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Contents

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PHARMACOLOGICAL INTERVENTIONS IN ANIMAL MODELS OF INTELLECTUAL DISABILITIES: TRANSLATIONAL IMPLICATIONS

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Strategies: As the genetic basis of developmental disabilities is being discovered, the molecular mechanisms underlying genomic disorders are gradually unraveled. The ability to rationally design animal models, mimicking the genetic disorder, is helping us to test novel therapeutic interventions, including pharmacologic tools which may positively affect specific clinical phenotypes.

Down syndrome (DS) is caused by the presence of an extra copy of human chromosome 21 (Hsa 21). The mouse is a premier model organism for DS because regions of Hsa 21 are syntenically conserved with three regions in the mouse genome, located on mouse chromosome 16 (Mmu 16), 10 and 17.

Two different strategies have been followed to model DS in mice.

1. Mouse trisomies (TS), which allow analysis of the neurobiology of phenotypes or the effects of increased dosage of specific chromosomal regions. They also facilitate the study of the efficacy of potential treatments and gene dose correction in a trisomic environment.
2. Single-gene transgenesis, which involves the increased expression of a single gene in a disomic environment. Some gene-specific effects may be more specifically dissected.

The first viable TS model of DS was the Ts65Dn mouse, formed from the translocation of the distal region of Mmu 16 into the centromere of Mmu 17. This region of Mmu 16 contains over 100 genes that are orthologous to Hsa 21 genes. Ts65Dn mice show abnormalities in various organs, including the brain.

Pharmacologic interventions:

1. *Trisomic mice.* Several drugs have been tested to improve cognitive abilities in the Ts65Dn and other DS mouse models. As a paradigmatic example, I will focus on one specific issue: the excitation-inhibition imbalance which may play a central role in brain malfunction in DS.

Excessive GABA-mediated neurotransmission is one of the underlying causes of the cognitive deficits in TS mice. They display fewer asymmetric synapses that mediate excitatory transmission in the temporal cortex and dentate gyrus (DG) and synaptic structural abnormalities in the hippocampus and cortex, including a selective reorganization of the inhibitory input. They also show a marked reduction in long-term potentiation (LTP) in the CA1 and DG areas. Previous studies showed that chronic administration of non-selective $GABA_A$ receptor antagonists (picrotoxin, pentylenetetrazol) reversed the deficits in LTP and hippocampal-mediated memory of TS mice. However, these drugs are anxiogenic and proconvulsant.

Among the different $GABA_A$ receptor subtypes, $GABA_A$ $\alpha5$ subunit-containing receptors, preferentially localized in the hippocampus, play a key modulatory role in cognition. Selective $GABA_A$ $\alpha5$ negative allosteric modulators (NAMs) (inverse agonists) have cognition-enhancing effects without anxiogenic or proconvulsant side effects.

It has been recently shown that RO4938581, a selective $GABA_A$ $\alpha5$ NAM, rescued cognition and behavioral deficits in adult TS mice without inducing anxiety, convulsions or over motor effects. In the hippocampus of chronically treated TS mice, RO4938581 rescued the deficits in LTP, adult neurogenesis and normalized the density of GABAergic synapses (1).

2. *Transgenic mice.* Dyrk1A, a serine-threonine kinase, is expressed in the developing and adult nervous system. The gene *DYRK1A*, located in Hsa 21, is triplicated in DS and Ts65Dn mice. Reducing the expression level of Dyrk1A in TS mice rescues some cognitive phenotypes. Treatment with epigallocatechin-3-gallate, a polyphenolic constituent of green tea that inhibits DYRK1A kinase activity, reduced brain morphogenesis alterations in DYRK1A-overexpressing transgenic pups, and normalized the levels of BDNF and TRKB in adult transgenic mice (2).

Translational studies: Clinical trials are in progress in individuals with DS, to test the activity of both, a selective $GABA_A$ $\alpha5$ NAM and epigallocatechine-3-gallate.

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MULTIPLE SCLEROSIS: PARADIGM OF THE DUALITY INFLAMMATION/NEURODEGENERATION. NEW TREATMENTS

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Multiple sclerosis (MS) is the most frequent immune-mediated, inflammatory, demyelinating and neurodegenerative disease of CNS in young adults. It is a heterogeneous disease from the immunological, neuropathological and clinical point of view. Almost 85% of whole cases begin with relapses (relapsing-remitting form). There are two definite phases in de evolution of the disease. Initially inflammatory events in CNS are predominant and relapses are its clinical expression. Although neurodegenerative process may be present from the start of the disease, it is more relevant in a second phase (secondary progressive form), when inflammatory phenomenon decreases in intensity and axonal loss is predominant.

MS is a serious disease, dates from natural history show that after 10 years of disease evolution, more than 60% of patients have reached the secondary progressive phase and most of them are dependent and need a wheelchair.

The relation between inflammation and neuro-degeneration remain unclear. Initial acute demyelinating and inflammatory events and relapses can cause high level of disability and axonal loss, but after a few years disability evolution seems to progress independently of acute inflammatory events and relapses. There are different theories about this evolution, as the existence of a sub-clinic chronic inflammatory process from the start of MS, a primary neuronal loss from de begin independent of inflammation, even a primary degenerative process as cause of immune alteration is proposed.

Over the last two decades, pharmacology has focused on developing drugs that are able to modify the course of this disease, with the aim of reducing the frequency of the relapses and disability progression. Nevertheless, today, there are no drugs with a curative effect, and neuroprotective and neuroreparative strategies are still in their early stages. Special attention is dedicated to two molecules with mechanism of action based on MS physio-pathology: Natalizumab and fingolimod. Nowadays both are the most effective treatment in MS although with different specific molecular target and different kinds of patient indication because they are not bio-equivalent.

Natalizumab, an anti-alfa-4 integrin monoclonal humanized antibody, binds to lymphocyte surface receptors to prevent transmigration of lymphocytes to areas of inflammation into the brain tissue. Furthermore, natalizumab appears to reduce T-cell activation following their infiltration of the brain parenchyma and may contribute to T-cell apoptosis in these tissues.

In AFFIRM study (phase III pivotal study -natalizumab vs. placebo-) Natalizumab reduced the annualized relapse rate (ARR) at 1 year by 68% and the risk of sustained progression of disability by 42% ($P < 0.001$). Patients under natalizumab treatment and positive serology to John Cunningan virus (JCV) have an increased risk of develop a progressive multifocal leukoencephalopathy (PML). Risk for develop PML is influenced by three factors: previous exposition to JCV, time under treatment with natalizumab and previous immune-suppressor treatment.

Fingolimod acts as an inverse agonist on sphingosine-1-phosphate receptors, inducing degradation of receptors. On lymphoid circulation, this effect causes retention in lymph nodes of naöve and central memory T cells. As a result, the level of activated circulating T cells is markedly decreased and aggression against CNS is reduced. Fingolimod enters the central nervous system and binds to receptors on glial cells and neurons. In experimental autoimmune encephalomyelitis, the therapeutic efficacy of fingolimod is associated with a direct effect on CNS, mostly on astroglial cells. In FREEDOMS study (phase III pivotal study -fingolimod vs. placebo-) a significant 54% reduction of the ARR was observed in the arm treated with fingolimod vs. placebo. In the other hand, a 37% reduction in the risk of disability progression and a significant reduction in MRI active lesions was observed in fingolimod arm.

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CLINICAL MANIFESTATION OF NEUROPATHIC PAIN. EFFICACY OF CURRENT TREATMENTS

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Introduction: The International Association for the Study of Pain (IASP) defines Neuropathic Pain (N.P.) as 'pain that appears as a direct consequence of an injure or a pathology that affects the somatosensory system' (1).

N.P. patients can present pain which is related to specific pathologies and also pain which is produced as a consequence of several treatments and diagnostic procedures. In general, N.P. is associated with an unexpected duration of a disease or surgery, and requires a fast identification in order to apply an specifically addressed treatment which aims at controlling pain. N.P. includes clinical syndromes such as diabetic neuropathy, postherpetic neuropathy, trigeminal neuralgia, radiculopathies, complex regional pain syndrome, phantom limb syndrome, and central pain, among others.

While a percentage of N.P. patients are controlled if the causal disease is correctly treated and the first analgesic procedure is properly administered, some of these patients are refractory to standard treatment. In these cases other strategies are required given that some patients presenting the same pathology do not equally respond to the same treatments. So, a multimodal-multidisciplinary approach is needed in order to significantly decrease pain and improve functioning.

Pharmacotherapy continues to be the key procedure in the treatment process. This treatment should be started with monotherapy, but combined treatments using drugs with complementary action mechanisms might be proposed when partial response appears with monotherapy.

Different pharmacological groups and techniques exist for the treatment of N.P. It is commonly said that neuropathic pain is polipharmacologic. In general, it is recommended for the pharmacological treatment to follow several steps, but it is often needed to use combined therapies.

Mainly tricyclic antidepressants, antiepileptics, nonsteroidal anti-inflammatory drugs (NSAIDs), and opioids are used. Other drugs which are sometimes administered are local anesthetics, NMDA receptor inhibitors, GABA receptor agonists, and substance P antagonists (2).

Frequent alternatives are blocks (nerve blocks and sympathetic blocks), transcutaneous nerve stimulation (TENS), iontophoresis, and both active and passive physiotherapy.

More aggressive invasive techniques can also be used with rebel cases or as an alternative to an inadequate answer to other treatments (intolerances or adverse non-controlled effects).

When all the above alternatives fail, it should be analyzed if an intrathecal bomb might be indicated.

So, the treatment of neuropathic pain is sometimes complex and a multidisciplinary treatment is needed, including pharmacology, rehabilitation, interventionist techniques, psychological treatment and other therapeutic interventions. Only then a comprehensive approach will be guaranteed, which is specially indicated in N.P. patients, whose quality of life is significantly deteriorated.

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CHRONIC POST SURGICAL (*NEUROPATHIC*) PAIN AND PAIN MEMORY

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The recognition that acute post-operative pain may become chronic in predisposed individuals has encouraged a great deal of research both in preclinical models (1) and humans. Thus, in surgical patients, acute postoperative pain is followed by persistent pain in 10–70% of patients after usual procedures; the pain has neuropathic features in approximately 39% of these patients, after certain surgeries.

In a mouse model of post-surgical pain we have investigated long-lasting pain vulnerability following surgery, when nociceptive threshold had returned to baseline values after complete healing of the surgical wound; pain vulnerability was substantiated by an increased susceptibility to develop hyperalgesia in response to new stimuli (2). This phenomenon is also known as *latent pain sensitization* (LPS) or *pain memory*, and *may reflect the transition from acute postoperative, to chronic post-surgical pain*. In our model, LPS can be evidenced by the naloxone test where the abrupt blockade of opioid receptors precipitates hyperalgesia. The effect is stereospecific, centrally originated, and involves the dynorphin/kappa opioid receptor system. The blockade of nor-binaltorphimine-induced hyperalgesia by MK-801, also suggests the implication of NMDA receptors. Drugs and drug-combinations that may decrease postoperative hyperalgesia and prevent LPS are under active investigation in our laboratory.

Among the possible mechanism involved in pain hypersensitivity, spinal cord (SC) and dorsal root ganglia (DRG) glial-cell activation have been consistently reported after inflammation, nerve injury and/or opioid exposure. However, up to date, glial contribution to postoperative LPS is unknown. In the mice model of post operative pain, we have investigated adaptative transformations in glial cells (SC and DRG) after surgery. We observed a transient microglia/macrophage and astrocyte activation in the immediate postoperative period, while increased immunoreactivity in satellite glial cells lasted 21 days; at this time point, a challenge with (−)naloxone blocked opioid receptors and triggered hyperalgesia and astrocyte and satellite glial-cell re-activation. The administration of (+) naloxone did not produce hyperalgesia,

but induced astrocyte-reactivation, also reversing the sustained satellite glial activation in the periphery (3). Our results show that surgery induces long-lasting morphological changes in astrocytes and satellite cells, involving opioid and toll-like receptors. These findings could contribute to establish future cellular and anatomical targets to prevent latent pain sensitization, and the development of chronic post surgical pain.

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NEW THERAPEUTIC TARGETS FOR NEUROPATHIC PAIN TREATMENT: THE SIGMA-1 RECEPTOR

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Neuropathic pain is a prevalent condition that affect around 7–8% of the population. Treatment of this kind of pain is performed with different types of drugs that are administered systemically (e.g., gabapentinoids, noradrenaline/serotonin reuptake inhibitors, carbamazepine, etc.) or topically (e.g., lidocaine or capsaicin patches). However, a significant percentage of neuropathic pain patients treated with the available drugs still suffer pain; therefore, the discovery of new drugs for its treatment is an unmet therapeutic need. Several new targets are now under study in the search for new antineuropathic drugs. Among then, studies performed in experimental animals have highlighted the sigma-1 receptor as a promising new therapeutic target.

The sigma-1 receptor has been cloned and shows no significant homology to any other mammalian protein. It is highly expressed in peripheral and central nervous system areas of great importance for pain control, such as dorsal root ganglia, spinal cord dorsal horn, periaqueductal grey matter and rostroventral medulla. Sigma-1 receptor antagonists (e.g., BD-1047, BD-1063 and S1RA) reduce neuropathic pain symptoms in different experimental models. Thus mechanical allodynia, thermal hyperalgesia and cold allodynia are inhibited by treatment with these drugs in models of chemotherapy-induced neuropathy (e.g., paclitaxel-induced neuropathy), as well as in models of neuropathy induced by mechanical lesion of peripheral nerves or dorsal root ganglia (e.g., partial sciatic nerve ligation, sciatic chronic constriction injury and chronic compression of the DRG). In these models, the protective effects against neuropathic pain symptoms are observed when the sigma-1 receptor antagonists are acutely or chronically administered after the neuropathy is fully established. Moreover, these antagonists are able to prevent chemotherapy-induced neuropathy when they are administered before each dose of the antineoplastic. In agreement with the results of the pharmacological studies, both mechanically- and chemically-induced neuropathic pain is markedly reduced or even abolished in sigma-1 receptor knockout mice.

The response to mechanical and thermal stimuli in control or sham-operated animals is not altered after either acute or chronic treatment with sigma-1 receptor antagonists or in animals with a sigma-1 receptor gene deletion. In contrast, pharmacological or genetic blockade of sigma-1 receptors inhibit pain in models of central sensitization such as capsaicin-induced mechanical allodynia and the second phase of the formalin test. These data suggest that normal transduction, transmission and perception of sensory and nociceptive inputs remain intact following blockade of sigma-1 receptors and that these receptors are specifically involved in the sensitization mechanisms that produce hyperalgesia and allodynia. In support of this view, it has been demonstrated that blockade of sigma-1 receptors interfere with several electrophysiological and biochemical mechanisms underlying the central sensitization processes involved in neuropathic pain facilitation. Thus, spinal wind up induced by repeated C-fiber electrical stimulation is reduced in sigma-1 knockout mice and in wild-type animals treated with sigma-1 receptor antagonists. Moreover, central sensitization in models of mechanically- or chemically-induced neuropathy is associated with an enhanced phosphorylation of both the NR1 subunit of NMDA receptors and the extracellular receptor kinase (ERK), and the pharmacological and genetic blockade of sigma-1 receptors reduced these biochemical changes in the neuropathic animals.

In conclusion, numerous studies performed in experimental animals have demonstrated that sigma-1 receptor blockade represent a new strategy for neuropathic pain treatment, probably because these receptors modulate several of the electrophysiological and biochemical mechanisms involved in pain facilitation. These results may have therapeutic interest in humans and, in fact, one of the new sigma-1 receptor antagonists, S1RA, is now under evaluation in a phase II clinical trial in patients with different types of peripheral neuropathic pain.

OVERVIEW OF OREXINS FUNCTIONS

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Orexins were discovered in 1998 as neuropeptides of the hypothalamus. Orexinergic neurons – as most thoroughly mapped in rat by immunochemistry – project from the lateral hypothalamic area (and nearby regions) to wide but distinct areas of the central nervous system (CNS). The propeptide prepoorexin is processed (in principle) to one each of the orexin peptides orexin-A and orexin-B; the details of the process are unknown. Orexin peptides act on two G-protein-coupled receptors called OX_1 and OX_2 receptors. Orexin receptor mRNA distribution follows the peptide distribution. Among the projection sites some show expression of both receptor subtypes, whereas other nuclei show clear dominance of one receptor subtype. Thus, the receptor subtypes may mediate specific functions, and definitely some differences are seen between different species (below). Orexin peptides and receptors are found in the vertebrate species from zebrafish to man.

Soon after their discovery, the functions of orexins were investigated in rodents by injection of orexins in the brain ventricle space. Two distinct responses were seen: firstly, feeding was stimulated, and secondly, wakefulness was enhanced at the cost of especially deeper sleep. Orexin involvement in these functions was also soon supported by many different types of data. It was, however, discovered that orexins probably are redundant as appetite stimulators and that their action possibly is more important for the regulation of metabolic rate. The regulation of sleep-wakefulness cycle, however, has been shown to be the single most important function of orexins. A null mutation in OX_2 receptor gene was demonstrated to be the cause of hereditary canine narcolepsy. This was followed by studies showing that human narcoleptics have low orexin levels in their cerebrospinal fluid and that different ways of eliminating either orexins peptides, orexinergic neurons or both orexin receptors cause a narcoleptic phenotype in rodent models. Orexinergic neurons thus seem to have a role in this circuitry by stabilizing the waken state. Human narcolepsy is thought to be caused by death of orexinergic neurons.

Orexin receptors are also found in several organs outside CNS, like gastro-intestinal tract, endocrine organs and the male reproductive tracts, as indicated by the presence of the receptor mRNA and responses to exogenous orexins. Preproorexin expression is also found

in some tissues, but not nearly as widely as the receptor expression. It is thus far not known, whether the peripheral orexins/orexin receptors have significant normal physiological role, except possibly for development of brown adipose tissue.

The physiological functions discovered for orexins have raised obvious hope for therapeutic targeting of this system. Currently, the most promising and most well-explored indication is insomnia. Several pharmaceutical companies hold orexin receptor antagonists at different stages of development. The first one to make it to the US market may be suvorexant from Merck, which has successfully completed phase III studies. Suvorexant, as most other compounds under evaluation, is a receptor subtype-nonselective antagonist. Man remains largely an open question what comes to the physiological roles of the receptor subtypes, and thus some differences may be revealed as compared to animals studied, once the antagonists become available. Other fields of interest for orexin receptor antagonists may be found in the treatment of other sleep-wakefulness disturbances, drug addiction, affective disorders and anxiety. In contrast, no small-molecular orexin receptor agonists, except for one vaguely described patent, are known. Such compounds could be useful for treatment of narcolepsy and other similar disorders and to enhance metabolic rate. It has also been shown that orexin receptor activation induces programmed cell death in recombinant cells and native cancer cells. Orexin receptor activators might therefore also find use in cancer treatment.

Further reading: Many expert reviews on the specific functions of orexin system have recently been published (please see references in Kukkonen, 2012; Kukkonen, 2013). For general reviews, please see (Gotter *et al.*, 2012; Kukkonen, 2012; Kukkonen, 2013).

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OREXINERGIC/HYPOCRETINERGIC NEUROTRANSMISSION IN SLEEP-WAKE REGULATION

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Orexins are peptides synthetized by hypothalamic neurons located in the perifornical area (PeF). The PeF orexinergic neurons project widely to many brain and spinal cord regions, and sustain relevant actions in feeding, energy homeostasis, reward and sleep-wake control by releasing Orexin to activate two different receptors (OxR1 and OxR2). Orexinergic neurons are maximally active during wakefulness and virtually cease firing in slow-wave sleep (SWS) and REM sleep. Insufficient actions of orexins (Ox), also named hypocretins (Hcrt), either by peptide deficiency, receptor alteration or signaling interference, are linked to narcolepsy, a sleep disorder characterized by excessive daytime sleepiness, sleep attacks and rapid onset REM sleep. Narcoleptics have very low or undetectable Ox/Hcrt levels in cerebrospinal fluid and a reduced number of PeF Ox/Hcrt neurons as compared with control subjects. PeF Ox/Hcrt neurons project to pontine tegmentum areas involved in sleep-waking control, namely cholinoceptive regions of the dorsal (dRPO) and ventral (vRPO) divisions of oral pontine tegmentum, respectively involved in wakefulness and REM sleep induction.

We have studied Ox/Hcrt distribution and actions in dRPO and vRPO using a multidisciplinary approach to uncover Ox/Hcrt regulation of sleep-wake states in those pontine areas and putative mechanisms underlying narcolepsy pathophysiology. Thus, we here report neural mechanisms by which dysfunction of Ox/Hcrt neurons innervating the pontine tegmentum could elicit narcoleptic signs. We used polygraphic sleep recordings in free non-anesthetized cats, extracellular unit recording in anesthetized rats, anatomical tracing and immunohistochemistry. Hcrt-1 microinjection in dRPO increased wakefulness and decreased SWS and REM sleep. In contrast, Hcrt-1 in vRPO suppressed REM sleep as sole significant effect without a definitive trend for changes in other states. The number of transitions from SWS to REM sleep was decreased after Hcrt-1 microinjection in dRPO or vRPO. To determine cellular mechanisms underlying such actions, we examined unit activity of dRPO/vRPO neurons after PeF stimulation and local hypocretin application. PeF stimulation elicited orthodromic responses in dRPO and vRPO. Accordingly, anatomical tracing showed PeF retrogradely-labeled neurons, some of which contained hypocretin, from both tegmental areas. Hcrt-1 application in dRPO provoked increase in dRPO neurons activity and decrease in EEG delta activity that were blocked by a Hcrt-1R antagonist. Quite the opposite, Hcrt-1 applied through a barrel micropipette in vRPO induced an inhibition, which was blocked by bicuculline, indicating that Hcrt-1inhibitory action may involve $GABA_A$ receptors activation in that region.

Both in dRPO and vRPO Ox/Hcrt was localized in thin unmyelinated axons with numerous varicosities and terminal enlargements containing small synaptic vesicles as well as dense-core vesicles. In dRPO many of those Ox/Hcrt axons made appositional contacts or synapses with neurons retragradely-traced from the cerebral cortex. In vRPO the Ox/Hcrt-containing axons established many appositional and synaptic contacts with GABA-immunoreactive neurons. Although the proportion of bona fide synaptic contacts was low compared with appositions, the synapses were always morphologically asymmetric (excitatory-type).

Collectively, these data suggest that PeF Ox/Hcrt neurons may enhance wakefulness by activating dRPO neurons with ascending projections, and also impair REM sleep generation by a GABA-mediated inhibition of vRPO neurons. Absence of Hcrt signaling in narcolepsy would impair those actions, thus leading in dRPO to somnolence and hypovigilance and in vRPO to REM sleep disinhibition. Further recent electrophysiological and anatomical studies in our laboratory suggest that PeF neurons, in addition to the excitatory action on dRPO and inhibitory effect on vRPO neurons described above, receive projection from both brainstem areas in order to create a neuronal network that may control the sleep-wake cycle. Therefore, both dRPO and vRPO regulate the activity of PeF neurons in order to create a feedback modulation of its activity contributing to the orchestration of sleep-wake cycle. In support of our results, recent pharmacological studies have shown that dual Ox/Hcrt receptor antagonists, which block both OxR1 and OxR2, promote sleep and are a promising therapy for the treatment of insomnia.

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INVOLVEMENT OF HYPOCRETIN/OREXIN RECEPTOR-1 IN CUE-INDUCED REINSTATEMENT OF NICOTINE SEEKING BEHAVIOUR

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Hypocretin/orexin signaling is critically involved in relapse to drug-seeking behaviors. In this study, we investigated the involvement of the hypocretin system in the reinstatement of nicotine-seeking behavior induced by nicotine-associated cues. Pretreatment with the hypocretin receptor-1 antagonist SB334867, but not with the hypocretin receptor-2 antagonist TCSOX229, attenuated cue-induced reinstatement of nicotine-seeking, which was associated with an activation of hypocretin neurons of the lateral and perifornical hypothalamic areas. In addition, relapse to nicotine-seeking increased the phosphorylation levels of GluR2-Ser880, NR1-Ser890, and p38 MAPK in the nucleus accumbens (NAc), but not in the prefrontal cortex. Notably, phosphorylation levels of NR1-Ser890 and p38 MAPK, but not GluR2-Ser880, were dependent on hypocretin receptor-1 activation. The intra-accumbens infusion of the protein kinase C (PKC) inhibitor NPC-15437 reduced nicotine-seeking behavior elicited by drug-paired cues consistent with the PKC-dependent phosphorylations of GluR2-Ser880 and NR1-Ser890. SB334867 failed to modify cue-induced reinstatement of food-

seeking, which did not produce any biochemical changes in the NAc. These data identify hypocretin receptor-1 and PKC signaling as potential targets for the treatment of relapse to nicotine-seeking induced by nicotine-associated cues.

AGONIST SIGNAL TRAFFICKING AT SEROTONIN 5-HT$_{2A}$ RECEPTOR IN HUMAN BRAIN: IMPLICATIONS FOR SCHIZOPHRENIA AND ANTIPSYCHOTIC TREATMENT

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Activation of serotonin 5-HT$_{2A}$ receptors (5HT$_{2A}$-R) is a necessary condition for the psychoactive effect of lysergic acid diethylamide (LSD) and other hallucinogenic drugs. LSD administration in humans resembles some of the clinical manifestations of psychosis and schizophrenia. On the other hand, the density of 5HT$_{2A}$-R is increased in brain of drug-free schizophrenic subjects when the active G-protein-coupled receptor conformation is quantified. Moreover, second-generation antipsychotic drugs such as clozapine and olanzapine display important affinity for the 5HT$_{2A}$-R. However, non-hallucinogenic compounds such as lisuride and ergotamine share structural similarities and pharmacological properties with LSD but lack of psychoactive properties.

Cell culture and animal studies have demonstrated that in cortical pyramidal neurones, both hallucinogenic and non-hallucinogenic 5HT$_{2A}$-R agonists induce c-fos by G$_{q/11}$-protein activation. In contrast, the signalling of hallucinogenic drugs acting at 5HT$_{2A}$-R induces egr-2 by G$_{i/o}$-protein activation [1]. The existence of agonist-specific receptor conformational states that preferentially engage distinct cellular pathways has been termed agonist-directed trafficking of signalling.

In the present study, the functional coupling of 5HT$_{2A}$-R to the different G-proteins induced by hallucinogenic and non-hallucinogenic drugs was evaluated in postmortem human brain cortex. The possibility of alteration in coupling of specfic G-proteins to 5HT$_{2A}$-R in brain cortex of schizophrenic subjects was tested.

The hallucinogenic drugs (±)DOI, DOB, TCB-2 and LSD (10^{-5} M) increased the coupling to G$_{\alpha q/11}$-, G$_{\alpha i1}$-, G$_{\alpha i2}$-, and G$_{\alpha i3}$-proteins (range E_{max} 113 ± 3–152 ± 5%, n = 4–22). These stimulations were antagonized by the 5HT$_{2A}$-R antagonists ketanserin and altanserin but not by the 5HT$_{2C}$-R antagonist SB242084. TCB-2 and LSD (10^{-5} M) also stimulated G$_{\alpha o}$-proteins (range E_{max} 105 ± 1–145 ± 4%, n = 4–9), although these stimulations were not sensitive to 5HT$_{2A}$-R antagonists. The non-hallucinogenic drug lisuride (10^{-5} M) enhanced the coupling to G$_{\alpha q/11}$-, G$_{\alpha i1}$-, G$_{\alpha i2}$-, G$_{\alpha i3}$-, and G$_{o}$-proteins (range E_{max} 133 ± 4–155 ± 7%, n = 9–15), but only the coupling to G$_{\alpha q/11}$−proteins was antagonized by ketanserin and altanserin. In knock-out mice for the 5HT$_{2A}$-R, the coupling of hallucinogenic drugs to G$_{\alpha i}$-, and G$_{\alpha q/11}$-proteins, and the coupling of non-hallucinogenic drugs to G$_{\alpha q/11}$−proteins were abolished when compared to wild-type animals. These results demonstrate a differential agonist-directed trafficking of G-protein signalling between hallucinogenic and non-hallucinogenic 5HT$_{2A}$-R agonists in human brain. The functional coupling of 5HT$_{2A}$-R to G$_{\alpha i}$-proteins –mainly G$_{\alpha i1}$-, and G$_{\alpha i3}$ subtypes- may contribute to the hallucinogenic liability of LSD and LSD-like drugs.

The high-affinity component of the [^{3}H]kentanserin binding (2 nM) displacement by (±)DOI (10^{-12}–10^{-4} M) represents the G-protein-coupled conformation of the 5HT$_{2A}$-R. This receptor fraction was increased in postmortem brain cortex of drug-free schizophrenic subjects (10 ± 1%; n = 29) when compared to matched controls (5 ± 1%) and with antipsychotic-treated schizophrenic subjects (8 ± 1%; n = 16) [2]. In schizophrenic subjects (n = 23), the coupling induced by the hallucinogenic 5HT$_{2A}$-R agonist (±)DOI on G$_{\alpha i1}$–proteins was 6% higher ($P < 0.05$) than in matched controls (n = 23) whereas no differences were found in (±)DOI-mediated coupling to G$_{\alpha q/11}$–proteins. Furthermore, the enhanced coupling of 5HT$_{2A}$-R to G$_{\alpha i1}$–protein was present in drug-free subjects and disappeared in subjects under chronic antipsychotic treatment. These findings demonstrate a selective increase of the 5HT$_{2A}$-R signalling through G-proteins subtypes sensitive to hallucinogenic drugs in schizophrenia, while non-hallucinogenic pathways do not seem to be altered. The results show a richer molecular pharmacology than previously thought. It might be possible, by designing ligands with specific pathway activation, to promote 5HT$_{2A}$-R-mediated therapeutic actions without eliciting associated side effects.

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GALPHAQ INTERACTOME AND NEW SIGNALLING PATHWAYS

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The cell is a dynamic entity with highly intertwined biochemical networks responding to internal and external perturbations in an orchestrated manner. The elements in those networks are usually proteins that build complex circuits through protein-protein interactions. G proteins are essential cellular components that initiate signalling cascades following the activation of G protein-coupled receptors (GPCR). This function is achieved through highly specific and evolutionary conserved interactions between activated G proteins and a number of cellular effectors. Particularly, the Galphaq/11 family of G proteins play a paramount role in cardiovascular physiopathology and have been classically shown to associate to and activate the enzyme PLCbeta, thereby initiating lipid- and calcium-dependent pathways. However, a growing body of evidence points at additional effector proteins that are responsible for some of the functions of Galphaq. We have recently reported that GPCR activation promotes the interaction between Galphaq and two novel effectors, PKCzeta and MEK5, that mediate the activation of the ERK5 pathway (García-Hoz et al., 2010). This seems to be an important mechanism in the cardiovascular system and in the development of cardiac hypertrophy (García-Hoz et al., 2012). We have described a biochemical characterisation of the Galphaq/PKCzeta protein complex with specific focus on the interaction surfaces and its negative modulation by the GPCR kinase 2 (GRK2), a well-known player in Galphaq signalling. We identified lysine 19, located in the PB1 domain of PKCzeta as the crucial aminoacid for the association with Galphaq. Similarly, two glutamic acids at positions 234 and 245 located in the switch III domain of Galphaq were reported to be essential for the interaction with PKCzeta. Most importantly, the introduction of the double mutation (E234A/E245A) in a constitutively active form of Galphaq completely abrogated ERK5 activation. Interestingly, we found that GRK2, through its interaction with Galphaq, it prevents the association to PKCzeta, impairing the downstream activation of ERK5 by Galphaq-coupled GPCR. In line with this, protein silencing of GRK2 enhanced the duration and amplitude of ERK5 activation. Overall, this study provides important biochemical insight into the activation of ERK5 by Gq-coupled receptors and also puts forward GRK2 as a key regulator of the pathway.

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STRUCTURAL INSIGHTS INTO OPIOID RECEPTOR FUNCTION

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The opioid receptor family is comprised of three members, the μ, δ and κ opioid receptors, that respond to classical opioid alkaloids such as morphine and heroin as well as endogenous peptide ligands like endorphins. They belong to the G-protein-coupled receptor (GPCR) superfamily, and are excellent therapeutic targets for pain control. I will discuss new insights into conserved elements of opioid ligand recognition and structural features associated with ligand subtype selectivity based on the crystal structures we obtained for μ-OR and δ-OR. These data also provide a structural explanation and validation for the 'message-address' model of opioid receptor pharmacology in which distinct 'message' (efficacy) and 'address' (selectivity) determinants are contained within a single ligand. Comparison of the 'address' region of the OR with other GPCRs reveals this structural organization may be a more general phenomenon, extending to other GPCR families as well. I will also discuss the μ-OR oligomeric arrangement observed in the crystal structure and its potential implication in opioid receptor function.

GOVERNMENT AGENCIES: MY TRACK TO THE SPANISH AGENCY OF MEDICINES AND MEDICAL DEVICES

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There is frequently a great curiosity linked to young researchers, also the possibility to study abroad in another country to complete their training with disciplines useful for their careers is a great opportunity to improve their skills, gain experience abroad and decided the best places to works in a global context. I studied pharmacy in Peru, at the National University of San Antonio Abad in Cusco. In 2000 after completing my Master in pharmacoepidemiology at the *Universitat Autònoma de Barcelona* Spain, I returned to Peru and worked in a hospital and at the General Directorate of Medicines and Devises of the Minister of Health where I had done activities of pharmacovigilance and clinical trials during 3 years. These institutions bring me the opportunity for experience the public health responsibility and social responsibility. In 2007 I decided to begin a European PhD in Spain and after 2 years I saw that Peru has made great strides in clinical trials (CTs) regulation in recent years. However, Peru did not have guidance on how to review the ethical and scientific aspects of clinical trials for use by research ethics committees (RECs). As a result, during my PhD research, I developed and validated a guide for reviewing the ethical and scientific aspects of CTs to be used as an institutional standard for the Peruvian RECs, in collaboration with multinational experts in ethics and clinical research, Peruvian NIH officials and members of 13 RECs. In 2011, I finished my PhD with European mention at the University of Seville Spain with a visiting research in the School of Law, Queen Mary University of London and I completed the Master in Bioethics and Law at the University of Barcelona Spain in 2010. All this formation leads me to my current job position. Since 2011, my task at the Spanish Agency of Medicines and Medical Devices (AEMPS) include building collaboration, cooperation, support and capacity building activities with 21 countries in the Ibero American Medicinal Products Authorities Network (EAMI) www.portaleami.org. My works for the AEMPS, in the international office as the liaison between Spain and Latin America presents a range of new challenges as the development of collaborative forms, shared practices and ways of working within the broader diversity of practice and values. In this context I am interesting in continuing my scientific career deepen into the idea of pursue a specific training to develop complementary skills in global health to better understanding, to propose important and innovative solutions to current issues related of medicines that have impact in public health.

A CAREER IN SCIENTIFIC PUBLISHING: AN EDITOR'S PERSPECTIVE

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Leaving the lab after many years of research can be daunting, but there are plenty of other ways to put your years of education to good use. During my PhD it became clear to me that what I enjoyed the most was reading and writing about science rather than doing experiments. When I graduated I applied for an internship at Nature and landed my first job. Eight years on I do not miss the lab at all and I still feel that I contribute to the field/community in a different capacity. During my talk I will give you an overview of what life is like working as an editor of Nature Publishing Group, the differences between publishing primary research and reviews publishing, the pros and cons of the job, and some pointers for how to get a job in scientific publishing or science communication.

RESEARCH IN THE PRIVATE SECTOR – 'PROS AND CONS OF RESEARCH IN THE PRIVATE SECTOR'

Rivera R.

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As PhD students we are rarely challenged to plan what our next move will be once graduation is over. However, from the beginning one normally knows how they feel about becoming a Principal Investigator (PI) and have your own lab: you either like it or not. Whatever that is, it will not change with the years at the bench and publications, although you may conform. One thing is true: future planning should never be approached with a passive attitude. Additionally, researching your options (inside and outside of Academia) is not a reflection of an unfocused or bad Scientist. The utopian challenge: find a job that allows you to do what you would do without getting paid while making the most out of your Academic accomplishments, time and money invested, titles, training and intellect. With an innate passion for Beauty and Fashion I always tried to keep my personal interests away from the bench, in fear of not sounding serious or intellectual enough. In the US I learnt that marrying a PhD in Neuroscience and two Postdoctoral positions (New York University and Columbia University) with what interests me on my spear time is not only feasible but smart. How? By bringing science to beauty at the world′s number 1 beauty Company, L′Oréal Paris in New York. With over 30 brands under its umbrella, L′Oréal has over 72,000 employees worldwide. A University on its own to grow, explore, learn from, contribute and feel accomplished. Pros: to have a platonic job, the irony of getting paid to do what you are passionate about, your accomplishments are rewarded in many ways (beyond publishing an article or an editorial piece), the professional growth, lack of monotony, salary, vacation, stability, benefits, etc. Cons: Rules, politics (which we do not get exposed as PhD students or Postdocs to until it is too late), fears of the unknown, promotions are fierce and not always fair, being told what to work on… However, if you love what you do, you will exceed your own expectations and will overcome the downsides. The challenges I have today are way more exciting than the fears of failure.

THE CHALLENGE OF BIOTECH ENTREPRENEURSHIP IN SPAIN

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A biotechnology company is a mix of business and science. The felling universally expressed from experienced biotech entrepreneurs is that starting a biotechnology company is exciting, stimulating, and frightening – all at the same time. The product development process contains unpredictable biological and technical risks. These risks arise from a core technology based upon promising yet unproven science. Entrepreneurs must be prepared for an extraordinarily long product development timeframe. The average time to reach commercialization for biologics, drugs, and other types of therapeutics can take upwards of 15 years to reach the market. In this talk a case of creation, development and expansion of a biotech company in Spain is explained.

RESEARCH IN PUBLIC RESEARCH ORGANISMS: 'IS FEASIBLE THE ACADEMIC CAREER?'

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There is often a great deal of uncertainty in the early stages of an academic career, before a permanent position is achieved (which may take from 5 to 10 years). The path towards an academic career after a PhD could be carefully planned ahead to increase the chance of success within the actual competitive market, this include consulting careers advisor at different levels, talk with supervisors about their experiences and advice, find out how the job market is like in other countries and enhance the experience with responsibilities such as teaching, committee membership and administration duties. However, mobility, together with top scientific publications, is nowadays emerging as the standard gold of an academic career. During the PhD it is expected at least one or two short research periods abroad, and after the PhD it is expected one or two postdoctoral stays abroad. After successful postdoctoral a candidate could be in position to obtain an independent fellowship to establish his/her own research group or to obtain the necessary accreditation for a lecturer position. Nevertheless the academic career incorporates an important element of luck: being in the right place at the right time, so patience and/or a contingency plan can be helpful.

MORPHINE EFFECTS ON NON CODING RNAs ACTIVITY

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Drug addiction is an extremely complex system in which there still remain many unknowns and where many empty spaces or missing links are still present. Recent studies have identified changes in the expression profiles of several specific miRNAs which affect the interactions between these molecules and their targets in various illnesses, including addiction, and which may serve as valuable targets for more efficient therapies. Here, we summarize results which clearly demonstrate that several morphine-related miRNAs have roles in the mechanisms that define addiction. In this regard, morphine has been shown to have an important role in the regulation of different miRNAs, such as miR-let-7 (which works as a mediator of the movement of the mu opioid receptor (MOR) mRNA into P-bodies, leading to translational repression), miR-23b (involved in linking MOR expression and morphine treatment at the post-transcriptional level), and miR-190 (a key post-transcriptional repressor of neurogenic differentiation, NeuroD). Fentanyl increases NeuroD levels by reducing the amount of miR-190, but morphine does not affect the levels of NeuroD. We also discuss the relationship between morphine, miRNAs, and the immune system, based on the discovery that morphine treatment of monocytes led to a decrease in several anti-HIV miRNAs (mir-28, 125b, 150, and 382). The zebrafish (*Danio rerio*) has been used as an experimental model to study not only genetics and development but also disease-related pathways, given its easy *in vivo* manipulation. In this sense, the zebrafish can be an important tool to analyze *in vivo* the molecular mechanisms related to the activity and function of the opioid system that cannot be fully established in other models. For instance, in contrast to mammalian embryos, which develop in the uterus and are influenced by the maternal biochemical processes, zebrafish embryos develop externally, avoiding the maternal effect on these embryos. This is essential when dealing with drug exposure, because the effects observed in mammalian embryos might be due to the susceptibility of the mother and not the embryo *per se*. The study of the direct effects of morphine in the embryos will provide a better understanding on the molecular mechanisms that underlie the physical and neurobehavioral defects shown in fetuses and offspring after maternal morphine consumption (Nasiraei-Moghadam et al., 2010). We show here, using the zebrafish as a model, that that after morphine exposure the levels of both the miR-212 and miR-29a are altered, in both qPCR and ISH studies. This work is centered on miR-133b and its possible involvement in addiction through the effects of morphine. We analyze the effect of morphine on the miR-133b regulatory pathway using zebrafish embryos, which have been widely used to study the role of miR on development, as a model. At 24 h after fertilization (hpf), the dopaminergic system begins its differentiation, and the first TH-positive neurons begin to be detected at this particular developmental stage Our previous studies also indicated that at 24 hfp, there is a robust expression of zfMOR, the putative target of morphine. Therefore, the use of 24 hpf zebrafish embryos not only will provide information on the implication of the opioid system in the maturation and differentiation of dopaminergic neurons compared with any other stages of development but also will demonstrate that the μ-opioid receptor is functional in the zebrafish and has a specific role in the development of the CNS and a possible pathway that leads to addiction. We establish the importance of miR-133b as a regulatory factor by summarizing its activity in different pathological processes, especially cancer. Using the zebrafish as a research model, we discuss the relationship between mir-133b, the dopaminergic system, and morphine, considering: (i) that morphine modulates the expression of miR-133b and of its target transcript Pitx3, (ii) the role of the zebrafish mu opioid receptor (zfMOR) in morphine-induced regulation of miR-133b, which depends on ERK1/2, (iii) that morphine regulates miR-133b in hippocampal neurons, and (iv) the role of delta opioid receptors in morphine-induced regulation of miR-133b. Our results suggest that the control of miRNAs levels may be a mechanism for the development of addiction to morphine, or other drugs of abuse that increase dopaminergic levels in the extracellular space.

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TEN YEARS OF NEW TECHNOLOGIES APPLIED TO TEACHING AND LEARNING

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Introduction: In the last 10 years major developments in technologies has led to a change in contents transmission to our students. Twenty year ago, we were using voice, blackboard and photographic slides as source of communication in the lecture theatre. At that time, computers started to be available, with appropriate software to create complementary

materials. At the classroom; overhead and slide projectors were the only support along with the blackboard, when once again, computers, with image projectors, marked a new way of performance.
We were peacefully assimilating changes, when suddenly something new came to us; the virtual learning environments (VLE). This powerful tool allow teachers to: Have easier content management; better curriculum planning and mapping, easier tracking of students' progress, and tools for communicating; in summary, the perfect teaching and learning tool.
Aim: The aim of this study is to perform a retrospective analysis on the use of the VLE, in the Medical Degree (MD) at Malaga University; with special attention to the contents provided by teachers and those most used by students.
Materials and methods: Data of use of VLE resources were obtained from the VLE database. All the subjects on the different courses of the MD were included in the study. The analysis was performed on aggregated data of the following: Number of VLE per year at MD. Number and type of resources provided. Resources most used. Evolution in number and type of resources provided by teachers.
Results: The number of VLE in the MD showed an increase from 17 (2005) to 74 (2012). The main resources provided were web links, video, programs, presentations, images, and documents.
In terms of number of items, the increase has been spectacular, from 194 (2005) to 2654 (2012).
Regarding the resources with highest use; forums were the most visited (rate of use 561%) and tasks the second one (490%). Documents received 214,459 visits (107%). Images were seen 4161 times (90%). Presentations were visited 29,956 times (83%). Video movies were accessed 11,925 times (65%); and finally, web links were used 1714 times (28%).
The type of resources provided has been the same along the time, with a clear progressive increase. In the last 3 years, forums, tasks and quizzes have gained place. It is also noticeable the decrease in the percentage of presentations (36% in 2005 vs. 14% in 2012), and almost the disappearance of programs.
Discussion: The use of VLE in the MD linearly increased with time, reaching a number higher than the subjects to be covered in the MD, this could be due to the existence of several VLE for the same subject, yet we believe that the number is quite reasonable for a MD.
In the very last years, quizzes, forums and tasks, gained place among the most used resources. The explanation could be that quizzes use to be compulsory for students, and most quizzes can be done more than once. Forums are not compulsory but serve as communication way. Tasks are the third most visited resources; most are compulsory, so all students will visit it.
Something remarkable is the evolution of documents and presentations. These items have a lower rate of use than others because students may download them. The number of documents available for students has grown with time from 43% in 2005 to 75% in 2012, whereas presentations has decreased from 36% in 2005 to 14% in 2012. This interesting behavior is probably due to the fact that presentation are offered in VLE as pdf format, so being considered as documents.
Regarding the use of videos, the number of visits decreased with time; probably related with the fact that most videos are embedded in VLE as links to external webs, so the use of videos needs a more cautious analysis.
Conclusions: We could conclude that VLE is a great tool in teaching and learning processes. The most used resources are documents and presentations. The particular point of view of students is that they feel overflowed by the huge amount of contents available for each subject through the VLE, so they decide to be practical and resort to the contents that probably most guaranty the pass of the subject with the minimum effort to do it… documents and presentations.

USING VIRTUAL REALITY IN THE PRACTICAL TEACHING OF PHARMACOLOGY IN THE DEGREE OF MEDICINE. STRENGTHS AND WEAKNESSES

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The main characteristic of virtual learning environments (VLE) is to facilitate the learning of the student and its management by the teacher. The use of these resources by teachers involves knowledge and skill in the design and management of virtual learning scenarios.
Since the 70s until the start of 21st century, the study of pharmacology in the third year of the Bachelor of Medicine at the University of Cantabria included both, theoretical classes and practical laboratory classes with animals (rat or cat), which allowed students to observe the drug effects in a living organism (influence of dose, routes of administration, desired effects and unwanted variability in response, etc.).
But in the recent years, the legislative changes together with the attitude of students and society, in general, towards the use of experimental animals have been forced to change our teaching methods. Thus why in the University Medical School of Cantabria we are now including virtual reality based computer programs to replace laboratory animals in practical classes: *Ciberpatient,* a simulator of time course of plasma levels of the drugs in the body in response to the change of specific pharmacokinetic parameters and developed by Michael B. Bolger at the University of Southern California, and the *Cardiovascular system* a simulated experiment on a 'pithed' rat, developed by Rat John Dempster in the University of Strathclyde. We must highlight that these VLE are a great tool as a substitute of experimental animals to 'see' the pharmacological effects of the drugs; indeed, the problems we are facing when using this technology in teaching are more 'logistic' than technical ones. From a pedagogical point of view, VLE simulators on their own do not add any educational value to a learning environment since it is essential to make use of them with a pedagogical proposal, being able to leverage their strengths and avoid their drawbacks. If we follow a similar methodology to that used in classroom training, no significant differences in student learning are found when using VLE. Even more, they provoke a greater personal involvement of students in the experience; enable the projection of the potential of the methodology to other areas and the development of autonomy. When a constructivist teaching approach is adopted, the level of achievement of the learning objectives and knowledge construction are clearly favoured.
From the point of view of learning achievements, the following features of VLE must be highlighted:

1. Provoke a more active and participatory learning.
2. Allow the development of autonomous learning.
3. Favours a critical attitude towards learning.
4. Improve student′s self-concept and self-esteem.
5. Students tend to believe everything said by the teacher or written in a textbook whereas this new model helps students to understand the need to expand their academic training by searching information from other sources and to maintain a critical attitude toward knowledge.
6. Students show interest, motivation and willingness to learn actively and autonomously.

The main limitations of VLE technology we have detected are:

1. The data obtained in the current simulators are very homogeneous, so that all classroom computers get exactly the same value of the parameter analyzed, this can confuse our students about the diversity of the response to drugs in a living organism.
2. The system does not detect if the dose is correct or not and then acting accordingly.
3. In many cases the price of these simulators is high. Although the final cost is less than the number of animals used every year, it is difficult to convince our institutions to make large initial investments.

4. There is a lack of training of the university teachers who use these programs so that the potential and capabilities of these simulators are not always adequately exploited.

Overall, virtual computer programs, rather than a collection of experimental recordings are a good alternative to animal experimentation as a teaching methodology in practical classes of Pharmacology to Medical students.

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DESIGN, IMPLEMENTATION AND FOLLOW-UP OF A LEARNING PROGRAM BASED ON ACTIVE METHODOLOGIES IN THE SUBJECT OF *CLINICAL PHARMACOLOGY* IN MEDICAL DEGREE

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Background: In past years universities have witnessed changes in the learning paradigm as the result of the convergence process within the European Higher Education Area. ECTS-based models have prompted teachers to assume active methodologies, which are intended to achieve better development of professional competences and abilities, greater motivation and involvement of students and improved integration between new and previous knowledge, theory and application.

Objective: The study was aimed to design, implement and evaluate a problem-based learning (PBL) methodology in the subject of *Clinical Pharmacology* that is taught in the fifth year of the Medicine Degree of the University of the Basque Country. This experience was proposed to facilitate a main competence of Medical Degree: '*rational prescribing of first-line drugs on the basis of well-structured scientific and ethical criteria*'.

Materials and methods: In 2009/10, the university offered a training program that attempted to assist professors to design and implement active methodologies in different subjects. The program consisted of a 'subject design' phase in 2009/10 and a subsequent 'implementation phase' in 2010/11, both supervised by one-to-one tutors and revised by independent international referees. Our study was framed within the schedule of this training program. In the second four-month period of 2010/11, the subject *Clinical Pharmacology* was followed by two cohorts of students in the Hospital Units of *Basurto* and *Donostia* (n = 65). Four types of learning indicators were evaluated: (i) the initial level of competence was assessed through a self-reported questionnaire filled out at the beginning of the course; (ii) the acquirement of specific and transversal competences along the course was evaluated by a continuous assessment of the proposed activities (personal interviews and filling rubric tables) and a multiple-choice test at the end of the course, (iii) the academic performance in the subject (final marks) was compared with that in the previous year, and (iv) the student′s opinion and satisfaction level about the PBL was obtained through two different questionnaire forms prepared by the Educational Assessor Service and the Teaching Evaluation Service of the university.

Results: *Design*: Team activities based on PBL methodology were scheduled in five sessions (3 h each), embedded in the 2.5 practical credits of the subject. Using clinical scenarios as triggers, each group had to identify the learning objectives, review the appropriate information and finally discuss the results. The sessions finished with the elaboration of a first-line drug formulary including prescription and safety information of the selected drugs. During the session, student had to follow and achieve a number of learning indicators, which were previously established by the teacher. Other practical activities and lectures were also included.

Implementation: In the initial questionnaire, students considered themselves to be proficient in transversal competences (scores: 60–70%), but unskilled in the specific competences of the subject (scores < 45%). Assessments at the end of the course confirmed the high scores obtained by students in transversal competences (>85%). Furthermore, PBL-based activities over the course enabled students to acquire new specific competences such as the ability to integrate the theory with the prescription practice or to identify the safety problems of the drugs (scores > 82%). However, some other specific competences (such as decision making on the basis of a risk/benefit analysis) were more poorly scored (72%). The academic performance was very good, with a high average mark (7.9 ± 0.2). The number of medium-high and very good grades (mark > 7) improved by 10–20% compared to 2009–10. The students′ opinion and satisfaction about the PBL tasks was successful for most of the evaluated domains (scores: 69–86%). More than 85% of the students felt satisfied or very satisfied with the experience, were prone to repeat it the next year and claimed to learn more than with conventional methodologies. The main limitation was the number of hours and sessions used.

Conclusions: Learning indicators used to measure the impact of a PBL methodology on a *Clinical Pharmacology* course show good performance and satisfaction levels of the students and high standards of medical competence development. We have included several improvements in 2011–13 that are now being analyzed.

OSTEOARTHRITIS: A MAJOR OR A MINOR PROBLEM?

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Osteoarthritis (OA) is a high prevalence, systemic degenerative disease that usually affects several joints. Poor quality of life, limitation of movement and high cost makes OA a major problem to patients, their families and society, with high direct and indirect cost to health systems. However, health authorities have not a clear perception of the problem and consequently, they do not implement programmes to educate patients and their care givers since they consider OA as a minor disease. On the other hand, many primary care physicians consider OA as an unavoidable age-linked problem difficult to combat therapeutically. Symptomatic treatment it is based on analgesics, non-steroidal anti-inflammatory drugs and so-called SYSADOA (Symptomatic Slow-Acting Drugs for Osteoarthritis). However, studies using X-rays have shown that chondroitin sulphate (CS) and glucosamine sulphate (GS) may have disease (structure) modifying effects in knee OA patients, so called DMOAD effect (Reginster et al., 2001; Michel et al., 2005; Kahan et al., 2009). In recent years, higher resolution MRI technology has been used to assess the structural changes in knee OA and identify risk factors associated with cartilage volume loss. For example, a randomized, double-blind, placebo controlled study has shown that CS reduces both cartilage volume loss and bone marrow lesions in knee OA, starting as early as 6 months after initiation of therapy (Wildi et al., 2011). But the guidelines from the regulatory agencies require that joints structure modification also translate into a significant clinical benefit for the patient before allowing the classification as DMOAD. To this end, the prevention of patient disability and prevention of the need for joint replacement has been suggested as possible clinically relevant outcomes (Altman et al., 2005). In this line, a prediction study of total knee replacement (TKR) using MRI, suggests that CS treatment was associated with fewer long-term occurrences of total knee replacement in a 4-year follow up period (Raynauld et al., 2013).

In conclusion, some previous X-ray studies, some more recent trials using MRI techniques and some cohort studies are adding supporting evidence on a DMOAD effect of CS alone or in association with GS, in OA patients. OA is a major health problem that is gradually finding pharmacological strategies to improve the long-term quality of life of patients.

Conflict of interest: AGG is director of CABICYC, a research programme in chronic inflammation and cytoprotection of Universidad Autónoma of Madrid, funded by Bioibérica, Barcelona, Spain.

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