were presented with pure tones (500 or 1000 Hz, 500 ms, 70 dB), which were delivered monaurally (ISI 2.5 - 3.5 s; ~ 600 repetitions per ear). The neuromagnetic field was recorded with a 306-sensor whole-head Elekta Neuromag® device. The patterns of activity observed over temporal regions at sensor level were on the whole consistent with the previous findings. In terms of evoked activity, a strong N100m peak was found in the time domain, considerable evoked tau-band increase showed up in the frequency domain, but no evoked gamma-band increase was observed in the grand average or individual participant data. In terms of induced activity, some evidence of late (after 0.3 s post stimulus onset) tau-band decrease and induced gamma-band increase was observed. Strong evidence of a contralateral advantage in the evoked N100m and the evoked tau-band increase was observed, as for induced activity, no conclusions about contralateral advantage could be made. The study confirms that evoked and induced activity reveal different aspects of the auditory response.

Title

Excitatory or inhibitory behavioral responses to a signal are modulated by temporal contexts

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Traditional associative and connectionist theories predict that if a signal receives excitatory conditioning during the first phase of an experiment, and inhibitory conditioning in a second phase, a so-called catastrophic interference will occur. This means that the excitatory response acquired during Phase 1 should become unlearned during Phase 2. However, more recent theories and many experiments with humans and animals suggest that the signal will actually preserve both properties (excitation and inhibition), even though only the most recently acquired response will typically be expressed (in this example, inhibition). This means that the previously acquired excitatory response can be reactivated through several manipulations. For example, if excitation was trained in Context A, and inhibition in Context B, presenting the signal in Context A during a subsequent test phase can reactivate excitatory responding to the signal. Therefore, contexts modulate the expression of inhibition or excitation when the same signal can trigger both types of conflicting behavior. Moreover, some researchers have suggested that contexts are not only the physical spaces in which the different training phases occur. The passage of time can also act as a context change. We have conducted several behavioral experiments with humans in which we manipulate those temporal contexts. Specifically, we assume that all the stimuli presented while one training phase occurs constitute the temporal context for that particular phase. Each stimulus and response gets linked to the temporal context and the temporal context to them. For different phases of the experiment those temporal contexts are necessarily different. In our experiments excitatory responding to a signal was trained in Temporal Context 1, and inhibitory responding was trained to the same signal in Temporal Context 2. After inhibition was complete, excitatory behavior was reinstated by presenting several of the stimuli that were part of Temporal Context 1 while excitation was trained. These results suggest that temporal contexts can have similar effects as physical contexts in modulating the expression of excitatory or inhibitory responses to an ambiguous signal that has been trained to trigger both types of conflicting responses.

Title

From EEG to WWW: How can neuroscientists take advantage from the Web?

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Traditionally, neuroscientists have used complex techniques and expensive equipment under a strict experimental control for the analysis of the physiological basis of the human behavior. This approach has produced a vast number of studies with a high degree of internal validity.

Title

The development of sound – shape correspondence in the monolingual and bilingual mind

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Sound-shape correspondence represents a bias in multisensory integration between acoustic and visual information, specifically between the name (auditory) and shape (visual) of an object.. For instance, participants tend to associate the pseudo-word (PW) kiki with angular objects, but the PW bouba with rounded objects. This bias is known as the bouba-kiki effect and it has been observed in both monolingual adults (Nielsen&Rendall, 2011) and infants (Ozturk, et al., 2012) from various language backgrounds. However, it is unclear whether this effect is specific to the combination of phonemes found in bouba and kiki or the effect can be overextended to other PWs specific to the participants' native language background(s). Therefore, the aim of the current set of experiments is to identify PWs that are associated with angular and round shapes by monolingual and bilingual users of Basque and Spanish; as well as to test whether infants exhibit similar biases. In Experiment 1, 6 Spanish, 6 Basque monolinguals and 6 Basque-Spanish bilinguals rated auditory PWs in four dimensions: roundness, angularness, Spanish–likeness, and Basque-likeness. Overall, the Spanish and bilingual groups rated the PWs as significantly more Basque–like than the Basque group; the Spanish group rated the PWs as significantly more round than the bilingual and Basque group. To test whether Basque-Spanish monolingual and bilingual infants exhibit similar sensitivities to shapes and sounds adults do, we have selected two pairs of native language-appropriate stimuli based on Experiment 1: rounded PW vs. angular PW; neutral PW vs. neutral PW. These pairs, in different experiments, have been presented to 4-month-old infants using a preference looking paradigm in response to congruent (shape-sound match based on adult ratings) and incongruent (shape-sound mismatch based on adult ratings). Preliminary results in relation to the adult findings will be discussed.

Title

Event-related brain potential (EEG) modulations associated to the processing of causal illusions

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The ability to learn about causal relationships between events becomes a crucial tool to environmental adaptation. Knowledge derived from this learning process enables the organisms to fit their behavior based on predictions about future outcomes, enhancing, in that way, their chances of obtaining those outcomes that are desirable and avoiding undesired ones. However, the cognitive system involved in that learning does not always lead to accurate causal estimations. There is a vast amount laboratory experiments showing that people systematically incur in biased causal estimations under specific conditions. It has been shown that the greater the probability of the outcome, the greater people's causal judgments will be about the relationship between the potential cause (e.g., their own action) and that outcome. This occurs even when that relationship is null, that is, when the potential cause and the outcome are causally unrelated. In these situations people tend to develop the illusion that the causal relationship exists and they act as if it in fact existed. This is known as the Illusion of Causality. Illusions of causality have been related with superstitious behavior and beliefs in pseudoscience. For example, the perception of an illusory relationship between an innocuous health treatment and spontaneous cures can lead people to believe that the treatment works. This would explain why people persist on believing in the effectiveness of pseudoscientific treatments. Given the implications for society at a practical level it is not surprising the large number of research exploring the cognitive processes involved in causal illusions. However, the neural mechanisms underlying the illusion of causality have not been to date specifically investigated. The aim of this work is to explore neural mechanisms involved in the illusions of causality. Causal learning models suggest that learning proceeds by fitting our expectations of occurrence of target events. The difference between what we expect to occur and what actually happens defines the amount of learning that can be developed in a specific situation. When a person has learnt that an outcome and a cause are causally related the expectation of occurrence of that outcome when the cause is present must be higher than the expectation of the outcome's occurrence in its absence. In that case, if the outcome does not occur in presence of the cause, the mismatch between what is expected (the outcome) and what actually happens (absence of the outcome) represents relevant information that makes the person learn and adjust future expectations. Existing literature in cognitive neuroscience has recognized several brain electrical indicators of this expectation with which neural correlates of causal learning can be explored. Interestingly, our predictions about the neural correlates of expectation of cause present and cause absent would be exactly the same regardless of whether the causal relationship perceived by the person is real or illusory. In the present work we make use of EEG recordings to explore the neural correlates of expectation in an experimental setting in which the relationship that the participants perceive between the potential cause and the outcome is illusory.

Title

Language Switch Costs: Production vs. Comprehension

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How are individuals that speak more than one language able to keep their languages separate and switch to the appropriate language as required? One approach used to gain insight into the control mechanisms underlying language processing in bilinguals examines the costs that arise when bilinguals switch between languages. Some studies report asymmetric switch costs (i.e. costs for switches from L2 to L1 are larger than for switches from L1 to L2) while others do not report this asymmetry. These studies differ on a number of characteristics such as the tasks and methodology employed and type of stimuli used. Recent findings suggest that these different patterns in switch costs are not caused by differences in methodology or stimulus type, thus suggesting that whether or not asymmetric switch costs are found depends on the nature of the task employed (production vs. comprehension). The current study aims to establish whether the switch cost asymmetry typically found in production tasks can also be observed on a comprehension task by directly comparing language switching performance on a production and a comprehension task with all other parameters held constant. To test this, unbalanced Spanish-French bilinguals were required to unpredictably switch between their first and second language while performing either a production task (naming) or a comprehension task (grammatical gender judgment). In accordance with the literature, language switches on a production task should yield an asymmetric switch cost (larger cost for switching to L1 than switching to L2), while switching between languages on a comprehension task should fail to show this asymmetry (comparable cost for switching to L1 and L2). However, the comprehension task employed in this study differs from the tasks typically employed in this field. That is, in tasks such as semantic categorization (e.g., is this object animate or inanimate) it is only the input that is language-specific (e.g., reloj, Spanish: clock) while the required response is language-neutral (the correct response is “inanimate”, irrespective of whether the stimulus was reloj (Spanish) or horloge (French)). In contrast, grammatical gender judgment requires participants to respond in a language-specific manner, since it is possible for a given noun to be masculine in one language (reloj) but feminine in the other (horloge). By making the response language-specific, the task becomes more easily comparable to a classical production task, which might affect the pattern of switch costs. Findings and their implications for theories of language control will be discussed.

Title

GIRK channels mediate the electrophysiological, behavioral and hypothermic effects of 5-HT_{1A} agonists and citalopram

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Background: It is well-known that GIRK channels containing GIRK2 subunits play an important role controlling excitability of several brain areas. These channels are coupled to inhibitory G-protein coupled receptors, such as 5-HT1A receptor, which inhibits serotonergic neurotransmission.

Objective: To study the implication of GIRK channels in the electrophysiological, behavioral and hypothermic effects of 5-HT1A agonists and citalopram.

Methods: Electrophysiological studies included in vivo single-unit extracellular recordings and in vitro patch-clamp recordings of dorsal raphe neurons. Behavioral tests were tail suspension test (TST) and novelty-suppressed feeding test (NSFT). Functional status of 5-HT1A autoreceptors was assessed measuring the hypothermic response to the 5-HT1A agonist 8-OHDPAT. Experiments were performed using wild-type and GIRK2 mutant mice.

Results: In vivo dose-response curves of citalopram (0.5-3 mg/kg, i.p.) were significantly shifted to the right in GIRK2 homozygous mice compared to that obtained in wild-type and GIRK2 heterozygous animals. This inhibitory effect of citalopram was blocked with the 5-HT1A antagonist WAY100365 (25µg/kg, i.p.). Moreover, when GIRK channels were pharmacologically blocked with tertiapin (100pmol, i.c.v.) dose-response curves of citalopram were also significantly shifted to the right as well as basal firing rate and the proportion of burst-firing neurons were increased. Whole-cell patch-clamp experiments revealed that 5-HT1A agonist 5-CT (100nM)-induced current was smaller in GIRK2 mutant groups. As expected, the hypothermic effect of 8-OHDPAT (0.5mg/kg, i.p.) was greater in wild-type mice comparing to GIRK2 mutant genotypes. Mutant groups showed lower immobility time in the TST and lower latency to eat in the NSFT. Interestingly, in TST, citalopram (10 mg/kg, i.p.) was less effective reducing the immobility time in GIRK2 mutant genotypes.

Conclusion: Mutation of Girk2 gene reduces the 5-HT1A-mediated signaling and improves the behavioral response to anxiety-related situations. Thus, GIRK channels could be candidates as a therapeutic target for neuropsychiatric disorders.

Title

PINK1 and LRRK2 are involved in the dysregulation of mitochondrial dynamics in Parkinson´s disease

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Parkinson´s disease (PD) is the second most common neurodegenerative disorder and is characterized by the degeneration of dopaminergic neurons in the substantia nigra. The majority of the cases are sporadic but genetic mutations account for ~10% of the patients. Mutations in PARK8 or leucine-rich repeat kinase 2 (LRRK2) are the most frequent cause of late onset autosomal dominant PD and are also frequently found in sporadic cases. Among the early onset recessive forms, mutations in PARK6 or PTEN-induced putative kinase (PINK1) have been described to affect to mitochondrial homeostasis. In this study we sought to investigate whether LRRK2 plays a role in the pathogenesis of all forms of PD and identify common pathways between LRRK2 and PINK1. To investigate how mitochondrial dynamics affect PD pathogenesis, we used primary dermal fibroblasts from healthy donors (n=4), 2 patients with c.1488+1G>A mutation (PINK1+/-) (carriers) and 2 compound heterozygous with c.1488+1G>A + c.1252_1488del double mutation (PINK1-/-) (affected) (Samaranch et al., 2010). In addition, we generated iPS cell lines from these fibroblasts and used iPS-derived neurons in some experiments. We analyzed the expression of LRRK2 in fibroblasts by qPCR and western blot. LRRK2 protein expression but not mRNA expression was significantly higher in PINK1-/- than in either PINK1+/- or control fibroblasts. This result suggests that LRRK2 turnover might be decreased in PINK1-/- fibroblasts. We then examined the mitochondrial morphology and quantified the percentage of cells in each genotype according to elongation and shape (tubular and round or tubular network) using mitotracker. Fibroblasts from affected patients presented higher percentage of cells with a tubular mitochondrial network compared with PINK1+/- and control counterparts. In addition, PINK1-/- fibroblasts showed a lower percentage of cells with tubular and round morphology. These findings suggest that PINK1 deficient fibroblasts present a dysregulation in the fission/fusion process. We analyzed the expression of fission protein MFF and observed that this protein was highly expressed in PINK1-/- and PINK1+/- but not in healthy controls. The lentiviral knockdown of LRRK2 in PINK1 defficient cells, reversed the mitochondrial phenotype increasing the cells showing a tubular and round morphology. In preliminary experiments using neurons derived from iPS cells we observed a trend to an increase in LRRK2, both at the mRNA and protein expression levels. Interestingly, following LRRK2 silencing the morphology of the mitochondria was normalized as found also in fibroblasts. Collectively, these initial findings suggest that LRRK2 may modulate mitochondrial dynamics in PINK1 mutations and perhaps in other forms of PD.

Title

DNA methylation analysis in a rat model of L-DOPA induced dyskinesia

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L-DOPA is still the most effective drug to treat the signs and symptons of Parkinson's disease (PD), but a long-term use causes a development of dyskinetic L-DOPA induced dyskinesia (LID) in the majority of patients treated. Moreover, once LID is developed it is very difficult to reduce or reverse it even if patients discontinue the treatment for a long period of time. This also occurs in animal models of LID where several molecular changes have been suggested. It is known that the expression of the DfosB protein, a truncated spliced isoform of the fosB protein, coded by the FOSB gene is highly increased in animals treated with L-DOPA which developed LID. The aim of this study was to assess whether the grade of methylation of the FOSB gene is involved in the development of LID. For the purpose, rats treated with 6-OHDA to induce unilateral dopamine-denervating lesion received a 3-week exposition to L-DOPA using a daily injection of 6mg/kg L-DOPA plus 12mg/kg benserazide. During this period LID was studied using behavioral tests. Rats were sacrificed 24h after the last L-DOPA injection and the striatum was collected for DNA methylation analysis. DNA was bisulfate-modified and seventeen CpG sites were pyrosequenced with PyroMark IQ96MD. According to behavioral and immunohistochemical assays, 6-OHDA lesioned animals treated with L-DOPA were divided into dyskinetic and non-dyskinetic groups. In addition, a control group was introduced. Furthermore, methylation levels were evaluated in three different zones of 5'UTR FOSB gene. No differences were determined in the level of methylation of the analysed CpG sites between subjects or experimental groups. In conclusion, DNA methylation of FOSB is not responsible for the development of LID. Further studies should be performed in order to assess if epigenetic mechanisms are involved in LID.

Title

Implication of serotonergic and dopaminergic systems in buspirone anti-dyskinetic effect

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INTRODUCTION: The chronic treatment of Parkinson's disease (PD) with L-dopa is severely limited by the development of motor complications including dyskinesia. It has been described that buspirone, a partial agonist of 5HT1A receptors and a D3-receptor antagonist elicits anti-dyskinetic effect. Although the mechanisms underlying the development of L-dopa-induced dyskinesia are not completely understood, prior studies demonstrated that this anti-dyskinetic effect is blunted by subthalamic nucleus (STN) lesion. OBJETIVE: To assess the mechanisms underlying the anti-dyskinetic effect of buspirone. METHODS: Behavioral and molecular approaches in 6-OHDA-lesioned rats treated with L-dopa, and single-unit extracellular recordings on the STN neurons of anesthetized rats were performed. RESULTS: The anti-dyskinetic effect of buspirone was dose-dependent (the dyskinetic score obtained with L-dopa alone was reduced by 33, 71 and 83% after 1, 2.5 and 4 mg/kg i.p.). WAY-100635 (1 mg/kg i.p.), an antagonist of 5HT1A receptors; and PD128907 (3 mg/kg i.p.), a D3-receptor agonist, blocked significantly the anti-dyskinetic effect of the highest doses of buspirone while GR103691 (1.5 mg/kg i.p), a D3-receptor antagonist, failed to modify it. WAY-100635 or GR103691 alone had no effect on dyskinesia. Buspirone (4 mg/kg i.p.) and buspirone (4 mg/kg) plus WAY-100635 (1 mg/kg i.p.) reverted the molecular changes described on dyskinetic rats; the increment of pDARPP32, pERK2 and pCreb on the DRD1 pathway and pGSK3β on the DRD2 pathway. Buspirone significantly decreased STN firing rate in a dose dependent manner (buspirone 4 mg/kg i.p. 35% and 8 mg/kg i.p. 67% vs the basal firing rate). We first evaluated the effect of WAY-100635 and PD128907 in combination with buspirone and blocked its effect. Given the efficient blockage, we then tested the effect of each drug separately and found that while WAY-100635 completely reversed the effect of buspirone, PD128907 only partially reverted it. In addition, buspirone increased the coefficient of variation and decreased the bursty activity of STN neurons. The acute and chronic administration of L-dopa (6 mg/kg plus benserazide 12 mg/kg i.p.) did not modify the inhibitory effect of buspirone (4 mg/kg i.p.). CONCLUSION: Buspirone administration induces a reduction of dyskinetic movements, avoides the expression of molecular changes observed in dyskinetic rats and reduces the firing rate of the STN neurons through DRD3 and 5HT1A receptors.

Title

Characterization of an experimental model of Parkinson´s Disease: Behavioral and electrophysiological study in 6-hydroxydopamine-lesioned mice.

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Parkinson's disease (PD) is characterized by a progressive loss of dopaminergic cells in the substantia nigra pars compacta that leads to marked impairment in motor function, which is commonly treated with the dopamine precursor, L-DOPA. Usually, 40% of the patients develop the so-called L-DOPA induced dyskinesia (LID) in 4-6 years of chronic L-DOPA treatment. The unilateral 6-hydroxydopamine (6-OHDA) -lesioned rat model has been widely used in PD research, but in the last years several knockout mice have been developed, opening a whole new world in research in this field. For this reason, the 6-OHDA-lesioned mouse model should be characterized for being able to evaluate the lesion degree and the consequent motor impairment induced. In this mouse model chronic L-DOPA treatment has also been demonstrated to induce LID. In addition, the subthalamic nucleus (STN) activity is increased in PD patients and in the 6-OHDA model dyskinetic rat and it may also be changed in dyskinetic mice. The aim of this study was to characterize the model of 6-OHDA and subsequent LID in mice using behavioural and electrophysiological techniques. To that end stereotaxic injections with 6-OHDA or vehicle in the right medial forebrain bundle of C57/16 mice were performed. The adjusting steps test showed that the use of the ipsilateral and the contralateral limb was equal for both lesioned and sham group. But in the spontaneous rotation test, the number of ipsilateral rotations in the lesioned group was higher than that measured in the sham group. After assessing motor impairment, the lesioned group was daily treated with an intraperitoneal injection of L-DOPA (10 mg/kg) plus benserazide (12 mg/kg) during 21 days. All parkinsonian mice treated with L-DOPA developed LID, which were measured and remained stable after two weeks of treatment lasting 100-120 min. Similarly to the results reported in rats, the peak of the LID was reached between 60 and 100 min after L-DOPA administration. Neuronal firing rate and coefficient of variation (7.76 ± 0.82 Hz and 71 ± 10 %) of STN neurons in control mice were similar to those reported in rats. However, the firing patterns were slightly different from the rat, showing an increased percentage of bursting neurons (13 % random, 27 % tonic and 60% burst firing neurons). In dyskinetic mice no difference were found compared to the rat model. Finally, the tyrosine hydroxylase immunochemistry confirmed the severe lesion induced in the 6-OHDA group. To sum up, in this work we optimized the protocols for performing 6-OHDA lesions in mice and evaluate the motor impairment after dopaminergic loss, pointing at the spontaneous rotation test like a good marker of highly lesioned mice. We also characterized the development of LID and studied the electrophysiological properties of STN neurons in control and dyskinetic mice.

Title

α-synuclein levels in the blood as a potential biomarker for PD

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The diagnosis of Parkinson's disease (PD) remains primarily a clinical issue, based mainly on phenotypic patterns. The identification of biomarkers capable of permitting the preclinical detection of PD is critically needed. a-Synuclein is a key protein in PD, with missense and multiplication mutations in the gene encoding a-synuclein (SNCA) having been reported in familial cases of PD, and accumulation of the protein identified in Lewy bodies (LBs) and Lewy neurites (LNs) in affected brain regions. With the objective of validating the use of a-synuclein as a clinical or progressive biomarker in an accessible tissue, we used an enzyme-linked immunosorbent assay (ELISA) to measure a-synuclein levels in the peripheral blood plasma of idiopathic PD and LRRK2 mutation carrier patients and compared our findings with healthy control subjects. Compared to healthy controls, we found a significant decrease in plasma total a-synuclein levels in idiopathic PD (iPD) patients (n = 134, p = 0.010). However, the reduction was less significant in patients who were LRRK2 mutation carriers (n = 32, p = 0.133). This lack of significance could be due to the small number of individuals employed in this group. No predictive value of total a-synuclein in the diagnosis of PD was found in a receiver operating characteristic (ROC) curve analysis. Although this is a pilot study requiring corroboration on a larger cohort of patients, our results highlight the possible use of plasma a-synuclein as a biomarker for PD.

Title

Apomorphine-induced abnormal involuntary movements in unilateral 6-hydroxydopamine-lesioned rats: Quetiapine effects

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The emergence of motor complications, such as levodopa-induced dyskinesias, complicates long term treatment of Parkinson's disease (PD). Its pathophysiology is not well understood but pharmacological and histological studies suggest that serotonergic receptors, specially the subtype 2A (5-HT2A), might have a role. 5HT2A receptor antagonists have reduced dyskinesias in parkinsonian monkeys, but data in PD patients are controversial. Thus, we investigated the effect of quetiapine, an atypical neuroleptic with 5-HT2A receptor antagonistic properties, on the dyskinesias induced by the dopaminergic agonist apomorphine (APO) in the 6-hydroxydopamine-lesioned (6-OHDA) rat model. The nigro-striatal pathway was unilaterally lesioned with 6-OHDA. After a dose-increase protocol of APO administration (escalating doses 0.1, 0.2, 0.5 mg/kg, sc., over three 5-d blocks), animals developed dyskinesias. On day 16, APO (0.5 mg/kg) and quetiapine (20 mg/kg, ip.) or vehicle were co-administered for 2 weeks. Dyskinesias and rotational behavior in response to APO were measured on days 1, 5, 6, 10, 11, 15, 16, 20, 25 and 30. In addition, PET studies with the radioligands 11C-PE2I (dopamine transporter) and 18F-FDG (metabolism rate) were performed under the influence of drugs. Quetiapine reduced the total number of APO-induced rotations in comparison with animals treated only with APO, indicating a motor inhibitory effect of quetiapine which could be explained by its D2/D3 dopamine receptor antagonist activity. Surprisingly, quetiapine increased the global score of dyskinesias. This could be either a consequence of the reduction of the rotational behaviour, and/or due to the action of quetiapine on other receptors such as adrenoreceptors or histamine receptors. A more complex interaction between dopaminergic and serotoninergic systems should also be considered. Dyskinetic animals had a reduction in FDG uptake in the striatum which was associated with the severity of the dyskinesias. In conclusion, the striatum is a key structure in the expression of dopaminergic induced dyskinesias as shown by PET studies. The role of the serotonergic system in the pathophysiology of dyskinesias needs to be further addressed although other receptors might also be implicated. Current studies using PET with the radioligand 18F-Altanserine specific for 5-HT2A could help to understand the underlying action mechanisms of quetiapine and to deeply analyze the role of the serotonin system (DFG11/019, PI11/02109).

Title

Characterization of blood Microparticles in an Experimental Autoimmune Encephalitis model.

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Microparticles (MP) are membrane fragments shed by activated cells after a variety of stimuli such as stress or inflammatory processes. Apparently they play a role in extracellular communication with no direct contact. They also have been shown to contain genetic material, mainly RNA and miRNA, that produces genotypic modifications in the target cell. Multiple Sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) characterized by myelin loss, axonal injury and progressive neurological dysfunction. There are studies that shown an increase of MP in MS patients in relapse compare to remission. MP has been postulated to be an interesting biomarker for monitoring, diagnosis and prognosis of autoimmune diseases such as MS. Experimental Autoimmune Encephalitis (EAE) is the murine model of the disease that reproduces the pathology of MS. The aim of the study was to analyse the leukocyte-derived MP (CD45+ MP) in peripheral blood from EAE and healthy mice. We performed three mice groups: EAE (Myelin Oligodendrocyte Glycoprotein [MOG] model), immunized (only adjuvant without MOG) and control group. The animals were monitored daily with weight and score. The sacrifice was carried out under inhaled anaesthesia and through hearth exsanguination in the 30th day post immunization. We used flow cytometry to analyse MP levels. EAE and immunized groups revealed similar CD45+ MP percentages (35.6% and 35.9% respectively). However a lower percentage (15.0%) was found in the control group. We propose EAE model as a plausible animal experimental model to investigate MP. Our hypothesis is that the similar MP levels in EAE and immunized groups may be due to migration of leucocytes through the blood brain barrier (BBB) into the CNS in EAE group, and finally a lower count of leucocyte-derived (CD45+) MP in peripheral blood. Leucocytes from the immunized group are not reduced in number as they do not migrate through an intact BBB. Cerebral spinal fluid studies are mandatory to confirm a probable higher MP level in EAE group than immunized group.

Title

Effect of Calpain 3 deficiency on calcium homeostasis

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Limb girdle muscular dystrophy type 2A (LGMD2A) is one of the most frequently occurring forms of recessive muscular dystrophies and it is characterized by primary wasting of scapular and pelvic muscles that result in progressive muscle weakness. Impairment of calcium transport is one contributing factor to the clinical phenotype of LGMD2A muscular dystrophy, which is caused by mutations in Calpain 3 (CAPN3), a non-lysosomal calcium-dependent cysteine protease. As we have recently shown in a collaboration study involving CAPN3 knockout mice and LGMD2A patients, impaired calcium-mediated signaling and weakened muscle adaptation are pathogenic mechanisms operating in CAPN3-deficient muscles. In the present study, we have used mouse and human myotubes to analyze a whole array of molecules associated with calcium handling by real time PCR, western blotting and calcium imaging techniques, and found significant reduction in a number of proteins essential for calcium homeostasis in muscle fibers. Our new findings are in line with the ones previously described in the LGMD2A mouse model and suggest novel targets that open new avenues for potential therapeutic approaches to treat LGMD2A muscular dystrophy.

Title

In vivo imaging of Dopaminergic and Serotoninergic Neurotransmission following long-term Brain Ischemia

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Objectives: Neurotransmitter systems may play a key role in the functional recovery process and brain plasticity in response to cerebral ischemia. The aim of the present study is to study by positron emission tomography (PET) both the dopaminergic and serotoninergic neurotransmission to elucidate their relationship with brain perfusion, metabolism and functional recovery after long-term brain ischemia. Methods: Longitudinal PET imaging with [13N]ammonia, [18F]FDG, [11C]raclopride, [11C]DASB and [18F]altanserin was performed to explore the changes in cerebral perfusion, glucose metabolism, dopamine D2 receptor, serotonine transporter (SERT) and serotonine 5-HT2A receptor binding before and after 1, 3, 7, 14, 21 and 28 days following transient focal cerebral ischemia in rats. PET imaging studies were conducted in parallel with in vitro autoradiography by using [3H]Raclopride and inmunohistochemistry with tyrosine hidroxilase. Function recovery was carried out using a battery of neurologic and behavioral tests for rats. Results: In the ipsilateral hemisphere, PET [13N]ammonia showed a decrease of the signal at 1 day followed by the uppermost binding at day 7 after reperfusion. Surprisingly, a similar time course was observed in the contralateral hemisphere. The glucose metabolism of the ischemic territory evaluated by [18F]FDG showed a decrease at day 1 followed by a slight recovery around day 7-14 that did not reach control values. The [11C]raclopride binding showed similar control values around day 1-7, after what signals dropped to 70% of control from days 14 to 28 in the ischemic striatum. Interestingly, a slight binding increase was observed at day 1-3 followed by the uppermost binding at day 7 in relation to control values in the contralateral to the lesion. In vitro binding of [3H]raclopride and immunohistochemistry verified the in vivo PET results. The serotoninergic system also showed changes following brain ischemia. PET with [11C]DASB and [18F]altanserin showed a dramatic decline of both SERT and 5-HT2A binding potential in the ischemic hemisphere from day 1 to day 28 after cerebral ischemia. Interestingly, a slight increase of [11C]DASB binding was observed from days 7 to 21 followed by the uppermost binding at day 28 in the ipsilateral midbrain raphe nuclei. In contrast, no changes were observed in the contralateral hemisphere to the lesion by using both radiotracers. Both functional and behavior testing showed major impaired outcome at day 1 after ischemia onset followed by a recovery later on. Therefore, animals experienced a quasi-normal recovery of functional outcome that run in parallel with (i) an increase of whole brain perfusion, (ii) glucose metabolism recovery in the ischemic territory, (iii) over-expression of D2 dopaminergic receptor in the contralateral hemisphere and (iv) SERT binding increase in the ipsilateral midbrain following long-term cerebral ischemia. Conclusions: These results may provide new information about the key role of brain perfusion and both dopaminergic and serotoninergic neurotransmission in the recovery of brain function and may elucidate mechanisms involved in brain plasticity after stroke.

Title

Cerebral white matter correlates of processing speed in multiple sclerosis

Author(s)

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Introduction Multiple sclerosis (MS) is associated with multiple and complex cognitive function impairments, but information processing speed (PS) is considered as the main and most common cognitive deficit in this disorder. PS has been related to white matter (WM) integrity in MS, other neurodegenerative pathologies and in healthy aging in several brain areas. While results obtained in some studies point out the influence of specific tracts in different brain areas, other studies sustain the influence of whole brain WM in PS. The aim of the current study is to investigate the relationship between performance on tasks of PS and whole brain WM fractional anisotropy (FA) in MS. Methods We recruited 29 patients with multiple sclerosis (mean age=43). Participants were given an extensive battery of neuropsychological tests and a diffusion tensor MRI on a Philips Achieva 3T TX. Total correct responses on the Symbol Digit Modalities test, Letter Portion of the Salthouse Perceptual Comparison Test, and Words and Colors Portions of the Stroop Color-Word Test were considered indicative of PS and were united in a composite score (α= .91). Whole-brain voxel-wise regression analysis of this composite score and cerebral WM FA data was performed controlling the influence of the gender and age using TBSS (Tract-Based Spatial Statistics) as implemented in FSL. Results A positive correlation between the composite score of processing speed and FA in a cluster of voxels (n=55397, pcorrected< .001) was found. This cluster involved WM of several lobes including the following cerebral white matter tracts bilaterally: inferior cerebellar peduncle, middle cerebellar peduncle, superior cerebellar peduncle, cerebral peduncle, medial lemniscus, corticospinal tract, inferior longitudinal fasciculus, superior longitudinal fasciculus, inferior fronto-occipital fasciculus, superior fronto-occipital fasciculus, cingulum WM, corpus callosum (genu, body and splenium), fornix, anterior thalamic radiation, posterior thalamic radiation, optic radiation, posterior corona radiate, sagittal stratum, anterior corona radiate, superior corona radiate, forceps major, forceps minor, uncinate fasciculus, internal capsule and external capsule. Conclusion Our results reinforce the previously mentioned findings about the relationship between PS and whole brain WM integrity, appearing this relationship in commissural, associative, projective and brainstem tracts and suggesting that the PS is related to widespread WM in MS.

Title

Parkinson's disease, processing speed and white matter connectivity: a diffusion tensor imaging study

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Introduction Parkinson Disease (PD) was considered a motor disorder, but cognitive functions such as processing speed (PS), memory, language, executive and visuospatial/constructive functions are also impaired. Diffusion tensor imaging (DTI) gives estimations of neuronal changes in patients with neurological diseases. DTI is used to assess the integrity of white matter (WM) tracts in the brain assessing, among others, fractional anisotropy (FA) values. In the specific case of PD, the significance of WM tract microstructure alterations for motor and cognitive function disease is still in debate. In this study we aimed to investigate the relationship between performance on PS and cerebral WM FA in PD patients. Methods We recruited 35 non-demented PD patients (mean age=69.03, mean of illness duration=6.51). Patients underwent a neuropsychological battery and were scanned with an imaging protocol that included DTI on a Philips Achieva 3T. PS was measured with Salthouse Perceptual Comparison Test´s (PCT) total correct score. Whole-brain voxel-wise regression analysis of PS and cerebral WM FA data was performed using TBSS (Tract-Based Spatial Statistics), part of FSL software. Results The group´s mean score in PCT was 19.94 (SD=12.24). A significant positive correlation (pcorrected =.04) was found between PS and a cluster including WM areas of right external capsule and right inferior-frontooccipital fasciculus. Other tracts found were right superior longitudinal fasciculus (temporal part), right anterior thalamic radiation, right acoustic radiation, right corticospinal tract white matter, right optic radiation, right uncinate fasciculus, anterior corona radiata and callosal body. Conclusion Our results show correlations between WM FA and performance on PS in PD patients. We conclude that those specific WM tracts contribute to PS in PD patients.

Title

Identification of novel biomarkers in carotid atherosclerosis: symptomatic versus asymptomatic

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Background: Stroke is the first cause of death in women and the third in men. It is also the leading cause of severe disability. Atherosclerotic plaque formation is a risk factor for stroke. Destabilization and rupture of those plaques may lead to ischemic attack. Currently it is not possible to predict whether a plaque will become symptomatic or when those symptoms will be detectable. Thus, understanding the mechanism of plaque destabilization is crucial for development of preventive treatments. Methods: 80 atherosclerotic plaques were obtained by carotid endarterectomy in Basurto Hospital, 45 of which symptomatic and 35 asymptomatic. Total RNA was extracted from carotid plaques and the levels of gene expression of 60 genes were analysed by QPCR with ABI7500Fast platform. The expression was quantified using the comparative Ct method and the QPCR results were analysed by the Mann-Whitney U test. Correlation analysis was performed with the Spearman's Rank Order correlation test. Total proteins were also extracted from carotid plaques using RIPA lysis buffer. Protein extracts were resolved by SDS-PAGE and analysed by western blot with specific antibodies (i.e. anti-LCB3 antibody, Cell Signaling Technology). Proteins bands were detected by chemiluminiscence using ChemiDoc™XRS Imaging System (Bio-Rad). β-Actin was used as housekeeping gene. Results: In this study we found mRNA expression levels to differ between the two groups of patients, i.e. symptomatic and asymptomatic. Among the 60 genes studied, 13 genes were identified with more than 1.5 fold increase in gene expression in symptomatic samples, and 12 genes demonstrated a fold decrease from -1,38 to -5 in the same group of plaques. Genes TIMP1 and ITPR1 were found to be over-expressed in the symptomatic group compared with the asymptomatic group (3-fold), while the MAP1LC3B and ERP27 genes were under-expressed (4-5 Fold). In addition, for MAP1LC3B, the western blot analysis demonstrated that the protein levels of MAP1LC3B were also lower in symptomatic plaques. Conclusions: We have identified novel biomarkers of potential relevance to the prediction of plaque rupture and destabilization. Pathway analysis revealed that endoplasmic reticulum stress, inflammation and autophagy-related pathways predominate among the differentially expressed genes.

Title

A mutation in the signal peptide of IL22RA2 is strongly associated with risk for multiple sclerosis: a genetic and functional study

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Multiple sclerosis is a chronic inflammatory disorder of the central nervous system, characterized by demyelination, aberrant remyelination and axonal degeneration, with a probable autoimmune etiopathogenesis, that affects predominantly young adults. IL22RA2, located on chromosome 6q25.1, codes for a soluble antagonist of IL-22. In a candidate gene study of cytokine and cytokine receptor genes carried out by our group in a Basque-resident population, a SNP in this gene, rs202573, proved significantly associated with the disease. In order to locate the causative variant, a fine mapping including 15 SNPs in a 47.8Kb region comprising the gene and flanking sequences was performed. Of these, a rare (MAF=0.02) exonic non-synonymous variant displayed a trend toward association (p=0.054). The minor allele of this SNP changes a Leu to Pro aminoacid position in the signal peptide. Because of its potential functional implication, and in order to increase the statistical power, this SNP was genotyped in further European populations (Madrid, Barcelona, Andalucía, France and Germany), comprising a total of 8636 cases and 7613 controls, and showed a strong association (ORminor allele= 1.274, P=2.5e-4) in the combined cohorts stratified by the Cochran-Mantel-Haenzel test. The protein encoded by this gene is a soluble class II cytokine receptor which lacks a transmembrane and intracellular domain, that binds with high affinity to interleukin 22 (I-L22), thus blocking the interaction of IL-22 with its cell surface receptor. It is therefore an antagonist of IL-22, a pro-inflammatory cytokine secreted by Th17 cells known to play a key role in MS. In order to test the functional effect of this mutation, two different expression vectors were constructed. A transfection-ready plasmid (TrueORF Gold Expression-validated cDNA Clone (Origene; code RC219095)) in which expression of a C-terminally myc-DDK-tagged ORF of human IL22RA2 is driven by the CMV promoter, was used to introduce the Leu to Pro mutation by site-directed mutagenesis (GeneArt® Site-Directed Mutagenesis System, Invitrogen). Moreover, wild.-type and mutant versions were cloned in the ponasterone A-inducible vector pEGSH vector, from which the protein is expressed with C-terminal DDK-tag. This battery of vectors is currently being used to test how this mutation affects translocation of the protein into the endoplasmic reticulum (RE) potentially preventing protein glycosylation and inducing retention of the protein in the ER. The full genetic and functional data elucidating IL22RA2 as risk factor for MS will be presented.

Title

The SOCS1 gene: a strong risk factor for multiple sclerosis in the Basque-resident population

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Background: Multiple sclerosis is the most prevalent demyelinating inflammatory disease of the central nervous system, and constitutes the first non-traumatic cause of disability in the young adults. Genetics plays a crucial role in the susceptibility to MS, and most of the genes associated to the disease are related to the immune system. SOCS-1 is a negative feedback regulator of cytokine signaling, which is induced upon cytokine activation of immune cells and blocks the JAK-STAT pathway. A SNP non-coding in the LD area of the SOCS1 gene, rs243324, was the most significantly associated in a candidate-gene associated study of cytokines and cytokine receptors including 368 SNPs in 55 genes and performed in a population of 463 MS patients and 470 healthy controls from Bilbao (Basque Country, Spain). Variations in this gene have also been independently reported as genetic risk factors for MS. Methods: To refine the association signal in this area, we selected 19 haplotype tagging SNPs, which covered a 60kb region around the gene, and genotyped them in 588 MS patients and 567 controls from Bilbao using the iPLEX MassArray techology. Additionally, a rare variant in the area, imm_16_11281298, which has been reported as associated with celiac disease, was also genotyped in the Bilbao cohort and in additional populations from Andalucía and Madrid, comprising a total of 2462 cases and 2973 controls, using a Taqman custom-made assay. To study the possible influence in SOCS1 expression of the most associated SNP in the fine mapping, total RNA was extracted from 45 MS patients and 15 healthy controls from Vall d'Hebron hospital in Barcelona, and SOCS1 expression was assessed by qPCR using the ΔCt method. Statistic analysis was performed with Plink v1.07 and Graphpad v.5. Results: The fine-mapping exercise resulted in 14 additional associated SNPs, some of these reaching P values of 10-7. The most strongly associated SNP (P=5.71E-7, OR=1.8) was rs423674, a non-coding SNP located at about 25kb upstream of SOCS1. The rest of the SNPs showing the most significant P values are in high LD (r2=0.9) with rs423674, and they lie in intergenic regions 20-40kb upstream of SOCS1. No association with imm_16_11281298 was detected in any of the cohorts, nor considering the 3 cohorts combined. qPCR analysis revealed that risk allele carriers have a significantly higher expression of SOCS1 in PBMCs (p=0.024). Conclusions: SNPs in SOCS1 are strongly associated with MS in a Basque resident population, with the strongest association signals located in non-coding areas 20-40kb upstream of SOCS1. The most significantly associated SNP, rs423674, is correlated with expression level of SOCS1 mRNA, therefore, it is possible that SNPs in this area influence MS risk by changing SOCS1 gene expression in immune cells. We did not detect association with a rare variant, imm_16_11281298, with MS in this population; however, due to the extremely low minor allele frequency of this variation, very large sample sizes would be needed to detect this association.

Title

Cost-effective mutation screening in retinal neurodegenerative diseases based on Axiom Exome genotyping array.

Author(s)

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Purpose: To develop a cost-effective, excel-based genetic screening strategy for the diagnosis of neurodegenerative retinal diseases based on DNA Axiom Exome Genotyping Array Plates (Affymetrix). Methods: Peripheral blood from a total of 190 individuals (80 affected and 110 non-affected relatives) was collected and DNA samples were analyzed using two Axiom Exome Genotyping Array Plates. Initial analysis and quality control test of raw data was performed using the genotyping console software 4.1.4 (Affymetrix). This array contains over 5,000 variants within 181 genes involved in retinal diseases. We developed an excel-based data mining strategy in order to screen those pathogenic variants in our population. All genetic findings were validated by Sanger sequencing. Results: A total of 10 genetic variants, either reported as pathogenic or with a prediction of probable pathogenic were found in 10 retinal disease-related genes affecting 30 patients. Conclusions: Using our excel-based data screening strategy we were able to extract relevant biological information out of the large amount of data generated by the DNA Axiom Exome genotyping array plates. Although non-specific for retinal diseases, these arrays proved extremely cost-effective for the molecular characterization of heterogeneous hereditary retinal diseases, for 1/50 of the cost involved when using retina-specific APEX arrays.

Title

High Resolution Melting (HRM) Analysis as a Diagnostic Tool in Retinitis Pigmentosa.

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Purpose: To test the feasibility of a high-throughput genetic screening approach for the molecular diagnosis of RP using a fast and cost-effective HRM analysis-based method, and to compare this methodology with two of the most widely used Next Generation Sequencing (NGS) platforms: Reversible dye terminator (Illumina) and Ion semiconductor (PGM) technologies. Methods: RP patients were clinically diagnosed at the Ophthalmology Department of Donostia University Hospital, San Sebastian, Spain. We applied a genetic high-throughput screening based on HRM analysis to non-syndromic RP probands (n=117) and to a set of syndromic RP probands that met clinical diagnosis for Bardet-Biedl syndrome (BBS, n=5). A total of 17 RP genes were analyzed: 10 genes of non-syndromic RP, that in aggregate account for about 17% of all cases of RP world-wide and 7 BBS genes, that account for about 70% of all cases of BBS. Inclusion criteria for gene selection were: 1) size: genes of less than 4 kb; 2) prevalence: genes reported to account for, at least, 0.4 % of total RP cases world-wide; and 3) number of exons: genes with up to 22 exons. For comparison purposes, RHO gene was also sequenced with Illumina (GAII; Illumina) and Ion semiconductor (PGM-Ion Torrent; Life Technologies) technologies. Detected mutant variants were confirmed by Sanger sequencing and tested for co-segregation in first-degree family members of the affected proband. Results: A total of 34 genetic variants were found, 15 of which in silico predicted as probably damaging or previously reported as pathogenic. Out of these 15 variants, 5 were novel. All variants found in the RHO gene were also detected by Illumina and PGM sequencing. Furthermore, additional RHO genetic variants were detected in distal non-coding regions by HRM analysis. Conclusions: In the present work, we have validated a high throughput (HTP) genetic screening method for mutation discovery in retinitis pigmentosa (RP), based on high resolution melting (HRM) analysis. This approach has proven to be a fast and cost-effective mutation screening approach in the context of small/medium size (up to 4 kb) RP genes. All variants detected by HRM analysis were also found by two of the most widely used NGS platforms (Illumina and PGM-Ion Torrent).

Title

Familial cortical myoclonic tremor, epilepsy, and parkinsonism due to a nonsense ACMSD mutation (p.W26X)

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Familial cortical myoclonic tremor associated with epilepsy is an autosomal dominant disorder phenotypically and genetically quite heterogeneous. Today, despite the numerous familial studies performed and the number of loci identified, there is no gene associated with this syndrome. However, it is expected that through the application of novel genomic technologies, such as whole exome sequencing and whole genome sequencing, a substantial number of novel genes come to light in the coming years. Here we describe the identification of a nonsense disease-segregating mutation in the ACMSD gene, which encodes for a critical enzyme of the kynurenine pathway of the tryptophan metabolism, in a large family featuring cortical myoclonic tremor, epilepsy, and parkinsonism. This finding not only reveals the identification of the first gene associated with familial cortical myoclonic tremor and epilepsy but also discloses the kynurenine pathway as a potential therapeutic target for the treatment of this devastating syndrome.

Title

LRRK2-R1441G prodromal Parkinson's disease

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Objective: To evaluate the prevalence of motor, non-motor symptoms and nigrostriatal abnormalities by DAT-SPECT in a cohort of asymptomatic carriers of LRRK2 R1441G mutation. Background: LRRK2 mutations are the most common known genetic causes of autosomal dominant Parkinson's disease (PD). The most frequent LRRK2 mutation, G2019S, has been observed throughout the world. The LRRK2-R1441G mutation is responsible for 46% of familial PD and for 2.5% of sporadic PD patients with Basque origin. Increasing evidence suggests that Parkinson's disease-related subtle motor deficits can be detected at prodromal stages, particularly when motor systems are challenged, and several non-motor features are present in patients with PD and some even predate the emergence of the classic motor features. Methods: A total of 30 asymptomatic R1441G-LRRK2 mutation carriers (R1441G+) and 27 relatives non-carriers of the mutation (R1441G-) were enrolled in the study and completed a standardized neurological and neuropsychological protocol. From the original sample, forty-six subjects (27 R1441G+ and 19 R1441G-) underwent DAT imaging with 123I-2β-carbomethoxy-3β-(4-iodophenyl)-N-(3-fluoropropyl)-nortropane (123I-FPCIT) SPECT. Clinical evaluation comprised motor symptoms, sleep complaints, olfaction and autonomic evaluation. Neuropsychological assessment included general cognition evaluation, attention, working memory and executive function, memory, visuospatial function, and emotion recognition. Neuropsychiatric assessment evaluated depression, anxiety, personality characteristics and novelty-sensation seeking. Psychomotor and motor evaluation contained complex visuo-motor coordination test, finger tapping test and up and go test. Dat Spect study was performed and striatal nuclei ^{123}I-FP-CIT uptake ratios were obtained. Results: There were not differences between R1441G+ and R1441G- in age, sex and years of education. There were significant differences in the UPDRS-III score and Rem Behaviour Disorder diagnostic criteria and the ^{123}I-FP-CIT SPECT showed less striatal tracer binding in the R1441G+ group than in non-carriers indicating a significant nigrostriatal dopaminergic dysfunction in the former group. Between-group comparison revealed no differences in the autonomic symptoms evaluation, olfaction, cognitive testing, personality and novelty-sensation seeking evaluation and in the motor and psychomotor skills assessment. Within the R1441G+ group, those with UPDRS-III= 0 were significantly younger and showed higher scores on executive function scale and lower nigrostriatal dopaminergic dysfunction than R1441G+ with a UPDRS-III>0. We observed a correlation (controlling for age) between some phsychomotor and motor tests, Grooved Pegboard and the tapping tests and the ^{123}I-FP-CIT SPECT uptake. There was also a strong correlation between age and the striatal nuclei ^{123}I-FP-CIT uptake ratios in the R1441G+ group. We found that the striatal uptake ratio decreases in a nonlinear way, objectifying a cut-off point about 52-56 years for the different striatal nuclei. Conclusions: R1441G mutation carriers have subtle bradykinetic features and RBD as only prodromal symptoms and a nigroestriatal denervation that not observed in relatives non carriers of this mutation. An association between the dopaminergic deficit with some psychomotor tests and age with an acute decrease at age 52-56 is observed suggesting that R1441G mutation carriers with these abnormalities and age could theoretically be at higher risk to develop PD. Thus, this profile of patients might represent prodromal PD in our cohort.

Title

APOE gene cluster and Alzheimer disease in the oldest-old.

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INTRODUCTION Currently there are notable differences in the aging of individuals of modern societies. While some enjoy a long healthy aging, others develop neurodegenerative diseases, such as Alzheimer's disease (AD). Environmental factors are critical, but clear evidence points to an underlying genetic cause, and much interest is focused on identifying genes associated with healthy cognitive aging. In addition, it has recently been postulated that longevity genes might also be neuroprotective. The aim of this study was to assess whether certain genetic variants can be associated with neuroprotective effect in aging. MATERIAL AND METHODS To identify such variants we developed a sequential three-step procedure. Firstly, we conducted an association study with individuals age ninety and older (demented and non-demented), AD patients and control subjects. For the purpose we analyzed sixty-four tagging SNPs distributed in thirteen candidate genes FOXO3, SIRT1, TOMM40, APOE, PICALM, COMT, CETP, CLU, CR1, IL-6, PCK-1, ZNF224 and ACE by Taqman Open Array. Secondly, we analyzed thirty tagging SNPs included in the TOMM40-APOE/C1/C4/C2 gene cluster by High Resolution Melting and Taqman assays on the same population sample. Finally, we selected clusters of samples to be sequenced in an enriched pool of target APOE gene cluster using Haloplex system and Next-Generation Sequencing to detect rare genetic variants. RESULTS No significant differences were observed between demented and non-demented nonagenarians in the candidate genes. Compared with AD and control subjects, the risk genotype APOE (E4, E4) is less frequent in nonagenarians, while some tagSNPs in FOXO3 and SIRT1 are overrepresented. The APOE gene cluster analysis shows underrepresented haplotype blocks and a frequency in tagSNP sets related to AD risk in the nonagenarian group. We also found that nonagenarians seem to carry genetic variants in the APOE gene cluster that could potentially confer neuroprotection. CONCLUSION APOE gene cluster is a cornerstone in AD risk and longevity, especially the nonagenarian samples have a genetic endowment that may confer less susceptibility to AD, and thus could be implicated in neuroprotection.

Title

Natalizumab therapy normalizes deregulated microRNA expression in multiple sclerosis patients.

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Multiple sclerosis (MS) is a common inflammatory and degenerative disease causing neurological disability in young adults\l ". MS is considered an autoimmune disease in which peripheral activated lymphocytes cross the blood-brain barrier to enter the central nervous system (CNS) and promote an inflammatory event\l ". As a consequence, demyelination and axonal damage occur leading to neurological disability\l ". In the last years, new drugs have been approved for MS therapy including several monoclonal antibodies such as natalizumab. It binds to the α4 subunit of integrins expressed in activated T cells and other mononuclear leucocytes, then blocking the adhesion to the endothelial cells and inhibiting the migration of leucocytes into the CNS. Gene expression profiles in peripheral blood cells have revealed that the treatment influence a subset of genes related to T and B-lymphocytes, neutrophils and erythrocytes functions. However, little is known about the effect of the therapy in microRNAs, which have been found deregulated in MS patients. In the present work we characterize microRNA expression profile during natalizumab therapy in 19 MS patients and three healthy donors as a control group. Natalizumab is administered intravenously every four weeks, and blood samples were collected in PAXgene tubes at baseline and monthly during one-year therapy prior to each drug infusion. Expression of 754 microRNAs was analyzed at three timepoints (baseline (t0), after six month (t6) and after one year (t12)) by quantitative PCR and TaqMan probe technology in an OpenArray platform. Relative expression was calculated with 2^-DDCT method taking the healthy control group as reference. We detected 183 (24%) microRNAs in 80% of the samples and a repeated-measures ANOVA test revealed 53 miRNAs having differential expression between either of the timepoints ($p<0.05$). Three of them passed false discovery rate correction (FDR >< 0.05): let-7c, miR-125a-5p and miR-642. Let-7c and miR-125a-5p were overexpressed at t0 comparing to controls, showing a decreasing trend towards the control state after six-month therapy and stable expression between t6 and t12. On the other hand, miR-642 exhibited an increasing trend, although the change in expression was not so evident. Validation experiments confirmed the normalization of altered expression levels of let-7c and miR-125a-5p after treatment (t-test $p=0.024$ and $p=0.001877$ respectively) and furthermore, this effect was detectable in let-7c even after the first natalizumab infusion (t1) ($p=0.0463$). Gene ontology analysis of let-7c and miR-125a-5p predicted targets resulted in several enriched terms related to post-transcriptional regulation of gene expression, microRNA biogenesis, signal transduction and filopodium assembly, among others. Thus, these two microRNAs might regulate some events of leucocyte migration by direct targeting members of these pathways or by targeting genes involved in post-transcriptional regulation and microRNA biogenesis machinery. In conclusion, we show with a time-series approach, how deregulated microRNAs in MS are normalized due to natalizumab therapy. Although in silico analysis give a hint of their function, further studies would be necessary to establish the biological role of these microRNAs in multiple sclerosis.

Title

TOR1A haplotype study in primary dystonia

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Dystonias are a group of movement disorders characterized by involuntary muscle contractions causing twisting movements and abnormal postures. Etiological classification of dystonias include: primary pure dystonia, primary plus dystonia, primary paroxisomal dystonia heredodegenerative dystonia and secondary dystonia. In this work, we have focused on primary pure dystonia, whose most remarkable clinical feature is torsion dystonia. The main genetic cause of early-onset primary dystonia is the GAG deletion on exon 5 of TOR1A (DYT1) gene but it shows a very low penetrance, around 30%. Most recently, THAP1 gene mutations have been involved as cause of DYT6 dystonia, an autosomal dominant primary form with about 60% penetrance, in the Amish–Mennonite population. Many groups have carried out association studies that revealed the presence of haplotypes associated to DYT1 dystonia. The aim of this work was to carry out an association study using five TOR1A common SNPs (rs13283584, rs3842225, rs1182, rs11787741 and rs2296793) in a sample of 60 primary dystonia patients and 50 controls. Genotyping for each SNP was carried out using sequencing approach and haplotype analysis was performed using the Haploview 4.2 package software. All the patients were negative for GAG deletion in TOR1A and for mutations in THAP1 gene. All five SNPs fit Hardy-Weinberg equilibrium and were in linkage disequilibrium. Three different haplotypes were inferred, but none of them showed statistical significant differences between patients and controls. The most frequent one was “CCCTG” with frequencies of 0.73 and 0.66 respectively; the second haplotype was “TdelACA” with frequencies of 0.217 and 0.320. The less frequent haplotype was TCCTG, with frequencies values lower than 0.020. We did not find a significant association between this five SNPs haplotypes and primary dystonia in our population.

Title

Relation between cognitive reserve and cognitive functions in Parkinson's disease

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Introduction Parkinson's disease has been traditionally considered a motor disorder but nowadays is known that is also related to impairment in cognitive functions, such as executive functioning, memory, attention, processing speed and visuospatial ability. In addition, in the last years special interest has been focused on the role of cognitive reserve and its capacity to slow the development of cognitive deficits that are produced by neurodegenerative diseases. The cognitive reserve is defined as brain ability for using preexisting cognitive resources in order to support bigger neuropathology before the disease appearance. The objective of this study is to find evidences of a positive correlation between a higher cognitive reserve and a better development of cognitive functions in Parkinson disease subjects with mild cognitive impairment (MCI). Methods We recruited 36 patients with Parkinson disease and mild cognitive impairment (MCI): 23 men and 13 women, aged from 53 to 78 years (mean age = 69 years). We tested the cognitive reserve with the cognitive reserve questionnaire and the different cognitive functions were tested with the following neuropsychological tests: Mini-Mental State Examination (MMSE); Word Accentuation test (TAP) and Pseudoword Reading test (Prolec); Brief test Attention (BTA), Digit Span from WAIS, the Salthouse test, Stroop test, Trail Making test (TMT-A and TMT-B) Verbal Fluency test (FAS), Hopkins Verbal Learning test (HVLT), Visual Memory Brief test (TBMV), Boston Naming test, Visual Object and Space Perception test (VOSP) and the Watch test (copy and order). Results There are significant and positive correlations between the cognitive reserve questionnaire and most of the neuropsychological tests. The results of this research show that the sustained ($r=0.39$, $p<0.01$), selective ($r=0.528$, $p><0.01$) and alternating attention ($r=-0.454$, $p><0.01$), processing speed ($r=0.475$, $p><0.01$), semantic verbal fluency ($r=0.587$, $p><0.01$) and fonetic verbal fluency ($r=0.548$, $p><0.01$), naming ability ($r=0.52$, $p><0.01$), immediate memory ($r=0.518$, $p><0.01$), working memory ($r=0.563$, $p><0.01$), verbal learning ($r=0.496$, $p><0.01$) and verbal recognizing ability ($r=0.452$, $p><0.05$), visual learning ($r=0.613$, $p><0.01$) and visual recall ability ($r=0.556$, $p><0.01$), visuo-perceptive ($r=0.443$, $p><0.01$) visuo-spatial ($r=0.336$, $p><0.05$) and visuo-constructive abilities ($r=0.43$, $p><0.01$) are related with a better cognitive reserve. Conclusion There is a clear and significant relationship between cognitive reserve of Parkinson's disease patients and their cognitive functioning. Indeed, the higher the cognitive reserve the better the cognitive performance in PD patients with MCI. These results show the importance of developing good cognitive habits to reduce the impact of neurodegenerative diseases in

Title

Autonomic nervous system in Parkinson ´s disease

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Autonomic nervous system (ANS) disorders are common in patients with Parkinson's disease (PD) and can precede the appearance of motor symptoms [1,2]. Myocardial MIBG scintigraphy and non-invasive cardiovascular autonomic function tests can evaluate the severerity and distribution of autonomic failure, even subclinical dysautonomia. The aim of this study was to compare autonomic function in idiopathic PD and genetic PD: symptomatic carriers of the LRRK2 mutation, symptomatic and asymptomatic carriers of the E46K alpha-synuclein gene (SNCA) mutations and park2 mutation symptomatic genetic carriers. Material and Methods: Autonomic function tests were performed in thirty one patients. Six are E46K mutation carriers, four of whom were symptomatic (ages: 46, 59, 52 and 28-years) and two were asymptomatic carriers (ages: 52 and 29 years). Autopsy studies were performed on an additional two symptomatic carriers not eligible for autonomic testing. Myocardial tissue sections removed from the three autopsied cases (2 E46K, 1 park2) were subjected to routine histological staining and immunohistochemical processing with monoclonal antibodies against tyrosine hydroxylase and alpha-synuclein. We studied also 12 carriers of the LRRK2 mutation (6 G2019S and 6 R1441G), 4 Park2 mutation carriers and 13 with iPD. Autonomic function test consist on blood pressure and heart rate monitoring during head up tilt, valsalva maneuver and deep breathing. We also recorded of sympathetic skin response (SSR) , cardiac MIBG scintigraphy and plasma levels of vasopressin and norepinephrine Results Both the four symptomatic and the older asymptomatic carrier of E46K mutations reported abnormalities in the SCOPA questionnaire and had markedly diminished cardiac MIBG uptake. Plasma norepinephrine in the supine and tilted positions was normal in all subjects. Only one E46K mutation carrier had significant orthostatic hypotension. There was a complete absence of tyrosine hydroxylase immunostaining in the myocardium of the two autopsied cases. Three of the patients with iPD and one of the LRRK2 carriers had orthostatic hypotension. Arterial pressure "overshoot" during phase IV of Valsalva manoeuvre was less pronounced in patients with iPD. MIBG late myocardial/ mediastinal uptake ratios were higher in LRRK2 mutation carriers then iPD and E46K mutation carriers. The MIBG scyntigraphy, autonomic functions tests and myocardium study of Park2 mutation carriers showed ANS mild abnormalities. Discussion: We have found imaging and histological evidence of cardiac sympathetic denervation in symptomatic and asymptomatic carriers of the E46K alpha-synuclein gene mutation The sympathetic denervation appears to be organ-specific, with selective affectation of the heart given that plasma norepinephrine levels and blood pressure were normal. Carriers of the LRRK2 and park2 mutations had less autonomic impairment than those with iPD as shown by higher cardiac MIBG uptake and less impairment of autonomic non-invasive tests These findings are notably different from observations in patients with mutations in the alpha-synuclein gene, in which the degree of sympathetic denervation is higher than expected, compared to idiopathic PD patients with no known genetic mutations. The potential variability in pleomorphic histological features in patients with LRRK2 mutations may explain the clinical findings to some extent.

Title

Impairments of Different Memory Processes in Patients with Parkinson's Disease Without Dementia

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Introduction Parkinson's Disease (PD) is characterized not only by motor symptoms such as tremor, rigidity, and bradykinesia, but also causes impairments in different cognitive functions. One of the most studied functions is the memory, but still there are many issues in this area that remain unanswered. Therefore, this research will focus on going into detail about deficits of different specific processes (encoding / learning, retrieval, recognition) involved in memory, and figure out which one is specifically responsible for the memory impairment in PD. Method The study involved 24 patients with PD and 24 control subjects matched according to gender, age and years of education. To assess verbal memory was used Selective Reminding Task (SRT) which is a list composed of ten words. Another test to assess short-term memory and processing speed has been the WAIS-R Digit and Salthouse respectively. Results Among the most important results we mention that there is a significant difference between the two groups in terms of the ability to learn ($p = 0.03$), but once the words are learned, patients with PD are not different from controls regarding delayed free recall ($p = 0.27$) and recognition ($p = 0.25$). Conclusions Memory deficits in PD is attributable to the learning process and the poor use of encoding strategies and not so much to the retrieval capacity as traditionally has been considered. Key words: Parkinson's Disease, memory impairment, Selective Reminding Task.

Title

Evaluation of the effect of norquetiapine on extracellular monoamine concentrations in rat locus coeruleus and prefrontal cortex: comparison with reboxetine and citalopram

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Quetiapine is an atypical antipsychotic with antidepressant efficacy. This antidepressant effect may be related in part to its metabolite norquetiapine (formed in humans but not in rodents) which inhibits the noradrenaline (NA) transporter. The aim of this study was to compare by in vivo microdialysis the effects of norquetiapine with those of the selective NA reuptake inhibitor (NaRI) reboxetine and the selective serotonin (5-HT) reuptake inhibitor (SSRI) citalopram on extracellular NA and 5-HT in the locus coeuruleus (LC), the main noradrenergic source of noradrenergic innervations in the brain, and in the prefrontal cortex (PFC). One microdialysis probe was implanted in the vicinity of the right LC and the other probe was implanted in the ipsilateral PFC of the rat (n=6-8/group). Approximately 20 h after probe implantation, animals were placed individually in a system to allow free movement and the probes were perfused with a modified CSF solution. Dialysates were collected every 35 min and NA and 5-HT were immediately quantified by HPLC with electrochemical detection. Three basal samples were collected previous administration of the different drugs. Maximal effects induced by each drug were evaluated by one-way analysis of variance (ANOVA). A low dose of norquetiapine (2 mg/kg s.c.) exerted a non-significant increase of NA in LC (139±58%; $p>0.05$) and a simultaneous decrease of NA in PFC (-48±6%; $p<0.05$). This effect in the PFC was abolished by local administration into the LC of the α2-adrenoceptor antagonist RX821002 (1 mM). Norquetiapine (4 mg/kg s.c.) increased NA in LC (213±62%; p><0.05) and PFC (203±8% p><0.05). Norquetiapine (2 and 4 mg/kg s.c.) induced a dose-dependent increase of 5-HT in LC (164±36% and 290±6%, respectively; p><0.05) and in PFC (197±28% and 241±13%, respectively; p><0.05). Reboxetine (3 and 5 mg/kg i.p.) increased NA in LC (174±7% and 246±21%, respectively; p><0.05) and in PFC (148±4% and 210±7%, respectively; p><0.05) but no changes in 5-HT were observed. Citalopram administration (5 mg/kg i.p.) did not modulate NA in either area. A higher dose of citalopram (10 mg/kg i.p.) exerted an increase of NA in LC (149±12%; p><0.05) and a simultaneous decrease of NA in PFC (-48±4%; p><0.05). In contrast, at 5 and 10 mg/kg i.p., citalopram evoked large increases of 5-HT in both areas (LC: 277±25% and 945±195%; p><0.05; PFC: 399±56% and 927±293%, respectively; p><0.05). The acute antidepressant effect of reboxetine and citalopram, as expected, seems to be mediated by a selective increase in NA and 5-HT, respectively. Norquetiapine presents a typical profile of a NaRI, showing a decrease in NA in PFC at low doses and an increase at higher doses. In addition, norquetiapine seems able to simultaneously increase NA and 5-HT in terminal areas. The increase in 5-HT concentration induced by norquetiapine could be related to its serotonin 5-HT2 antagonist and 5-HT1A partial agonist properties. The enhancement of noradrenergic and serotonergic transmission in the LC and PFC induced by norquetiapine might be relevant for the antidepressant action of quetiapine in mood

Title

G protein activation induced by hallucinogenic vs non hallucinogenic 5-HT2A receptor agonists in postmortem human brain

Author(s)

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Studies in rodents have shown serotonin 5-HT2A receptor (5HT2AR) hallucinogenic agonists (such as LSD and DOI) are able to activate Gi/o together with Gq/11 proteins, while non-hallucinogenic 5HT2AR agonists (such as lisuride and ergotamine) just activate Gq/11 proteins, which correspond with the canonical and preferred signalling pathway for this receptor [1]. This differential response has become a remarkable hallmark of 5HT2AR, but until now, there were no data about the role of each specific G protein subunit subtypes in this response in human brain tissue. The existence of agonist-specific receptor conformational states that preferentially engage distinct cellular pathways has been termed agonist-directed trafficking of signalling or biased agonism. The use of [35S]GTPγS binding stimulation followed by immunoprecipitation using specific antibodies allows the functional study of agonist-mediated activation of different Gα protein subtypes. This approach was used to evaluate biased agonism as the central signalling mechanism that distinguishes between hallucinogenic and non-hallucinogenic 5HT2AR agonists in postmortem human brain prefrontal cortex. Brain samples from subjects with premortem absence of neuropsychiatric disorders were obtained at autopsy and membrane P2 fractions of tissue homogenates were prepared for the assays. Specific antibodies against Gαi1, Gαi2, Gαi3, Gαo and Gαq/11 protein subtypes were used. The hallucinogenic 5HT2AR agonist drugs (±)DOI, DOB, TCB-2 and LSD (10-5 M) stimulated the [35S]GTPγS binding to Gαq/11, Gαi1, Gαi2, and Gαi3 proteins (range Emax 113±3% to 152±5%, n=4-22). These stimulations were significantly reversed by the 5HT2AR antagonists ketanserin and altanserin (10-5 M) in case of Gαq/11, Gαi1 and Gαi3 proteins. 5HT2AR antagonists did not fully reverse the effects of LSD, being the remaining LSD stimulation component sensitive to the serotonin 5HT1A receptor antagonist WAY 100635 (10-5 M). TCB-2 and LSD (10-5 M) also stimulated Gαo proteins (range Emax 105±1% to 145±4%, n=4-9). This [35S]GTPγS binding stimulation to Gαo induced was not inhibited by 5HT2AR antagonists, but was reversed by WAY 100635. All the stimulations for the different G protein subunits and drugs were abolished by the non-selective serotonin receptor antagonist methiothepine (10-5 M). In contrast to these previous findings with hallucinogenic 5HT2AR agonists, the non-hallucinogenic 5HT2AR agonist drug lisuride (10-5 M) stimulated [35S]GTPγS binding to Gαq/11, Gαi1, Gαi2, Gαi3, and Gαo proteins (range Emax 133±4% to 155±7%, n=9-15). Noteworthy, only the coupling to Gαq/11 proteins was significantly antagonized by ketanserin and altanserin, whereas the stimulation by lisuride of [35S]GTPγS binding to other Gα protein subtypes was sensitive to the serotonin 5HT1AR antagonist WAY 100635. Taking together, these findings support a mechanism of biased agonism through which hallucinogenic and non-hallucinogenic 5HT2AR agonists induce ligand-specific receptor conformations that result in differential activation of G protein-dependent signalling. These results also suggest an involvement of the inhibitory Gαi1 and Gαi3 protein subtypes in the psychoactive properties induced by hallucinogenic 5HT2A receptor agonists. In conclusion, is possible to design ligands with specific pathway activation that could promote 5HT2A-R-mediated therapeutic actions without eliciting associated side effects. [1] González-Maeso et al. Hallucinogens recruit specific cortical 5-HT2A receptor-mediated signaling pathways to affect behaviour. Neuron 2007;53:439-452.

Title

Neurochemical and behavioural characterization of the metabotropic glutamate 2 receptor knock-out mice

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A functional heterocomplex between serotonin 5-HT2A receptors (5HT2AR) and the group II metabotropic glutamate receptors mGlu2R has been described in brain. 5HT2AR agonists need of the mGu2R to induce their hallucinogenic properties. In this context, mGlu2R could represent a new drug target for the treatment of schizophrenia. Moreover, a dysregulation of the 5HT2AR-mGlu2R complex is proposed to be present in brain cortex of schizophrenic subjects. Administration of NMDA antagonists represents a model of psychosis-like behavior because it reproduces many sensory and cognitive deficits seen in schizophrenics. These effects are partially blocked by antipsychotic drug such as clozapine, a dopamine and 5HT2AR antagonist drug. The aim of this study was to evaluate the functional role of mGlu2R on the psychosis-like behavior and the modulation of neurotransmitter release in prefrontal cortex (PFC) of mGlu2R knock-out mice (mGlu2R-/-). Dopamine (DA), noradrenaline (NA) and serotonin (5-HT) concentrations were obtained by in vivo microdialysis and measured by HPLC. Simultaneously, locomotor activity was recorded. The sensorimotor gating process, highly altered in schizophrenia, was evaluated by the prepulse inhibition of startle reflex (PPI) technique. Data were analyzed by using unpaired Student's t-test, one-way or two-way ANOVA tests for repeated measures. Under basal conditions, mGlu2R-/- mice exhibited a significant decrease in the PPI when compared to wild-type animals (mGlu2R+/+) ($F[1,36]=7.37$, $p<0.05$). The deficits of PPI were not abolished by previous acute treatment with the antipsychotic clozapine (1.5 mg/kg, i.p.). No differences in PFC basal values of DA and 5-HT concentrations between mGlu2R+/+ and mGlu2R-/- mice were observed. In contrast, mGlu2R-/- exhibited higher NA basal values ($t=4.57$, $p><0.01$) than mGlu2R+/+ mice. The NMDA antagonist MK-801 increased DA and 5-HT in mGlu2R+/+ (Emax=425±64%; Emax=126±9%, respectively) and in mGlu2R-/- (Emax=439±64%; Emax=149±13%, respectively) mice, without differences between groups. MK-801 increased NA in mGlu2R-/- (Emax=156±19%) but not in mGlu2R+/+ mice ($FI[14,210]=2.54$, $p><0.01$). The mGlu2R-/- mice showed a deficit in basal motor activity when compared to mGlu2R+/+ ($t=5.57$; $p><0.0001$). Administration of MK-801 increased motor activity both in mGlu2R+/+ (Emax=203±24 counts) and in mGlu2R-/- mice (Emax=146±18 counts) ($FI[14,420]=2.19$, $p><0.01$). These results demonstrate that the lack of mGlu2R evokes profound alterations in sensorimotor gating processes. These PPI alterations represent a psychosis-like behavior. The present data also show that mGlu2R is necessary for the antipsychotic effect of clozapine. Therefore, the 5HT2AR-mGlu2R complex seems to be necessary for the antipsychotic activity of 5HT2AR antagonists. In addition, the lack of mGlu2R could facilitate the activation of noradrenergic pathways associated with NMDA antagonism and promote NA release in the PFC. Further studies are necessary to characterize the phenotype of the mGlu2R-/- mouse and its interest in psychosis-like

Title

Expression and density of serotonin 2A receptor in postmortem prefrontal cortex of schizophrenic subjects

Author(s)

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Several neuroimaging and postmortem studies have reported alterations of the serotonin 2A receptor (5-HT2AR) in the brain of schizophrenic subjects. However, striking differences have been obtained in relation to the high diversity of methodologies used and certain intrinsic confounding factors. In this sense, the aim of the present study was to evaluate the 5-HT2AR mRNA and protein expression in the prefrontal cortex (BA9) of schizophrenic subjects (n=45) and controls (n=45) matched by gender, age and postmortem delay. Quantitative real time PCR assays were performed to determine the 5-HT2AR mRNA expression. Western blot experiments in total homogenates and [3H]ketanserin (10 nM) binding assays in membrane enriched fractions were carried out to assess 5-HT2AR protein expression. Displacement curves of [3H]ketanserin binding (2 nM) by the 5-HT2AR agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) were performed in order to delineate the high-affinity state of 5-HT2AR. Subjects who gave negative results for antipsychotic drugs in the postmortem toxicological screening were considered as antipsychotic (AP)-free (n=29). To evaluate the potential effect of suicide completion on [3H]ketanserin binding, an independent cohort of suicide victims (n=13) —with a variety of psychiatric diagnoses different from schizophrenia— and matched controls was also included. Decreased 5-HT2AR mRNA expression was observed in AP-treated schizophrenics (-37±9%; n=9; $p<0.05$) compared to matched controls, whereas no change was shown in AP-free subjects (n=18). Immunodetection of 5-HT2AR was found to be unaltered in both AP-free and AP-treated schizophrenic subjects compared to their matched controls. [3H]Ketanserin binding (10 nM) was increased in AP-free schizophrenics (+28±11%; n=29; p><0.05), but not in AP-treated ones (n=16) or in suicide victims. Notably, and in comparison to matched controls, an increase in the fraction of high-affinity sites for DOI displacing [3H]ketanserin binding was found in both AP-free (12±1% vs 6±1%; p><0.001) and AP-treated schizophrenics (9±1% vs 5±1%; p><0.001). However, and importantly, these changes with the agonist DOI were significantly more pronounced in AP-free as compared with AP-treated schizophrenics (p><0.05). [3H]Ketanserin binding correlated negatively with age in schizophrenic, suicide and control subjects. This effect of aging was more pronounced in AP-treated (slope=-8.0±2.8) than in AP-free (slope=-3.6±2.6) or control subjects (slope=-2.3±0.8). These results suggest that the active conformation of 5-HT2AR is upregulated in schizophrenia, a modulation that tends to be reversed by chronic treatment with antipsychotic drugs. Progressive aging makes the identification of the upregulation more difficult in schizophrenic subjects under treatment. The lower expression of 5-HT2AR in older subjects may also underlie an association between fewer positive symptoms and increased

Title

NGF and BDNF as markers of long-term functionality in first-episode psychosis patients with / without cannabis comsumption

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INTRODUCTION One hypothesis for the pathogenesis of schizophrenia is the abnormal early development of the tissues of the CNS. Neurotrophins are a group of dimeric proteins envolved in the development of the CNS of mammals, so it has been considered that they can be candidates to be markers of the disease. In fact decreased levels of the neurotrophins BDNF and NGF were found in incipient psychosis and in patients with schizophrenia NGF levels were altered in patients with chronic cannabis comsumption. Cannabis use is associated with amotivational symdrom , in addittion to trigger psychosis in vulnerable subjects. If the neurotrophins were associated to the functionality of the patient, the stop of cannabis comsumption could explain the improvement of the disease in the short term, while if comsumption continues the patient would worsen crearly. OBJETIVES The objective of the study is to compare the levels of the neurothrophins BDNF and NGF in patients with a FEP with and without cannabis comsumption with a control group and to explore the relationships of these neurothrophins with the long-term functionality of patients. METHODS We included 35 FEP patients no cannabis consumers, 31 FEP patients cannabis consummers and 68 healthy controls. Blood plasma samples were taken at admission, 1 month, 6 months and 1 year later in FEP patients and only at baseline in healthy controls to assess NFG and BDNF levels. At baseline evaluation time and 1 year after the illness onset, patients functionality was evaluated with the Strauss Scale. Using a linear regression model we evaluated the influence of peripheral levels of NGF and BDNF in the functionality of these patients along the follow-up. RESULTS Basal NGF levels were significantly related to the annual score on the Strauss scale ($p = 0.010$) in the FEP patients cannabis consumers, not the case in nonusers. A higher level of basal NGF, a better functionality, 1 year after the first episode of psychosis. In the other hand, baseline level of BDNF gave a statistically significant result and also directly related to the functionality 1 year after the debut of the disease ($p = 0.044$) in the FEP patients non cannabis users. CONCLUSSIONS The baseline level of NGF could serve as a prognostic marker of long-term functionality for FEP patients cannabis consumers while the baseline level of BDNF would be useful as a prognostic marker of functionality in FEP patients without cannabis consumption.

Title

The Brain Derived Neurotrophic Factor as mediator of the cognitive reserve in first psychotic episode.

Author(s)

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Introduction: Cognitive deficits have been described in patients with psychoses. The Brain-derived neurotrophic factor (BDNF) promotes growth and maintenance of connections and participates in plasticity mechanisms such as long-term potentiation and learning. BDNF is highly expressed in the cerebral cortex and hippocampus, brain areas known to regulate functions such as memory and emotion (1) Usually, under this cognitive performance there are neurobiological underpinnings that might underlie these changes (2). In this study, we analyse the relation between the BDNF, cognitive performance in patients with recent psychosis. Methods: Forty five patients with a FEP were selected from the Basque Country catchment area. The diagnoses were made using the Structured Clinical Interview for Diagnostic (SCID-I) and met the DSM-IV criteria for psychotic disorder. Plasma BDNF levels were measured using the BDNF Sandwich ELISA Kit during the acute episode and after six months of treatment and a year follow-up. All patients were assessed clinically three times over a year using the following scales: PANSS, GAF. Also, a battery of cognitive tests (Wechsler Memory Scale and Wechsler Adult Intelligence Scale, WAIS-III) was applied six months after the acute episode when the patients were clinically stabilized. Results: We observed a positive association between BDNF levels after six months of treatment and five cognitive scales: (ß=0.468), digit-symbol coding (ß=0.397), logical memory learning curve (ß=0.559), verbal paired associates learning curve (ß=0.409) and verbal paired associates II (ß=0.382). The pharmacological treatment and the drug use or abuse is not related with the cognition, only the BDNF and the IQ of the patients could be a protective factor for the function and cognition. There was a significant positive correlation between plasma BDNF levels and cognition and GAF ratings across the study ($r=0.30$; $P<0.05$) Conclusions: Our results suggest that BDNF is associated with cognitive impairment seen after the psychotic episode. The levels of BDNF play an important role in the brain cognitive reserve especially in reasoning, processing speed, learning capacity and delayed memory. The cognition is related to functionality of the patients and is a predictive factor of the prognosis of the illness. It can be hypothesized that treatments which increase BDNF levels could be useful for improving cognition, although this hypothesis must be tested in a long-term follow-up study. Further investigations of the role of this neurotrophin in the symptoms associated with onset of psychosis are warranted. These neurotransmitters could be offer us a relevant key for the pharmacological treatment in the psychotic diseases. 1. Kapczinski F, Frey BN, Kauer-Sant'Anna M, Grassi-Oliveira R: Brain-derived neurotrophic factor and neuroplasticity in bipolar disorder. Expert Rev Neurother 2008, 8(7):1101–1113.
2. Ruiz de Azua S, Matute C, Stertz L, Mosquera F, Palomino A, de la Rosa I, Barbeito S, Vega P, Kapczinski F, González-Pinto A. Plasma brain-derived neurotrophic factor levels, learning capacity and cognition in patients with first episode psychosis. BMC Psychiatry. 2013 Jan 15;13:27.

Title

Behavioural effects of RS 67333 in the depression/anxiety mice model of bilateral olfactory bulbectomy

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Olfactory bulbectomy is a valuable animal model to assess potential antidepressant drugs due to its predictive validity. 5-HT4 receptor agonists have been reported to exhibit a faster onset of antidepressant and anxiolytic-like actions but their behavioral effects in animal models of chronic depression/anxiety have been poorly evaluated. In this study, we have compared the behavioral effects induced by the 5-HT4 receptor agonist RS 67333 (1.5 mg/kg/day, i.p.) and fluoxetine (SSRI, selective inhibitor of serotonin reuptake, 25 mg/kg/day, oral) in olfactory bulbectomized (OB) mice. Subchronic administration of RS 67333 for only 1 week reduced OB-induced locomotor hyperactivity in the open field (OF) test (peripheral distance=15.7±1.6m vs 19.3±1.9 in OB-vehicle). Interestingly, 7day treatment with RS 67333 also induced a significant anxiolytic effect in OB mice as evidenced by an increase in the OF central activity (distance=2.9±0.3m vs 1.5±0.3m in OB-vehicle, $p<0.01$; entries= 13.6±1.5 vs 8.0±1.2 in OB-vehicle, p><0.01). This anxiolytic effect was also present in sham-mice (central distance=3.8±0.4m vs 2.0±0.4m in sham-vehicle, p ><0.01; number of entries= 17.0±0.7 vs 9.1±1.9 in sham-vehicle, p><0.01). In addition, OB mice also exhibited a decrease in the sucrose preference test (% sucrose intake/total= 63.0±3.1), reflecting and anhedonic state, that was significantly reversed after 7 days of RS 67333 administration (% sucrose intake/total=73.0±3.5; p><0.05 vs OB-vehicle). However, 28day treatment with fluoxetine was needed to observe antidepressant efficacy either in the OF locomotor activity (peripheral distance= 12.5±1.1m; p><0.01 vs OB-vehicle) and in the sucrose preference test (% sucrose intake/total= 81.1±2.1%). Our findings indicate that the partial 5-HT4 receptor agonist RS 67333 exhibits antidepressant and anxiolytic actions with a faster onset of action than the prototypical SSRI fluoxetine, what it could be reflecting differences in their neurochemical and/or molecular mechanisms of

Title

Measuring plasma cotinine levels in patients with first episode psychosis

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Intro and purpose of the study: Patients with psychotic disorders exhibit extraordinary high rates of tobacco use compared with controls and patients with other mental disorders. In light of this, researchers have tried to understand the underlying causes of this phenomenon, resulting in different theories, such as the self-medication hypothesis. However, since traditional information obtained by self-report regarding smoking status can be misleading, biochemical validation seems to represent a more objective alternative of quantifying tobacco consumption with a higher specificity[1]. As cotinine levels detected in plasma are considered steadier above the ones detected in any other fluid such as urine or saliva, for this study, a technique was developed for the efficient determination of plasma cotinine levels. The purpose of the present study was to validate the technique in a sample of patients with a First Episode Psychosis (FEP). Methods: A total of 84 patients with FEP were originally enrolled in the study, of whom just the ones who completed the 12 month follow-up and had plasma cotinine measures were selected (n=36). Cotinine levels were quantified by using high-performance liquid chromatography with tandem mass spectrometric (LC/MS/MS) detection methods. A mixed-effects linear regression model was performed in STATA 12.1. Dependent variables were cotinine levels at months 2, 6 and 12, and the covariates entered into the model were age, gender, number of cigarettes per day, weight, diagnose (schizophrenia vs. other FEP), and antipsychotic medication dose measured in chlorpromazine equivalents. The variance-covariance matrix for cotinine levels was estimated by the model. Results: In the sample analyzed the mean age was 27.74 (SD=8.36). Of the variables under study, just the following ones turned out statistically significant: cigarettes per day (β=5.36; SE=1.75; p=0.002); gender (β=-160.29; SE=37.78; p<0.001); and antipsychotic dose (β=0.24; SE=0.087; p=0.007). Conclusions: Results are partly consistent with previous literature, since in Haley's study the authors did not find a significant relationship between cotinine levels and chlorpromazine equivalences[1]. Nevertheless, we can conclude that our study validates the recently created laboratory technique, given that the number of cigarettes per day was able to predict cotinine levels. Other factors such as gender and antipsychotic dose might also predict the dependent variable cotinine. The present study is the first which has analyzed plasma cotinine levels in patients with FEP. Studies with bigger samples are needed to confirm these results. In future research, we will try to replicate the results our team obtained in previous studies in which the different approaches to the self-medication hypothesis were analyzed[2], adding this time cotinine levels (as a biochemical marker of tobacco consumption) and their possible interactions with neuroleptic plasma levels and medication side effects. References: [1] Haley, N.J., Axelrad, C.M., Tilton, K.A., 1983. Validation of self-reported smoking behavior: biochemical analyses of cotinine and thiocyanate. Am J Public Health 73(10): 1204–1207. [2] Segarra, R., Zabala, A., Eguiluz, J.I., Ojeda, N., Elizagarate, E., Sánchez, P., Ballesteros, J., Gutiérrez, M., 2011. Cognitive performance and smoking in first episode psychosis: the self-medication hypothesis. Eur Arch Psychiatry Clin Neurosci 261(4),

Title

Relationship between negative symptoms and plasma levels of insulin-like growth factor 1 in first-episode schizophrenia and bipolar disorder patients

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Insulin-like growth factor 1 (IGF-1) is a 70-amino acid peptide, mainly produced by the liver, which plays an important role in human growth and development. IGF-1 crosses the blood-brain barrier and is thought to influence human brain development with roles ranging from neuroprotection following neuronal damage to neurogenesis, myelination, synaptogenesis and dendritic branching. Consequently, abnormal signaling of IGF-1 can influence neuronal differentiation and synaptic function leading to altered brain development and functioning. In addition, IGF-1 not only exerts its effects during neurodevelopment but also is an important antiapoptotic factor after brain damage. Lower IGF-1 blood levels were reported in antipsychotic-naïve schizophrenics several years after the onset of disease as well as in schizophrenic patients receiving clozapine. Thus, low levels of IGF-1 may underlie associations of markers of pre- and post-natal growth and development with schizophrenia. Here, we have analyzed the plasma level of IGF-1 in patients who suffered a first psychotic episode (FPE) and during a 1-year follow-up when patients were re-diagnosed with schizophrenia or bipolar disorder. We also studied if IGF-1 levels were related to clinical symptoms. 50 patients and 43 healthy controls matched by age, gender and educational level were selected from the Basque Country catchment area in Spain. Patient symptoms were assessed at the same disease stages using the Positive and Negative Symptoms Scale (PANSS), the Global Assessment of Functioning (GAF), the Hamilton Depression Rating Scale (HDRS21) and the Young Mania Rating Scale (YMRS). A statistically significant increase in the plasma levels of IGF-1 was found in the whole cohort of patients one month after FPE compared to matched controls (219.84 ng/ml vs 164.15 ng/ml; p=0.014), as well as in schizophrenia patients alone at that stage (237.60 ng/ml vs 171.60 ng/ml; p=0.039). This is in accordance with previous studies that found an increase in IGF-1 serum levels after short-term antipsychotic treatment. Plasma levels of IGF-1 did not change significantly after 1-year of antipsychotic treatment, which is in line with previous studies in rats and humans. In addition, negative symptoms in both groups of patients were positively correlated with IGF-1 levels both at FPE (β=0.521; p<0.001) and after 1 year (β=0.659; p=0.001), being patients diagnosed with schizophrenia the main contributors to this relationship. This finding is relevant to disease, as negative symptoms tend to be chronic and resistant to antipsychotic treatment as well as more frequent in patients with schizophrenia than in patients with bipolar disorder. These results indicate that there is a significant change in the plasma levels of IGF-1 at the initial stages of schizophrenia, and suggest that IGF-1 could have role in the pathophysiology of negative

Title

Studies of normalization and standarization of neuropsychological psychometric instruments in Spanish healthy adults.

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Introduction: The NORMACOG project aims to standardize a battery of neuropsychological tests used in neuroscience and clinical practice. Most of them assess cognitive functions relevant to the symptoms of neurological conditions for which we are not provided with well standardized norms. In this study, we included the TAP (Test de Acentuación de Palabras), PROLEC, MoCA, Taylor Complex Figure (TCF), Brief Visuospatial Memory Test (BVMT), Trail Making Test (TMT-A and TMT-B) and Grooved Pegboard Test (GPT). Therefore, the aim of this study is to provide normative data, adjusted by age and education for these instruments based on a Spanish sample. Method: We recruited a sample that consisted of 224 healthy participants from 18 to 81 years old (M=34.49; SD=16.642). The participants were distributed in six different age ranges. Results: We offer tables showing the accumulated frequencies which were removed from direct scores, then the ranges of percentiles and scalar scores. Age and education adjusted scores, are also provided by applying linear regressions and using the regression coefficient of each variable. Controlling the age ranges and years of education gives a higher reliability for the clinical and neuropsychological scores. Results provide evidence of the relevant influence of age and education for each test, leaving out the relevance of the gender. Conclusions: The current normative data provide clinically useful data for evaluating Spanish adults and making relevant clinical decisions in neurological conditions. Key words: Normative Data, Age, Education, TAP, PROLEC, MoCA, TCF, BVMT, TMT-A, TMT-B, GPT

Title

Long-term implications of oxidative stress damage during the first-episode of psychosis

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Purpose of the study. Oxidative stress supposes disequilibrium between pro-oxidant processes and the antioxidant defence system and will lead to a free radical attack of protein, DNA and lipids [1]. These processes have been reported to be involved in neurodegeneration [2], and in the pathophysiology of some severe mental illness such us schizophrenia [3]. Indeed, oxidative stress may disturb the important signal pathway in the cell called “reduction / oxidation” signalling that play an important role in the regulation of apoptosis and cell cycle. Oxidative stress has been linked to the pathophysiology of psychosis. It has been described that neurological symptoms are trait markers in schizophrenia and that some cognitive disturbs depend on neurological dysfunction which can affect the general functioning. Our objective was to assess the influence of oxidative stress at baseline in cognition, functionality and neurological symptoms at baseline and after 2 years, in a sample of early-onset psychotic patients. Method. The CAFEPS sample included 110 patients (aged 9-17) with a first psychotic episode. These patients were recruited from child and adolescent psychiatry units at six university hospitals located in Spain. We evaluated cognition, functionality and neurological symptoms in 110 patients, at baseline and after 2 years. The battery of neurocognitive tests was designed to assess five cognitive domains: global attention, speed of processing, working memory, learning and executive functioning, by combining selected individual measures from different tests. The Neurological Examination Scale (NES) was used to assess neurological symptoms and patient functioning was evaluated with the Global Assessment of Functioning Scales (GAF). We also determined at baseline their peripherical blood levels of total antioxidant status (TAS), glutathione (GSH), lipid peroxidation (LOOH) and the activities of the enzymes glutathione peroxidase (cGPx), catalase (CAT) and superoxide dismutase (SOD) Summary of Results. We found that, at baseline, patients who are worse in terms of neurological symptoms, also have a significantly lower antioxidant capacity. We don´t find this association with neurological symptoms at 2 years. At baseline we found no relationship between the variables of oxidative stress and the functionality of patients, but these variables appear to be associated with patient functioning after 2 year. Specifically a better antioxidant capacity (TAS and GSH) at admission is related with a better general functioning after 2 years. In patients we found that, at baseline, a higher level of TAS was associated with better attention, processing speed and memory. Taken the global cognition score, the model remains statistically significant. Indeed, we observe the same association between TAS at baseline and cognition domains measured after 2 years of the illness onset. Conclusions. A better antioxidant capacity at illness onset is related to a better cognitive functioning (baseline and after 2 years) and to a better general functioning after 2 years. Less neurological symptoms appeared at baseline in those patients with a higher TAS. [1] Young J et al. 2007. Biomarkers of oxidative stress in schizophrenic and control subjects. Prostaglandins Leukot Essent Fatty Acids 76:73-85. [2] Cui K at al. 2004. Role of oxidative stress in neurodegeneration: recent developments in assay methods for oxidative stress and nutraceutical antioxidants. Prog Neuropsychopharmacol Biol Psychiatry 28:771-99. Fendri C at al. 2006. Oxidative stress involvement in schizophrenia pathophysiology: a review. Encephale 32:244-52.

Title

Nicotine consumption predicts cognitive performance and clinical symptoms in Schizophrenia

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Introduction There are very few published studies about gender differences in patients with schizophrenia and his relationship with cognitive performance and clinical symptoms. Given that, smoking and the course of the disease differ between the sexes, this study sought to analyze gender differences in nicotine consumption, cognitive performance, and clinical symptoms in people with schizophrenia. Methods We recruited 144 patients with schizophrenia (99 males & 45 females) who reported smoking habitually. All patients were hospitalized at the time of evaluation. In both groups we evaluated nicotine consumption (FTND), clinical symptoms (PANSS), and cognitive performance on tasks of attention, memory, working memory, executive functions, and processing speed. We used stepwise regression analysis to assess the potential contribution of nicotine consumption to clinical symptoms and cognition in men and women separately. Results Nicotine consumption/dependence was very high in both groups (20 cigarettes/day) and there were not significant differences between sexes in nicotine consumption (t=1.157; p=.25). In general, this consumption was predictive of both cognitive performance and specific clinical symptoms. However, this predictive value was different for each gender. Regarding men, greater consumption predicted better executive functioning (β=-.515; p=.024), reduced disorders of volition (β=-.531; p=.019), and more hostility (β=.485; p=.035). In women however, greater consumption was associated with worse visual memory (β=-.629; p=.038) and a higher incidence of suspiciousness/persecution (β=.659; p=.027) and blunted affect (β=.012; p=.008). Conclusion Nicotine consumption/dependence was predictive of specific clinical symptoms and cognitive performance. However, gender differences mediated this association, as nicotine consumption improved both clinical symptoms and cognition in men, but was associated with worse cognitive performance and symptomatology in women. Therefore, our results only partially support the self-medication hypothesis in men.

Title

Social cognition predictor of functional outcome in schizophrenia

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Introduction Patients with psychosis show alterations in neurocognition and social cognition domains. These impairments have negative effects on their social life. It remains unclear; however, to what extend these domains represent independent cognitive domains. The purpose of this study was to investigate the relationship between these domains and the social outcome in patients with schizophrenia. Methods Twenty patients with schizophrenia were recruited according to DSM-IV criteria and clinical interview. All subjects underwent a full neuropsychological evaluation (for attention, memory, executive functions, visuospatial memory) and a functional outcome (Clinical Global Impression CGI and Personal and Social Performance PSP). Social cognition was evaluated several subcomponents such as emotion perception (Mind in the Eyes), theory of mind (FAUX PAS) and attribution style (Internal, Personal and Situational Attribution Questionnaire IPSAQ). Results Patients presented significantly low scores in several cognitive, social cognitive and functionality domains. A significant correlation was found between cognitive and social cognitive domains and different sub-items of the PSP scale and its total score. Specifically, regression analyses showed that total score of PSP scale was predicted significantly by attention (B=.50), assessed with the Continuous Performance Test (CPT), and internal positive attributional style (B=-.46). Attention explained up to 27% and attributional style 21% of the variance. Respect to the sub-scales of PSP, in one hand attention, assessed with the “Brief Assessment of Cognition in Schizophrenia (BACS) - Digit Sequencing” of Matrics, was a good predictor of the self-care functioning in PSP scale (B=-.48) explaining up to 24% of the variance. On the other hand, relational capacity was predicted by attention (B=.52), assessed with CPT, and with 17% of the variance; and internal positive attributional style (B=-.42), with 29% of the variance. Finally, visuospatial memory (B=-.57) and internal positive attributional style (B=.48), both predicted the social abilities explaining up to 56% of the variance. None of the variables included in the regression analyses predicted significantly behavior capacity. Conclusions Social cognition and some variables of neurocognition predicted functional outcome in our sample of schizophrenia patients. Attribution style was the stronger predictor of the functional capacity of the patients measured by psychiatrics with the PSP scale. Future studies should consider studying this variable as predictor of functional outcome in patients with schizophrenia.

Title

Do first episode psychosis patients smoke in an attempt to alleviate their clinical symptoms?

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Intro and purpose of the study: While tobacco consumption has decreased in the general population, prevalence in patients with schizophrenia is still alarmingly high (70%-90% vs. 25%-30% in the general population). The self-medication hypothesis constitutes a multidimensonal attempt to explain this phenomenon [1]. Of the three approaches considered by this theory, the present study explores the one that suggests that patients with schizophrenia would smoke to alleviate their positive and negative symptoms, since it is the approach that presents the smallest number of conclusive data so far. Methods: Patients with a first-episode psychosis and a diagnosis of a schizophrenia-spectrum disorder were evaluated at baseline, month 1, 6 and 12. Patients were grouped in two categories which remained stable throughout the study: non-smokers (0 cigarettes per day, n=80), and smokers (≥15 cigarettes per day; n=61). Symptoms were evaluated with the PANSS and compared between groups using a multivariate general linear model with gender as a covariate. Results: Significant differences between smokers and non-smokers with regard to sociodemographic data were not detected, except for gender (p

Title

New evidence on speech illusions and aberrant salience in psychoses

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The hypothesis regarding the psychosis continuum has gained support in these last decades. General population can show psychotic symptoms, including auditory hallucinations. One relevant aspect of these hallucinations is the emotional value, or salience, that the person attributes to them. This salience can be anomalous or aberrant, resulting in greater difficulties in interactions with their environment and perhaps even contributing to clinical psychosis. One previous study establishes a correlation between the intensity of the psychosis and the aberrant salience in psychotic patients and in the general population. The goal of this study is to explore this relationship. We have evaluated a group of patients with a first episode of psychosis (N=43) and a control group of healthy subjects (N=50) using a brief task developed to analyse the variation in the detection of speech illusion in neutral white noise. Socio-demographic, drug use, cognitive function and personal and family history data have been obtained. PANSS and the GAF scales were used for measuring psychopathology in psychotic patients, and sub-clinical psychotic symptomatology was measured through CAPE and SIS-R scales. Between groups, significant differences were described in relation to IQ, years of education and socio-economic class. A higher rate of speech illusion was obtained in the patients (46.5% versus 20%, $p<0.01$; OR 22.62). This difference was maintained independent of other factors of confusion (educational level, IQ , socioeconomic status, age and sex) that were analysed. The possibility of speech illusion was associated with a greater positive symptomatology in patients and there was a relation with the intensity of positive symptoms (PANSS scale), but not in the healthy subjects (CAPE and SIS-R scales). We did not observe any differences between the patients and the control subjects in the prevalence of aberrant salience. Further exploration is necessary in this area in order to resolve these

Title

A multidisciplinary approach to the catatonic syndrome

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The catatonic symptoms have been established as a syndrome which can have multiple etiologies, both medical and psychiatric. Toxic, pharmacological and psychiatric precipitant factors have also been described. A multidisciplinary approach is required to avoid underdiagnosis. Previous experiences in our hospital have shown opposite approaches to these patients depending on the treating services. A clinical protocol was developed unifying diagnostic criteria and treatment algorithm, involving neurologist, internal medicine doctors and psychiatrists. 20 patients with a final diagnosis of catatonic syndrome were studied with this diagnostic and treatment protocol focusing on delay on the diagnosis, diagnostic criteria, precipitant factors, evolution and treatment received. With an appropriate treatment that included electroconvulsive therapy in some cases, syndrome remission was observed in all cases.

Title

Cognitive improvement in elderly population after cognitive rehabilitation with REHACOP

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INTRODUCTION. The aim of the present study is to investigate whether a cognitive rehabilitation program can produce improvements in cognitive functions in elderly people. REHACOP is a program validated on psychosis and schizophrenia patients. Our hypothesis is that the subjects that have participated in the neuropsychological rehabilitation program will show a significant improvement in their cognitive abilities compared with the group that has not received any cognitive stimulation. METHOD. Participants: a sample of 20 subjects (11men, 9women). Age mean of 80,7 (TD=6,7; range=63-91) and the years of education mean is 7,6 years (TD=3; range=0-12). The experimental group received cognitive rehabilitation with REHACOP program, while the control group, did not receive any cognitive training. Instruments: Digits from WAIS-III, Brief Test of Attention (BTA), Salthouse test, Hopkins Verbal Learning Test (HVLT), REHACOP rehabilitation program. Procedure: Longitudinal Study with two measurement times, before and after the implementation of the rehabilitation program. During the baseline, a battery of neuropsychological test, which evaluated memory, attention and processing speed, was administrated to the entire sample. Then, only the experimental group participated in the REHACOP rehabilitation program during three months, three times per week in sessions of 90 minutes. After the intervention, both groups were evaluated once again in order to investigate the possible changes on the cognitive functioning. It was used the SPSS statistics program (lineal general model: repeated measures). RESULTS: The experimental group showed a significant improvement in processing speed (Salthouse) and immediate verbal learning (HVLT) during the three month period comparing with the control group (Time*Group in Salthouse: $p=0,045<0,05$; and Time*Group in HVLT-4E: $p=0,026><0,05$). It has not been found statistically significant differences between group and evaluation time in long-term memory, correct recognition answers and false positives in recognition (HVLT), and in working memory(WAIS-III Digits) and attention (BTA) either, although it is important to mention a positive tendency on working memory (Time*Group;$p=0,054>0,05$) and long-term verbal memory (Time*Group; $p=0,131>0,05$). DISCUSSION: The results demonstrate the efficacy of the REHACOP cognitive rehabilitation program directed to normally aged population, especially in the improvement on immediate verbal learning memory task related and processing speed.

Title

Executive function in schizophrenia and its Diffusion Tensor Imaging correlates

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Background: Schizophrenic patients are supposed to differ from controls in their measures of fractional anisotropy (FA) in the Cingulum bundle (CB). FA is a measure of the directionality and integrity of brain white matter (WM) fibers. Furthermore, evidence suggests that the Wisconsin card sorting test (WCST), which is a measure of executive functions, could relate with FA values for the CB and that it could be decreased in schizophrenic patients. Furthermore the corpus callosum (CC) has also been related with executive functions and we wanted to test if it will also relate with the scores of the WCST. Method: We used diffusion tensor imaging (DTI) and maps of FA were generated to quantify diffusion within the left and right CB and the CC. This was done for a sample size of 43 healthy controls and 76 schizophrenic patients. Our statistical analysis included a sociodemographic analysis, and ANCOVA, group comparison in FA values and WCST, Correlations and linear regressions. Results: Patients showed significantly lower FA values in the CB compared with controls but not in the CC. Also patients scored significantly lower in the WCST correct value and higher in errors. Furthermore FA values and WCST measures were significantly correlated. Finally the linear regressions were also statistically significant and the WCST could account for around 7% of the variance of the FA values. Conclusion: As found in previous studies patients show lower scores in WCST correct answer and higher scores in error and pervasive errors than controls, furthermore, FA values of the CB are also lower in patients than in controls, finally, this two measures show a strong correlation. Keywords: DTI, FA, Executive functions, Schizophrenia, WCST

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