Instituto Juan March
de Estudios e Investigaciones

103 CENTRO DE REUNIONES
INTERNACIONALES SOBRE BIOLOGÍA

Workshop on

The Neural Mechanisms of Addiction

Organized by

R. C. Malenka, E. J. Nestler and
F. Rodríguez de Fonseca

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P. Calabresi
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Introduction

R. C. Malenka, E. J. Nestler
and F. Rodríguez de Fonseca

Drug addiction is a medical illness that has devastating health, social, political and economic consequences. Indeed, it is one of the most important health problems facing the world today. Addiction has this devastating impact despite extensive political effort and social/behavioral research aimed at its reduction. Social and psychological approaches to the treatment of addiction - while beneficial to many - have been only partially successful in alleviating the worldwide burden of substance abuse.

Based on recent scientific advances, attitudes toward addiction and approaches to its treatment and prevention are rapidly changing. It is now clear that addiction should be conceptualized as a brain disease that occurs in susceptible individuals as a consequence of cellular and molecular changes in nervous system function. It is well established that certain brain circuits, like those involving mesencephalic dopaminergic neurons, are particularly important for mediating the behavioral and psychological actions of drugs of abuse. This has led to the development of more sophisticated molecular hypotheses to explain critical features of addiction such as sensitization, tolerance, withdrawal and dependence. Genetic studies in inbred strains of rodents and the achievement of genetically modified animals have facilitated the identification of these molecular targets of major abused drugs. Illustrating this approach, quantitative trait locus mapping has established strong associations between alcohol preference drinking and specific chromosomal regions containing the genes for the serotonin 1_B receptor, the dopamine D_2 receptor or the GABA-A receptor g_2 subunit, while gene targeting in mice identified the Φ-opioid receptor and the dopamine D2 receptor as necessary elements for the morphine-induced reward.

Additionally, other molecular mechanisms, recruited by drug stimulation as a second stage in cellular signaling, have been found to contribute to the acute and chronic effects of drugs. Specifically , second messengers such as cAMP and specific transcriptions factors such as FosB and CREB participate in the transition to the drug-dependence status resulting from repeated drug exposure. The actions of these transcription factors on specific genes such as those coding for voltage-gated channels or local mechanisms regulating synaptic transmission such as those involved in neurotransmitter storage and release contribute to the adaptive plastic alterations in synaptic transmission in reward-relevant circuits characterized in drug-dependent animals. The convergence of multiple transmitter systems in reward-relevant synapses (such as glutamate, acetylcholine or endocannabinoids) have been also identified as mechanisms contributing to the psychoactive effects of abused drugs.

Different forms of synaptic plasticity, including long-term depression and long-term potentiation, have been described in the main synapses of the reward-processing circuitry, including the nucleus accumbens and the ventral tegmental area. These synaptic modifications may also contribute to the neural adaptations that mediate addiction. Drug-induced plasticity is further modified by environmental factors that may amplify drug effects, eventually leading to marked morphological changes in reward-relevant synapses. Yet another common and important consequence of abused drugs is activation of the physiological stress response leading to the release of glucocorticoids. These hormones are activators of a family of transcription factors that regulate the expression of specific genes in the brain and may also contribute to the neuroadaptions underlying addiction.

Thus, studies on the cellular, biochemical and molecular adaptions that occur in the brain following acute and chronic exposure to drugs of abuse indicate that drugs and drug-associated experiences converge to modify critical neural circuitry and configure the phenotype of the drug-vulnerable subject. The physical and dynamic alterations in the circuits that process motivation and emotion are likely critical substrate for the long-term changes in behavior that are characteristic of drug users: conditioning, reward expectancy, loss of control of drug intake, drug craving and relapse. The main neural circuits that process these different elements of addictive behavior have been elucidated. One of the most important circuits is the mesolimbic dopaminergic system which appears to be critically important for processing reward information and the motivational salience of external objects and events. This system is activated by all drugs of abuse, as well as drug-associated stimuli.

These findings and hypothesis, based on work using laboratory animals have allowed the establishment of neurobiological models of drug addiction that can be verified or disproven in human studies. In fact, preliminary functional brain imaging analysis of the effects of drugs of abuse on the brains of addicted (and non-addicted) subjects has generally confirmed this model in which drugs with distinctly different chemical structures and initial molecular targets may ultimately activate the same "final common pathways" in the brain. The further elucidation of the adaptive changes that occur in these pathways in response to drugs of abuse and which ultimately lead to addiction should enormously facilitate efforts to diminish the dire medical and societal consequences of this chronic brain disorder.

R. C. Malenka, E. J. Nestler and F. Rodríguez de Fonseca

Session 1: Molecular Genetic Approaches to Addiction

Chair: Eric J. Nestler

What did we learn from opiod receptor knock-out mice?

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Opioid receptors mediate the strong analgesic and addictive properties of opiate drugs. These receptors and their endogenous ligands modulate numerous physiological functions, including pain and mood control. Pharmacological studies have long described three receptors classes, mu, delta and kappa, each participating differently to opioid function. Three genes refered as to MOR, DOR and KOR have been cloned, which encode mu, delta and kappa sites, respectively (see ref 1). Availability of these genes and gene targeting in mice allow now to investigate the implication of each receptor type in opioid function in vivo, to redefine the specific mode of action of classical opiate drugs, to address the molecular basis of opioid receptor heterogeneity at the pharmacological level and to examine the possibility of functional interactions between opioid receptors. We have produced mice lacking the MOR (2, 3, 4), KOR (5) and DOR (unpublished) genes, as well as combinatorial mutant mice, and the above issues will be discussed based on our biochemical; pharmacological and behavioral studies of mutant mice. For example, our results show that none of the disrupted gene is essential for growth and survival, that each gene product is differentially involved in the perception of acute pain, that the MOR and DOR genes encode all the described mu- and delta-opioid receptor subtypes, and that the mu-receptor is an essential target for both therapeutic and adverse effects of morphine. Our recent results also suggest a role of the DOR gene product in modulating mood states. Opioid receptor-deficient mice have now been generated in several laboratories, as well as mice lacking endogenous opioid peptides (see ref 6). Studies of behavioral responses of these mutant mice will be reviewed. Comparative analysis should in the future provide detailed information on homeostatic mechanisms that regulate opioid neurotransmission in response to threatening stimuli and should help the development of novel therapeutic strategies for the treatment of pain and drug addiction.

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Recent advances in the neurobiological mechanisms of opioid and cannabinoid dependence

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The neurobiological mechanisms involved in the development and expression of the different components of opioid dependence have been investigated by using mice with a genetic disruption of genes encoding mu, delta and kappa opioid receptors. Antinociceptive responses induced by morphine were completely abolished in mice deficient in mu opioid receptors, as well as the rewarding effects induced by repeated morphine administration in the place conditioning paradigm. Besides, chronic morphine did not develop any behavioral manifestation of dependence in these mu-deficient mice (Matthes et al., 1996). Antinociceptive and rewarding effects of morphine were preserved in mice deficient in kappa opioid receptors. These mice presented an increase in the rewarding properties induced by morphine when this opioid was administered at a high dose. In contrast, the manifestation of the behavioral signs of morphine dependence was slightly attenuated in kappa-deficient mice (Simonin et al., 1998). Antinociceptive and rewarding properties of morphine were also preserved in mice lacking delta opioid receptors. However, these mice showed a slight increase in the expression of some of the behavioral signs of morphine withdrawal. These results clearly indicate a crucial role of the mu opioid receptors in the different components of morphine dependence, whereas the role of kappa and delta opioid receptors does not seem to be important.

On the other hand, we have recently validated a behavioral and biochemical model of physical dependence to δ-9-tetrahydrocannabinol (THC) in mice. The administration of the selective CB-1 receptor antagonist SR141716A in animals chronically treated with THC precipitated several somatic signs that included wet dog shakes, frontpaw tremor, ataxia, hunched posture, tremor, ptosis, piloerection, decreased locomotor activity and mastication which can be interpreted as being part of a withdrawal syndrome. An increase in basal, forskolin and calcium/calmodulin stimulated adenylyl cyclase activity was induced by SR141716A in these THC-dependent mice. This adaptive modification on the cyclic AMP pathway was specifically observed in the cerebellum but not in other brain structures. THC withdrawal had no motivational consequences on the place conditioning paradigm since no change in preference to the environment associated to withdrawal was observed. Besides, no changes in body weight and temperature were observed in THC withdrawal mice (Hutcheson et al., 1998). Now, we have further investigated the functional role of the cyclic AMP pathway in the cerebellum in the establishment of cannabinoid withdrawal. After SR141716A precipitation of cannabinoid abstinence, basal and calcium/calmodulin stimulated adenylyl cyclase activities, as well as active PKA in the cerebellum increase in a transient manner with a temporal profile that matches that of the somatic expression of abstinence. Selectively blocking the up-regulation of the cyclic AMP pathway in the cerebellum by microinfusing the PKA inhibitor Rp-8Br-cAMP in this region markedly reduces both PKA activation and the somatic expression of cannabinoid withdrawal. These results directly link the behavioral manifestations of cannabinoid withdrawal with up-regulation of the cyclic AMP pathway in the cerebellum, pointing towards common molecular adaptive mechanisms for dependence and withdrawal to

most drugs of abuse. The cerebellum seems to play a major role in this neurobiological substrate for cannabinoid dependence (Tzavara et al., 1999).

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Neurogenetics of addiction

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It has been known for many years that individual differences in susceptibility to drug addiction are in part influenced by genetics. Most evidence has come from studies of alcoholism, but other drugs of abuse share similar genetic risk and protective factors. Animal model research has progressed the furthest toward finding specific genes in the area of alcohol responses. For example, the tendency to prefer alcohol solutions is a quantitative (continuously distributed) trait in mice, and identifying which genes might underlie the genetic predisposition to drinking has been a major area of activity.

During the past few years, the technique of quantitative trait locus (QTL) mapping has been undertaken by several groups to suggest specific chromosomal regions (loci) in mice that can be shown statistically to contain genes that influence drug responses (see Crabbe et al., 1999a, for review). In essence, these studies seek associations of drug response with allelic status at genetic markers that are mapped to a specific chromosomal region. Many of these studies used C57BL/6J and DBA/2J inbred mice, the BXD recombinant inbred strains and B6D2F2 mice derived from their crosses, and lines selectively bred for high or low alcohol responses. Several chromosomal regions have been found to harbor a gene or genes that influence alcohol preference drinking (Phillips et al., 1994, 1998; Belknap et al., 1997; Tarantino et al., 1998).

Once QTLs are statistically verified, there are two basic approaches to isolating and identifying the responsible gene. First, one can identify candidate genes in the region. These are functional genes (in distinction to the markers used for the association studies) whose products are known to have effects consistent with the drug response trait. For example, genes for the dopamine D2 receptor and serotonin 1B receptor also map to the region of chromosome 9 where the strongest QTL association for alcohol drinking has been found. The logic of such candidate gene studies is that a stringent effort must be made to rule out the candidate gene as a potential source of the QTL association. The gene coding for the GABA-A receptor g_2 subunit maps to a region of chromosome 11 where a QTL for alcohol withdrawal was mapped (Buck et al., 1997). However, when mice genetically engineered to be null mutants at the g_2 gene were tested for alcohol withdrawal severity, they did not differ from wild-types (Homanics et al., 1999). This is a strong argument against the possibility that the g_2 gene was responsible for the QTL association with withdrawal.

For the alcohol preference drinking QTLs, studies with 129/Sv-ter inbred mice and a transgenic line from which the 5-HT_{1B} receptor gene had been deleted showed that knockout mice drank twice the concentration of alcohol as controls, but had normal preference for saccharin, sucrose, and aversion for quinine solutions (Crabbe et al., 1996). This result could not exclude the 5-HT_{1B} gene as a candidate. Although the current data are still consistent with the fact that the alcohol preference gene indicated by QTL mapping is the *Htr1b* gene, definitive proof of this will require more specific studies. When the background genotype carrying the *Htr1b* gene deletion was altered slightly, the alcohol drinking difference was no longer evident (Crabbe et al., 1999b). This

shows the importance of epistatic gene effects when interpreting data from null mutant studies (Phillips et al., in press). Epistatic effects (where allelic state at one gene influences the effect of allelic state at another) are also important in QTL mapping studies, although they are just beginning to be explored (Hood et al., submitted). Studies with mice in which the dopamine D2 receptor gene had been deleted showed that these null mutants had significantly lower ethanol consumption (Phillips et al., 1998). Both, or neither, of these receptor genes could be at the basis of the association with drinking.

The interpretation of studies with null mutants is also complicated by the high degree of specificity of behavioral assays. On example is the serotonin null mutant, where studies using several indices of ethanol reinforcement other than drinking revealed different patterns of genetic differences (Risinger et al., 1996, 1999). This emphasizes the fact that the construct "drug reward" is not genetically monolithic, and the different behavioral indices of reward likely represent distinct neurobiological substrates Similarly, the effect of the *Htr1b* gene deletion on response to ethanol using several measures of ataxia produced a spectrum of effects in the knockout and wild-type genotypes (Boehm II et al., in press). Other well-known limitations of the null mutant method will be addressed through studies using tissue-specific promoters and antibiotic treatment in double-transgenic lines to inhibit receptor gene expression in adult animals. These conditional knockouts should help investigators to avoid developmental compensations in response to receptor knockout, and should further identify relevant brain regions of interest.

The alternative to candidate gene studies is positional cloning, which is currently infeasible due to the rather large size of the confidence intervals surrounding each QTL location. One approach to facilitating positional cloning of the gene is to increase the certainty of exact map location through fine-grained genetic mapping, using strategies such as congenic strains or advanced intercross lines. These congenic strategies can reduce the size of the QTL confidence interval as much as 10-fold, which will make the prospects of searching the remaining possible candidate genes much more likely to be successful.

Finally, the near-completion of the initial stages of the Human Genome Project has led to the availability of partial sequences of nearly all genes in the genome. These sequences are beginning to become available arrayed on chips or filters, where the expression of the gene can be assessed. By comparing tissue from animals treated with drug to tissue from controls, these expression arrays can reveal the large number of genes whose expression is altered by drug exposure. Gene expression methodologies are complementary to the QTL mapping strategies in that QTL mapping can lead the investigator to genes that differ in sequence. The ultimate understanding of behaviors as complex as addiction will doubtless require knowledge of genetic determinants of both types. Supported by NIH (AA10760, AA11322, AA06243, DA05228, DA10913) and the Dept. of Veterans Affairs.

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Molecular mechanisms of drug addiction: ΔFosB as a sustained molecular switch for addiction

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A key challenge in addiction research is to identify neuroadaptations that underlie the relatively stable behavioral changes that characterize addiction. One mechanism that could play a major role in such stable neuroadaptations is the regulation of gene expression. This talk will focus on one particular transcription factor, ΔFosB, which appears to be part of the molecular mechanisms that underlie addiction.

ΔFosB, a product of the fosB gene, is a member of the Fos family of transcription factors. Members of this family are induced rapidly and transiently in specific brain regions in response to diverse acute stimuli. However, in contrast to other Fos family members, biochemically modified isoforms of ΔFosB accumulate in a region-specific manner in brain uniquely in response to many types of chronic perturbations. Prominent among these are drugs of abuse, which after repeated but not acute administration induce the ΔFosB isoforms in the nucleus accumbens (NAc) and related striatal regions. These brain regions are known to mediate many behavioral effects of these drugs. Importantly, once induced, the ΔFosB isoforms persist in brain for relatively long periods of time due to their extraordinary stability.

We have studied the functional role played by ΔFosB in addiction by generating transgenic mice in which ΔFosB can be induced selectively within the same subset of NAc and striatal neuron in which drugs of abuse normally induce the protein. Expression of ΔFosB dramatically increases the animal's sensitivity to the rewarding and locomotor-activating effects of cocaine. Some of these effects of ΔFosB appear to be mediated via altered expression of specific glutamate receptor subunits. Expression of GluR2 (an AMPA receptor subunit) but not of other glutamate receptor subunits is increased in the NAc upon expression of ΔFosB. Moreover, overexpression of GluR2 in the NAc by viral-mediated gene transfer increases an animal's behavioral responsiveness to cocaine, thereby mimicking the effect seen in the ΔFosB expressing mice.

Together, this work supports a scheme in which ΔFosB functions as a sustained "molecular switch" that gradually converts acute responses into relatively stable adaptations that contribute to drug addiction.

Alterations of morphine and cocaine induced behavior in mice with a mutation in the gene encoding 12-lipoxygenase.

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INTRODUCTION: Drug addiction is a brain disease, which is characterized by compulsive drug use in the face of negative consequences. Early studies have focused on understanding the cellular mechanisms of drug addiction. Work at the level of receptors and second messengers has helped define important signaling pathways initiated by drugs such as morphine and cocaine. In particular alterations in levels of cAMP have been shown to contribute to the tolerance, sensitization, and dependence exhibited upon exposure to these drugs. The role of arachidonic acid metabolites in cell signaling is well documented, however the relationship of these signaling messengers to the mechanism of action of drugs of abuse is not well characterized.

Recent studies have demonstrated that the opioid inhibition of GABAergic synaptic currents in the PAG is controlled by a presynaptic voltage dependent K conductance by a pathway involving Phospholipase A2, arachidonic acid and 12-lipogegenase (12-LO). Thru a series of experiments using specific inhibitors of this cascade it was demonstrated that only inhibition of the 12-LO pathway selectively blocked opioid inhibition of GABAergic synaptic currents. If however one blocked either the 5-LO pathway or Cox pathway, there is a potentiation of the opioid inhibition of GABAergic synaptic currents.

Mice have been generated which lack the 12-LO enzyme. These mice develop and grow normally and are fertile. There appears to be an enhanced utilization of the 5-lipoxygenase pathway in stimulated macrophages. We investigated the role of 12-LO in behavioral responses to two drugs of abuse: morphine and cocaine.

RESULTS: Our results indicate that 12-LO mutant mice have an enhanced response to morphine Mice were given acute injections of saline, and 2 doses of morphine (3 and 9 mg/kg). 12-LO mutant mice show a greater analgesic response to morphine compared to their littermate controls. Furthermore the baseline level of analgesia (saline injection) is also greater in mutant mice. Following a sub-chronic exposure to morphine (5mg/kg for 5 days), the difference in baseline analgesia is gone, but the difference in morphine induce-analgesia is enhanced especially at the lower doses of morphine.

Mice made dependent on morphine exhibited increases in 4/5 behavioral manifestations of naloxone precipitated withdrawal. Current studies are evaluating the rewarding properties of morphine in conditioned place preference. When administered repeatedly the motor stimulant effects of cocaine augment. The behavioral sensitization is long lasting. Over time, 12-LO mutant mice show a significant enhancement of locomotor activating effects of cocaine compared to their wild type controls.

CONCLUSION: 12-LO is the most active LO in the CNS and its metabolites 12-HPETE (HETE) are unstable intermediates that are further metabolized by a variety of enzymes. 12 HPETE can act as a second messenger by directly modulating a specialized K+ channel in Aplysia and moreover acts by binding to the outside of this channel. The ability of this metabolite to easily cross the cell membrane would allow it to leave cells where they are produced and act as first messenger for neighboring cells thus setting up a very novel means of transsynaptic modulation of neuronal activity. The role of AA metabolites in the CNS is poorly understood, especially with regard to the mechanism of action of drugs of abuse. Our studies will help to elucidate the functional significance of these molecules in behavioral responses to drugs of abuse.

Session 2: Molecular Targets of Drugs of Abuse

Chair: Marc G. Caron

MULTIPLE GENETIC BASES OF COCAINE SENSITIZATION.

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Catecholamines are important modulators of synaptic neurotransmission and influence complex behaviors in the CNS such as locomotion, cognition, emotion and affect as well as reward mechanisms. Plasma membrane transporters of the Na^+/Cl^- dependent transporter family calibrate the duration and intensity of catecholamine transmission in the CNS by reuptaking various monoamines into presynaptic terminals whereas the proton-dependent non-selective vesicular monoamine transporter (VMAT2) repackages neurotransmitters into synaptic vesicles. To assess the role these transporters play in CNS function, we have produced strains of mice in which the genes for these various transporters have been genetically deleted. Deletion of the dopamine transporter (DAT) produces spontaneous hyperactivity in mice homozygous for this deletion. DAT knockout mice, despite their high levels of extracellular dopamine, paradoxically still self-administer cocaine. Activation of serotonergic systems in these mice by cocaine suggests that interaction of cocaine with targets other than DAT, possibly the serotonin transporter, can initiate and maintain cocaine self-administration.

Deletion of the VMAT2 gene is lethal shortly after (2-3 days) birth. Lack of functional VMAT2 leads to the absence of stored monoamines and a lack of calcium dependent exocytic release of neurotransmitters. Interestingly, mice heterozygous for the VMAT2 gene are viable and show modest changes in presynaptic homeostasis of the dopamine system which are associated with a supersensitivity to the locomotor-enhancing effects of psychostimulants. These effects are indistinguishable from that obtained with a cocaine sensitization paradigm. In addition, we have recently produced a line of mice in which the norepinephrine transporter gene (NET) has been inactivated. Interestingly, homozygous NET knockout mice, which are viable and slightly hypoactive, show markedly elevated locomotor responses to cocaine and amphetamine. This phenotype may be mediated through changes of the dopaminergic system. The results with these three genetic mouse models point to involvement of the three monoamine systems, dopamine, norepinephrine and serotonin, in the locomotor and reward properties of psychostimulants. The availability of several different genetically manipulated mouse lines which all show the same sensitization properties to psychostimulants may provide an interesting substrate to explore gene display techniques to identify common candidate genes underlying the phenotype.

Neurophysiology of cocaine addiction: focus on dopamine receptors and voltage-gated channels

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Repeated administration of psychostimulants such as cocaine and amphetamine leads to a number of behavioral changes, including sensitization, tolerance, dependence and withdrawal. Many of the changes arise as a result of neuroadaptations within brain circuitry known to be involved in the positive reinforcing (rewarding, hedonic) effects of psychostimulants and other drugs of abuse. Both the nucleus accumbens (NAc) and medial prefrontal cortex (mPFC) are major nuclei in reward circuitry and considerable evidence implicates these brain regions in psychostimulant self-administration and withdrawal effects in animal models of dependence. In particular, there appears to be an important role for dopamine (DA) receptors in these effects. Using a combination of current-clamp recordings in brain slices and whole-cell patch clamp recordings from freshly dissociated neurons, we originally reported that medium spiny neurons (MSNs) from the NAc are less excitable in cocaine withdrawn rats due to a novel form of plasticity - reduced whole-cell Na^+ currents (Zhang et al., 1998). Three days after discontinuation of repeated cocaine injections, NAc neurons recorded in brain slices were less responsive to depolarizing current injections, had higher action potential thresholds and lower spike amplitudes. Freshly dissociated NAc neurons from cocaine-pretreated rats exhibited diminished Na^+ current density and a depolarizing shift in the voltage-dependence of activation. These effects appear to be related to enhanced basal phosphorylation of Na^+ channels because of increased transmission through the DA D1 receptor/cAMP-PKA pathway. DA D2 receptors (D2Rs) also modulate whole cell Na^+ current, but the direction of the modulation depends upon $[Ca^{2+}]_i$, such that when $[Ca^{2+}]_i$ levels are not buffered, enhancement of Na^+ current is the dominant effect. The D2R modulation is mediated through a purtussis toxin-sensitive inhibitory G protein that results in mobilization of $[Ca^{2+}]_{in}$ and activation of calcineurin to de-phosphorylate voltage-sensitive Na^+ channels (Hu et al., 1999). Remarkably, after repeated cocaine administration, D2R modulation is almost completely abolished. Recently we have extended our work on Na^+ conductance to the mPFC. Recordings from freshly dissociated pyramidal neurons from layers 5-6 of rat mPFC reveal that D1Rs also suppress whole cell Na^+ conductance in these neurons and that, as in the NAc, withdrawal from repeated cocaine administration is associated with a marked reduction in Na^+ current density and a depolarizing shift in the voltage dependence of activation. In contrast, withdrawal from repeated amphetamine administration is accompanied by a slight reduction in current density with no alteration in activation voltage.

In addition to Na^+ channel down-regulation, we have also observed a reduction in whole-cell Ca^{2+} conductance in cocaine-pretreated NAc neurons. D1Rs suppress both N and P/Q type Ca^{2+} channels in NAc neurons through a signaling pathway that leads to de-phosphorylation via protein phosphatase 1. Following repeated cocaine treatment, there is marked reduction in the ability of D1R stimulation to suppress Ca^{2+} conductance, perhaps due to the 33% reduction in available Ca^{2+} conductance. The loss of basal Ca^{2+} current appears to result primarily from a reduction in N-type Ca^{2+} current. Because these channels are intricately involved in transmitter release from nerve terminals, our findings suggest that, even when less

excitable NAc neurons are driven to fire during cocaine withdrawal, transmission would still be depressed due to the reduction in N-channel conductance and a resulting decrease in transmitter release. Because NAc neurons are normally excited by glutamate released from mPFC neurons, a reduction in excitability of both mPFC and NAc neurons would greatly diminish excitatory transmission in this pathway. Given that NAc neurons are recruited to coordinate response patterns of movement and affect, the decreased excitability and transmission during cocaine withdrawal may be related to symptoms such as anergia, anhedonia and depression.
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Convergent mechanisms for cannabinoid and dopamine signaling in reward circuits

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The endogenous cannabinoid system is a new signaling system composed by the central (CB-1) and the peripheral (CB-2) receptors, and the lipid transmitters anandamide, and 2-arachidonylglycerol. This system is the target of natural cannabinoids, the psychoactive constituents of cannabis sativa preparations (marijuana, hashish). Acute and chronic cannabis exposure has been associated with subjective feelings of pleasure and relaxation, but also to the onset of psychiatric syndromes, a decrease of the efficacy of neuroleptics and alterations in the extrapyramidal system regulation of motor activity. These actions points to a tight association of the cannabinoid system with the brain dopaminergic circuits involved in addiction, the clinical manifestation of positive symptoms of schizophrenia and Parkinson's disease. In the present work we will present anatomical, biochemical and pharmacological evidences supporting a role for the endogenous cannabinoid system in the modulation of dopaminergic transmission. Cannabinoid CB-1 receptors are present in dopamine cells of the A8, A9 and A10 mesencephalic cell groups, as well as in hypothalamic dopaminergic neurons controlling prolactin secretion. CB-1 receptors co-localize with dopamine D-1 receptors in dopamine projecting fields. Manipulation of dopaminergic transmission is able of altere the synthesis and release of anandamide as well as the expression of CB-1. Administration of an anandamide uptake blocker or a CB-1 antagonists change the response to dopamine agonists. Chronic CB-1 stimulation results in sensitization to the motor effects of direct and indirect dopaminergic agonists, whereas chronic stimulation or blockade of dopamine receptors change the behavioral response to cannabimimetics.The dynamics of these changes indicate that the cannabinoid system is an activity-dependent modulator of dopaminergic transmission, an hypothesis relevant for the design of new therapeutic strategies for dopamine-related diseases such as addiction, the psychosis and Parkinson's disease.

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Nicotine receptor inactivation decreases sensitivity to cocaine

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The reinforcing properties of nicotine and psychomotor stimulants are thought to be mediated through the mesolimbic dopamine (DA) system. The present study investigates the role of high affinity nicotinic acetylcholin receptors (nAChRs) in cocaine place preference, and examines some of the neurochemical changes in the mesolimbic DA system that might account for the interaction between nicotine and cocaine. 5 mg/kg is the lowes dose of cocaine able to condition a place preference in C57Bl/6 mice. Co-treatment with the nicotinic antagonist mecamylamine (1.0 mg/kg) was able to disrupt place preference to 5 mg/kg cocaine. In addition, mice lacking th high affinity nAChR containing the β2 subunit showed decreased place preference to 5 mg/kg cocaine, although higher doses of cocaine could condition a place preference in these knock out animals. In contrast, co-administration of a low dose of nicotine (0.2 mg/kg) potentiates place preference to a sub-threshold dose of cocaine (3 mg/kg). Dopamine turnover was monitored in several brain regions using tissue levels of DA and its primary metabolite DOPAC as an indication of DA release. Wild type mice showed a decrease in DA turnover following treatment with 5 mg/kg cocaine, whereas this response was not seen in mice lacking the β2 subunit of the nAChR. Induction of chronic fos related antigens by cocaine was also reduced in mutant mice compared to their wild type siblings, implying that downstream actions of cocaine were also affected by inactivation of the hig affinity nAChR. These data indicate that the high affinity nAChR is likely to contribute to cocaine reinforcement.

DIFFERENTIAL DISTRIBUTION AND PHENOTYPIC CHARACTERIZATION OF STRIATAL NEURONS THAT EXPRESS D4 OR D5 RECEPTORS IN THE STRIATUM..

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Striatal dopamine is implicated in motor control, cognitive and emotional procceses as well as in drug-sensitization and addiction (Moratalla et al, 1996, Neuron, 17, 147-156). Alterations in dopaminergic transmission are associated with Parkinson's disease and schizophrenia. There are five dopamine receptor subtypes, classified in two families: D1-type (D1 and D5) stimulate adenylyl cyclase, and D2-type (D2, D3 and D4) either have no effect or inhibit adenylyl cyclase. We have developed specific antibodies against the rat D4 and D5 dopamine receptors and studied their general distribution in the rat brain (Khan et al, 1998, J. Comp. Neurol., 402, 353-371; Gutiérrez et al. 1999, Soc. Neuro. Abst., 25: 1219). We have now studied the regional and cellular distribution and phenotypic characterization of striatal cells that express these receptors in the rat.

D4 receptors were found highly expressed in the striatum, with higher density in the dorsal part than in the ventral. There was a lateromedial density gradient within the dorsal striatum. In addition, D4-receptor expression was enriched in patches that were in good register with the striosomes, identified in the same or adjacent section by immunocytochemistry for the mu-opioid receptor. There were more heavily stained D4-positive cells and fibers in striosomes than in the matrix. At the ultrastructural level, D4 receptors were observed mainly in spines and dendrites in the postsynaptic compartment. D5 receptors were homogeneously distributed in caudate-putamen and nucleus accumbens throughout the striatum and were detected mainly in large neurons and in a very low density in medium spiny projection neurons.

Double labelling experiments using immunofluorescent secondary antibodies showed that all cholinergic neurons (ChAT-positive neurons) and about 80% of somatostatin- and 80 % of parvalbumin-positive neurons in the striatum expressed D5 but did not express D4 receptors.

These results suggest that dopamine-regulated signalling via D4 receptor is preferentially processed in the striosomal compartment in the striatum and that D4 receptors are exclusively expressed by the projection neurons while D5 receptors are expressed by both types projection neurons and interneurons.

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Session 3: Cellular and Synaptic Actions of Drugs of Abuse

Chair: Robert C. Malenka

The influence of non-pharmacological factors on drug-induced neurobehavioral plasticity

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The repeated administration of psychostimulant drugs induces long-lasting changes in brain and behavior that are thought to contribute to the long-term sequelae associated with stimulant use and abuse, including tolerance, sensitization, dependence and addiction. However, some of the persistent effects of stimulants, such as sensitization, are powerfully modulated by the context in which the drugs are administered. Studies illustrating the ability of environmental context to modulate the development of psychomotor sensitization produced by amphetamine or cocaine will be presented, including recent studies in which we have specifically compared the ability of contextual versus discrete stimuli to modulate sensitization. Evidence from our initial attempts to explore the neurobiological mechanisms by which environmental context modulate sensitization will also be reported. We have found, for example, that the ability of both amphetamine or cocaine to induce mRNA for c-*fos* in brain reward systems is modulated by environmental context in a similar manner as is psychomotor sensitization. Indeed, it appears that amphetamine engages different cell populations in the striatum as a function of the circumstances surrounding its administration. Recent experiments addressing the nature of the persistent neurobiological sequelae associated with repeated exposure to psychostimulants also will be presented. We have already reported that repeated treatment with either amphetamine or cocaine alters the structure of dendrites on medium spiny neurons in the nucleus accumbens and pyramidal cells in the prefrontal cortex, changes that are still evident up to a month after the discontinuation of drug treatment. The psychostimulants increase dendritic branching, the density of dendritic spines and, in the accumbens, the number of branched spines (i.e., spines with multiple heads). We recently sought to determine if self-administered cocaine has comparable effects on dendritic morphology, and new data will be presented indicating that it does. (Research supported by NIDA.)

Synaptic mechanisms mediating acute opioid withdrawal

John T Williams

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Upregulation of the cAMP dependent cascade following chronic treatment with opioids was first reported by Klee and Nirenberg in experiments using NG108-15 cells and has become one of the cellular hallmarks of opioid withdrawal. It is only recently that the consequences of that upregulation have been identified using electrophysiological methods. GABA- and glutamate mediated synaptic transmission was studied at three synapses on dopamine cells in the Ventral Tegmental Area during acute withdrawal from morphine.

At the GABA synapses, there was evidence of an upregulation of the cAMP cascade that (1) increased GABA release (Bonci and Williams, 1997) (2) increased the inhibition produced by opioid agonists (Shoji, Delfs and Williams, 1999). An additional result of the increased cAMP production was (3) a rise in adenosine tone. This adenosine resulted in the activation of presynaptic A1 adenosine receptors to reduce GABA-B-mediated IPSPs during withdrawal.

Opioids also caused a presynaptic inhibition of glutamate-mediated synaptic currents measured in dopamine cells (Manzoni and Williams, 1999). During opioid withdrawal the release of glutamate was unaffected by a cAMP-dependent mechanism. The acute presynaptic inhibition caused by opioids was not changed during withdrawal, however, presynaptic inhibition mediated by both GABA and glutamate was facilitated in withdrawn tissues. Thus during opioid withdrawal, the activation of presynaptic GABA-B and mGluRs are more effective at decreasing excitatory synaptic drive of dopamine cells. Decreased excitatory drive, along with enhanced release of GABA, promote a potent inhibition of the firing of dopamine cells.

Similar observations have been made in the periaqueductal grey and dorsal raphe suggesting that the regulation of transmitter release is a critical consequence of acute withdrawal from opioids. The results also indicate that the upregulation of cAMP processes induced by chronic morphine treatment is responsible for the change in synaptic release at some, but not all sites.

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Cellular and molecular mechanisms underlying corticostriatal synaptic plasticity

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A complex chain of intracellular metabolic events is activated by the stimulation of dopamine (DA) receptors in striatal neurones. This chain of events is critically important in motor control and in the neural mechanisms of addiction. DA receptor activation, among the various physiological effects in the striatum has also been found to represent a critical factor in the formation of two alternative forms of neuroplasticity at corticostriatal synapses, long-term depression (LTD) (Calabresi et al., 1996) and long-term potentiation (LTP) (Centonze et al., 1999). Corticostriatal LTD, in fact, is prevented by unilateral nigral lesion, by D1- and D2-like DA receptor antagonists (Calabresi et al., 1996), and by genetic disruption of D2 receptors (Calabresi et al., 1997). Similarly, DAergic denervation of the striatum blocks LTP (Centonze et al., 1999). Striatal LTD and LTP are elicited *in vitro* by the repetitive activation of corticostriatal glutamatergic terminals, respectively in the presence and in the absence of external magnesium from the bathing solution. Whereas LTD is also expressed in the presence of NMDA receptor antagonists, LTP is fully prevented by these pharmacological agents, indicating that corticostriatal LTP, but not LTD is dependent on NMDA glutamate receptor activation. Noticeably, these enduring changes in the efficacy of corticostriatal transmission might profoundly affect the pattern of firing discharge of striatal spiny neurones, since, in the intact brain, it mainly depends on the release of glutamate from cortical terminals. More recently, we have provided evidence that the D1-like receptor dependent activation of DARPP-32, is a crucial step for the induction of two opposite forms of synaptic plasticity at corticostriatal synapses, LTD and LTP. To produce LTP, however, the activation of postsynaptic PKA is also required, whereas LTD induction is dependent on PKG stimulation. These kinases appear to be stimulated in a direct and indirect manner respectively, by the activation of D1-like receptors. Here we also highlight the role of PKC in corticostriatal LTP and the possible functional interaction with the DARPP-32/PP-1 activity to further clarify the role of the events initiated by the activation of D1-like DA receptors in this form of synaptic plasticity.

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Synaptic plasticity in the mesolimbic dopamine system

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Behavioral sensitization in response to administration of psychomotor stimulants such as cocaine and amphetamine is used as an animal model for the changes that occur during the development of certain forms of addiction. It is blocked by antagonists of excitatory amino acid receptors suggesting that, like many other forms of behavioral plasticity, psychomotor stimulant-induced behavioral sensitization involves long-lasting activity-dependent modifications of synaptic strength at critical sites within the neural circuitry that mediates the behavioral changes. We have studied the occurrence and mechanisms of synaptic plasticity in the nucleus accumbens and ventral tegmental area, two structures implicated in the behavioral responses to drugs of abuse. Excitatory synapses in both structures can express both long-term potentiation (LTP) and long-term depression (LTD) but their underlying mechanisms may be somewhat different. In the nucleus accumbens, the induction of both LTP and LTD requires NMDA receptor activation. In contrast, in the ventral tegmental area while the triggering of LTP appears to require NMDA receptor activation, the triggering of LTD does not.

We have also examined the actions of dopamine on excitatory synaptic transmission and synaptic plasticity. In the ventral tegmental area, dopamine has no effects on basal synaptic transmission but blocks the generation of LTD. In the nucleus accumbens, dopamine depresses excitatory synaptic transmission via a presynaptic D1-like receptor and may also influence the triggering of synaptic plasticity. The occurrence of different forms of synaptic plasticity in the nucleus accumbens and ventral tegmental area and their modulation by dopamine provide potentially important mechanisms for mediating the changes in neural circuitry that underlie addiction.

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Cocaine Disrupts Glutamate-Driven Burst Firing in the Nucleus Accumbens via D1 Receptor Activation: Relationship to Cocaine Seeking Behavior

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The majority of adult nucleus accumbens (NAc) medium spiny neurons have a bistable membrane potential that fluctuates between a hyperpolarized (Down) state (–80 mV) and a depolarized (Up) state (–50 mV) near firing threshold. Using *in vivo* extracellular recording from NAc neurons, we microiontophoretically applied glutamate to select those cells firing in bursting patterns reflecting a subthreshold bistable membrane potential. The average frequency of bursts events was 0.85 Hz. The average burst duration was 392 ± 3.5 msec, with an average of 13.4 spikes and an average spike frequency of 30.6 ± 3.1 Hz per burst.

Our first aim was to determine how activation of D1 receptors influences the bursting of NAc cells. The D1 agonist SKF 81297 was microiontophoretically applied to test for D1 receptor modulation. Most (80%) NAc neurons responded to D1 stimulation with excitation and a corresponding increase in burst duration. This response was blocked by microiontophoresis of the D1 receptor antagonist, SCH 23390. After first screening the cells with the D1 agonist, we tested the their response to intravenous cocaine (COC) (0.5 to 4.0 mg/kg). COC's effects matched those of the D1 agonist, producing excitation and prolonging the burst duration. However, shortly after (< 3 min) COC injection, the characteristic 0.8 Hz network bursting pattern was disrupted, replaced by continuous spikes. The loss of network bursting continued for 15 min at doses up to 4.0 mg/kg iv. Systemically administered SCH 23390 (0.1 mg/kg iv.) reversed the excitatory effects of COC and restored the network bursting pattern indicating the response was D1 receptor mediated.

Our second aim was to investigate the mechanism underlying the increased excitability and enhanced burst durations. Recently, D1 receptor stimulation has been shown *in vitro* to enhance an L-type Ca^{++} channel conductance, so we microiontophoretically applied the L-type calcium antagonist diltiazem with SKF 81297. Diltiazem blocked the effects of SKF 81297 suggesting a necessary role for the L-type channel in D1 receptor-mediated excitation. Currently, we are investigating whether systemically administered L-type Ca^{++} antagonists block the excitatory effects of COC.

Our third aim was to establish a model of COC-seeking behavior that would allow us to test whether L-type Ca^{++} channel blockers would decrease COC-seeking behavior. Rats were trained to self-administer COC (0.5 mg/kg) for 7 days and, after 7 –14 days of withdrawal, the rats were placed back in the operant chambers, but without the opportunity to administer COC. Noncontingent nose poking in the drug-paired hole was the index of COC-seeking. The drug-associated stimulus light was lit every 3 min to provide a COC-associated cue. During the first hour of the extinction test the COC-trained rats nose-poked significantly more than the saline group. During the second hour both groups extinguished responding. A noncontingent injection (i.p) of COC, but not saline, produced reinstatement of responding during the second hour. Tests examining the effects of an L-type antagonist on contextual and cue-elicited COC-seeking during the first hour and COC-primed COC-seeking during the second hour are currently underway.

Session 4: Behavioral Models of Addiction

Chair: Roy A. Wise

Neural plasticity in brain reward systems

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Two decades ago, the physiological psychologist Gordon Mogensen wrote a landmark paper that described the nucleus accumbens, a ventral extension of the basal ganglia, as a "limbic-motor interface." It was here, he postulated, that "motivation is translated into action." He noted that the nucleus accumbens receives extensive input from limbic and cortical structures that code emotional and cognitive processing, and he hypothesized that the accumbens integrated affective information with adaptive motor output. Our recent research supports and extends this theory. The talk will provide an overview of two related areas of research. In the first series of studies, we have shown that activation of N-methyl-D-aspartate receptors (NMDA) in the nucleus accumbens core is critical for instrumental appetitive learning. Animals do not learn to bar-press for food if these receptors are blocked during learning. We have recently extended these results and found that activation of NMDA receptors in the amygdala and medial prefrontal cortex is also required for this behavior, suggesting that glutamate-dependent plasticity in corticostriatal networks is critical for motor learning. Further experiments with the accumbens core show that a D1-NMDA receptor interaction, as well as optimal levels of protein kinase A, contribute to the learning process. The demonstration of neural plasticity in these circuits provides insight as to why drugs of abuse are powerfully addictive: they induce long-term neuroadaptations in the brain's response-reinforcement learning system. In the second series of studies, we have investigated how drug-associated conditioned cues may contribute to long-term neuroadaptations in cognitive and reinforcement circuits. When rats that have received repeated pairings of morphine with a specific environment are put into that environment without drug, there is a strong activation of the immediate early gene Fos in prefrontal, cingulate, septal, and ventral striatal areas. This profile, which has also been found in human neuroimaging studies, suggests that drug-associated cues alone are able to alter transcription factors and gene expression in pathways subserving motivational and cognitive functions. Recently we have also found that repeated association of highly palatable food reward, chocolate chips, with a specific environment, also results in prefrontal Fos activation when animals are exposed to the environmental alone. Our conclusions are that both drugs and natural rewards are able to induce long-term changes in cognitive circuits that may reflect conditioning, reward expectancy, and perhaps drug craving.

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Conditioned reinforcement and drug addiction: the role of limbic-striatal systems

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Drugs of abuse are often said to have rewarding or reinforcing properties, although little attention has been paid to the psychological mechanisms underlying such effects. One plausible hypothesis for stimulants such as cocaine and amphetamine is that they enhance the value of environmental stimuli that normally govern behavior, including not only unconditioned 'natural' rewards themselves, but also (more usually) previously neutral stimuli which predict their availability or occurrence. Such environmental stimuli can become conditioned reinforcers, by virtue of their repeated pairing with 'unconditioned' reinforcers, such as food, or sex. Thus, the initial impetus to drug-taking behaviour can be understood in part as the motivation to experience enhanced affective properties of significant environmental events. Of course, drugs of abuse themselves may become associated with environmental stimuli, and so these stimuli will also be subject to the 'amplifying' effects of the drug, in terms of their control over behaviour as conditioned reinforcers, leading to what has been interpreted as increased their 'incentive salience'.

This presentation reviews the evidence that the enhancement of effects of conditioned reinforcers by psychomotor stimulants has a degree of behavioural, neural and neurochemical specificity (see Sutton and Beninger 1999), using a range of behavioural techniques to measure conditioned reinforcement. The main effects are shown to depend on dopamine-dependent mechanisms of the nucleus accumbens, with recent data indicating a dual role for both the core and shell sub-regions. Evidence for a limbic influence is provided by demonstrations for distinct contributions by the basolateral amygdala complex (BLA) (projecting to the ventral striatum) and by the central nucleus of the amygdala. Thus, the associative aspects of conditioned reinforcement are shown to depend on the BLA, but not on other 'limbic' areas projecting to the ventral, including the subiculum of the hippocampal formation, and the ventromedial (infralimbic) frontal cortex. Similar results are found for conditioned reinforcers produced by pairing with cocaine itself. Moreover, in a model of drug-seeking behaviour in the absence of their pharmacological effects, BLA lesions are shown to block the acquisition of second order schedules of i.v.self-administration of cocaine, maintained in part by contingent presentation of brief stimuli ultimately paired with cocaine, even though the self-administration of the drug is itself unimpaired. The special utility of this second-order schedule procedure for studying neural and psychological mechanisms underlying cocaine and heroin abuse and relapse will be further explored, as well as its significance for understanding the sequence of processes by which drug-seeking behaviours potentially become learned, and somewhat autonomous, habits.

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Dopamine and reward: Simple as that?

Roy A. Wise

The hypothesis that brain dopamine plays an important role in the habit-forming properties of drugs of abuse arose from the previous noradrenergic theory of reward and was first advanced on evidence that selective dopamine but not selective noradrenaline receptor antagonists block or attenuate (depending on dose) the rewarding effects of amphetamine (1975) and cocaine (1977). Lesion studies (1976, 1979) quickly confirmed that it is nucleus accumbens dopamine terminals that are most importantly involved. Subsequent studies extended the hypothesis that dopamine is important for psychomotor stimulant reward; dopamine antagonists were found also to attenuate the rewarding effects of hypothalamic brain stimulation (1974), food (1978), water (1981) and opiates (1981). The fact that such diverse rewards were affected by dopamine blockade, taken with the findings that nicotine, ethanol, cannabis, and phencyclidine can, like psychomotor stimulants and opiates, elevate dopamine levels in nucleus accumbens, has led to generalization of the dopamine hypothesis and, subsequently, a variety of criticisms of and challenges to some of the variants. Quite a number of caveats are now in order.

First, there are several dopamine hypotheses and they should not be treated as equivalent. One hypothesis is that many drugs of abuse rely for their rewarding effects on the ability of these drugs to elevate extracellular dopamine levels. This conservative hypothesis that elevation of brain dopamine is a *sufficient* condition (in an otherwise intact animal) for drug reward stands up reasonably well to present evidence. Second, there is the hypothesis that *all* addictive drugs rely for their rewarding effects on the ability to elevate brain dopamine. This stronger hypothesis—that brain dopamine is a *necessary* condition for drug reward—has been questionable from the start and is clearly falsified by our recent finding that phencyclidine has dopamine-independent rewarding properties. While phencyclidine, benzodiazepines and barbiturates may be rewarding without elevating brain dopamine, these drugs may act on synaptic targets of the dopamine system. However, caffeine, for example, may be habit-forming through actions quite independent of the circuitry through which these other drugs act. In any case, generalization to all addictive drugs has been premature. Third there is the hypothesis that elevations in brain dopamine are essential for the *hedonic* impact of various rewards. This hypothesis is challenged by evidence from human subjects that the hedonic impact but not the rewarding effectiveness of cocaine and nicotine undergo rapid within-session tolerance. Thus the rewarding impact and the hedonic impact of addictive drugs can be dissociated. Fourth, there is the hypothesis—not yet formally stated in the literature—that *all* elevations in brain dopamine are rewarding. This view is troublesome for two reasons. It does not sit well (largely because of the assumption just mentioned that hedonic and rewarding effects are one and the same) the fact that aversive stressors can elevate nucleus accumbens dopamine levels. Moreover, it is falsified by the recent finding that rats *extinguish* the tendency to respond for psychomotor stimulants or opiates when their nucleus accumbens dopamine *exceed* certain levels. Small elevations of nucleus accumbens dopamine are associated with both drug craving and drug reward, but higher levels of dopamine temporarily satisfy craving and render psychomotor stimulants and opiates non-rewarding. Finally, there is the hypothesis that cocaine owes its rewarding properties solely to its ability to block the dopamine transporter. This hypothesis is falsified by the finding that

mice devoid of dopamine transporters still learn to work for intravenous cocaine. At least in these mutant animals, it appears likely that cocaine's ability to block the norepinephrine or serotonin transporter is sufficient to give this drug rewarding properties.

It is clear that brain dopamine plays an important role in the habit-forming properties of several drugs of abuse and that it has a potential role in the habit-forming properties of others. However, it should not be presumed—as is suggested in at least one recent clinical publication—that a drug is addictive if it activates the dopamine system and that it is not addictive if it fails to activate the dopamine system. The addition liability of a drug is proven behaviorally, not neurochemically. In addition, the dopamine hypothesis should not be confused with the dopamine *transporter* hypothesis. The dopamine transporter is not the only mechanism through which cocaine can elevate extracellular dopamine levels.

Animal models of long-lasting vulnerability to relapse

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Drug addiction is a chronic relapsing disorder characterized by compulsive drug-seeking and use [1,4,6]. Considerable advances have been made in the understanding of the cellular and molecular mechanisms of action of drugs of abuse and the identification of neural substrates mediating the reinforcing actions of drugs of abuse, both in the nondependent and dependent state. However, only limited information is available about the mechanisms controlling long-lasting vulnerability to relapse following withdrawal and abstinence.

One important element that has been implicated in enduring vulnerability to relapse are conditioning factors. Environmental cues repeatedly associated with the subjective effects of abused substances can elicit autonomic responses and drug craving that may lead to relapse in recovering drug addicts. Indeed, such learned responses have been proposed to be among the most important factors responsible for the high rates of relapse associated with drug and alcohol addiction, although direct experimental support for this hypothesis is sparse [5]. Progress in this area of addiction research has been hampered, in part, by a lack of appropriate animal models, but effective behavioral procedures have become available in recent years to model this aspect of the addictive cycle in rats. Data from operant response-reinstatement methods implemented to investigate drug-seeking behavior associated with exposure to drug-related environmental cues in rats, indicate that drug-predictive discriminative stimuli can reliably elicit strong recovery of extinguished drug-seeking behavior in the absence of further drug availability. The response-reinstating effects of these stimuli show remarkable resistance to extinction with repeated exposure and can still be observed after four months of forced abstinence. Moreover, in the case of ethanol, cue-induced drug-seeking behavior is enhanced in genetically alcohol-preferring rats and in rats with a history of ethanol dependence. Neurochemical, neuroanatomical, and pharmacological investigations implicate dopamine-rich forebrain regions including the medial prefrontal cortex, basolateral amygdala, and nucleus accumbens in the mediation of the motivating effects of cocaine-related environmental stimuli. The behavioral effects of ethanol-associated stimuli are sensitive to pharmacological manipulation of opioid receptors. In addition, there is an additive interaction between the effects of ethanol cues and both physical and conditioned stressors that may involve endogenous opioid mechanisms as well as alterations in the extrahypothalamic corticotropin-releasing factor system within the central nucleus of the amygdala [2,3,7,8]. Overall, these findings provide direct experimental evidence for a role of conditioning processes in the long-term addictive potential of drugs of abuse, and support the hypothesis that conditioned responses to drug-related stimuli are an important factor in relapse. The data also suggest that enduring neuroadaptive disturbances in brain reward and stress systems may augment the susceptibility to cue-induced drug-seeking behavior. Finally, the response-reinstatement methods employed in these studies provide a promising tool for the investigation of neurobiological mechanisms leading to drug-induced behavioral plasticity that may underlie vulnerability to relapse.

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The effects of nucleus accumbens core and shell lesions on intravenous heroin self-administration and the acquisition of heroin-seeking behaviour (under a second-order schedule)

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Evidence has implicated an important role of the nucleus accumbens (Nacc) in drug seeking and taking behaviours. To investigate the function of the Nacc core and shell in heroin seeking behaviour rats were trained to self-administer heroin (0.12mg/kg/infusion) under a continuous reinforcement (CRF) schedule. After stable responding rats were given excitotoxic lesions of either the Nacc core or shell (a sham lesioned group was also prepared) and after recovery were assessed for their retention of heroin self-administration under CRF. Post-lesion performance of self-administration was similar in each lesion group with slight reductions in self-administration levels in the core lesioned group in the early training sessions. After 7 training days all the groups achieved self-administration rates similar to their pre-lesion performance. At this point a second-order schedule was introduced with the response requirement increasing for each drug infusion starting at FR10: (FR1:S) (such that each lever press resulted in the presentation of a CS light (previously paired in the drug infusion) and 10 lever presses were required for presentation of the drug infusion) . The schedule was increased in every successive session (over 5 sessions) from FR10 (FR 2:S), up to FR10: (FR10:S), that is, 10 lever presses were required for presentation of the CS and 10 CS presentations were needed for the activation of drug infusion. As the schedule increased a relative deficit in responses was observed in core lesioned rats. No difference in the response rates was observed between shell and sham lesioned groups. These results, taken together with previous finding using core or shell lesions in rats self-administering heroin, indicate a important role for the core of the Nacc in the acquisition of heroin seeking behaviour and indicate that the core of the Nacc is critical in reward-related learning.

Session 5: Mesolimbic Reward Circuits

Chair: Gaetano Di Chiara

Drugs inside the human brain

Nora D Volkow

Positron Emission Tomography (PET) is an imaging method that enables to measure biochemical, metabolic and pharmacological processes in the living human brain. Because of the chemical flexibility of the short-lived positron emitters, PET has proven to be a highly valuable tool to study the effects of drugs of abuse. One of its strengths is its ability to non-invasively track the regional distribution and kinetics of labeled compounds in the human brain and to measure functional and neurochemical effects of drugs. It can also be used to assess the consequences of chronic drug exposure in brain and in this way can provide with information on possible toxic consequences of drugs of abuse as well as on cerebral processes that change with repeated drug exposure that may pertain to addictive behaviors. Because PET measurements are done in awake subjects it also allows to investigate the relation between the behavioral and the biochemical and functional effects of drugs as well as the temporal relation between the pharmacokinetics of the drug in brain and the temporal course of its effects. Because multiple PET measurements can be done on the same subject it can be used to monitor effects of drug intervention and with the use of multiple tracers it can also be used to assess the relation between biochemical parameters and/or functional variables. The investigation of drug abuse can be approached from the perspective of understanding the properties of the drug itself and the other is that of understanding the biochemical characteristic of addicted subjects.

PET can be used to investigate the properties of drugs of abuse by assessing its pharmacokinetics and pharmacodynamic properties in the human brain. Its pharmacokinetics can be measured using the labeled drug itself which allow to measure the absolute uptake, regional distribution and kinetics of the drug in the brain. Pharmacokinetics can also be investigated by monitoring the duration of drug's effects on specific biochemical or metabolic parameters. For example, PET has been used to compare the pharmacokinetics of cocaine and methylphenidate, two drugs with very similar pharmacological properties but with different addictive liability. PET has also been used to compare the pharmacokinetics and the regional distribution in the human brain of the two enantiomers of methylphenidate. Pharmacodynamic properties of a drug can be investigated with PET using different labeled tracers. For example, with appropriate radiotracers, the effects of a drug of abuse on metabolism, neurotransmitter activity, blood flow, enzyme activity or other processes can be probed. We will illustrate these applications of PET in drug research with studies that evaluated the efficacy of cocaine and methylphenidate at the molecular target (dopamine transporter) in the living human brain and to assess the effects of methylphenidate in the changes in brain dopamine concentration that result from the blockade of the dopamine transporters.

PET can also be used to investigate the biochemical characteristic of addicted subjects. Taking advantage of multiple tracers it can be used to assess the involvement of the various components of a particular neurotransmitter (s) system From the perspective of understanding the process of addiction PET has been used to assess the effects of chronic drug administration on brain function and biochemistry that relate to the loss of control and the compulsive administration of drugs, the biochemical changes that results from neurotoxic effects of drugs and the effects of drug withdrawal on those changes. PET has also been used to investigate the

significance that differences in the levels of molecular drug targets (i.e receptors) between subjects have on the behavioral responses to drugs of abuse.

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The Generalized Human Circuitry of Brain Reward and its Functional Dissection

Hans Breiter, MD

To produce behavior, motivational states necessitate at least 3 fundamental operations, including (1) selection of objectives focused on goal-objects, (2) compilation of goal-object information, and (3) determination of physical plans for securing goal-objects. The second of these general operations has been theorized to involve three subprocesses: (a) feature detection and other perceptual processing of putative goal-object "rewards", (b) valuation of goal-object worth in the context of potential hedonic deficit states, and (c) extraction of incidence and temporal data regarding the goal-object[1]. Through animal research and beginning efforts with human neuroimaging, it appears that a number of subcortical brain regions are involved in these three informational subprocesses, in particular the amygdala[2,3], sublenticular extended amygdala (SLEA) of the basal forebrain[4], and nucleus accumbens (NAc)[1,5]. These subcortical regions are further interconnected with a number of paralimbic regions, such as the anterior cingulate and insula; discrete sets of these subcortical and paralimbic cortical regions have been strongly implicated in the processing of information related to potential rewards. Functional magnetic resonance imaging (fMRI) studies of humans have recently begun to localize these subcortical and paralimbic regions during specific experimental conditions. In this presentation, two human cocaine infusion studies, one human morphine infusion study, one monetary reward experiment, and one cognitive psychology experiment will be reviewed in relation to their pattern of fMRI activation within subcortical and paralimbic regions. These projects necessitated disparate technical expertise across a number of intellectual domains, which was provided by collaborators such as Peter Shizgal, PhD, Danny Kahneman, PhD, Lino Becerra, PhD, Itzhak Aharon, PhD, Bruce Rosen, MD, PhD, and David Borsook, MD, PhD.

The work of these collaborative projects suggests two primary theses regarding human brain reward circuitry and its involvement with the resolution of motivational states. First, data across these studies suggest that there is a generalized circuitry of reward in humans which responds irrespective of rewarding stimulus. Thus, activation in the NAc, amygdala, and SLEA, along with

the dopaminergic output source of the ventral tegmentum (VT)[6] and a number of paralimbic regions such as the anterior cingulate/medial prefrontal cortex, and insula, are observed in both drug-naive and drug dependent subjects, and across disparate categories of drugs and non-drug rewards. Second, the data across these studies argue that we can begin, with functional neuroimaging, to dissect human reward circuitry into its functional subprocesses. In particular, the current fMRI data suggest that one function of the NAc and associated brain regions may be the evaluation of goal-object incidence data for the computation of conditional probabilities regarding goal-object availability. Further work is clearly warranted to test hypothesized functions for all subcortical and paralimbic regions implicated in the mediation of motivational states, and to integrate these regional functions into a larger understanding of motivated behavior.

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Reward processing in primate basal ganglia and orbitofrontal cortex

Wolfram Schultz

Biological organisms need to obtain a number of substances from the environment in order to survive. These substances are contained in liquids and food objects which acquire the function of rewards, as they are repeatedly approached and consumed once their survival value is known. Thus, rewards are prime motivating factors for selecting and initiating behavioral acts and serve as goals of voluntary, intentional behavior. Behavioral choices are made according to the motivational value of reward (quality, magnitude and probability of outcome), the current motivational state and the presence of available alternatives. Many forms of goal-directed behavior are based on intentions directed at upcoming, predicted outcomes which influence decisions between competing alternatives, and the relative frequencies of competing behaviors depend on the relative expected outcome of each alternative. In a separate function, rewards contribute to learning and maintenance of approach and consummatory behavior which serves to more efficiently obtain crucial substances from the environment. This function is based on the discrepancy between the prediction and occurrence of rewards. Behavior is modified when rewards occur differently than predicted (reward prediction error), and learned behavior is maintained when rewards occur as predicted. In another function, rewards induce subjective emotional states, which is difficult to investigate in animals.

Convergent lines of evidence suggest that the basal ganglia and frontal cortex are important for the control of voluntary, goal-directed behavior and the processing of rewarding outcomes. Psychopharmacological, lesioning and electrical self-stimulation experiments strongly indicate that dopamine neurons, ventral striatum and orbitofrontal cortex serve prime motivational functions. Major addictive substances, such as cocaine and heroin, act on the dopamine system, ventral striatum and frontal cortex.

In order to assess the mechanisms underlying the role of basal ganglia and orbitofrontal cortex in reward functions, we recorded from single neurons in these structure while monkeys performed controlled behavioral tasks for obtaining liquid or food reward. We found (1) neurons signalling reward information, including reward prediction errors; (2) neurons that process the behavioral preference (motivational value) for rewards relative to available alternatives; (3) an influence of expected reward on neuronal activity related to behavioral reactions directed at these rewards (neuronal 'knowledge' of outcome at the time of the behavior directed at this outcome).

Thus, although there are no specialized receptors for rewards like for sensory stimuli, brain structures extract various components of reward information from environmental stimuli in order to control behavioral reactions. It appears that mammalian brains contain dedicated systems that process several aspects of reward information necessary for the different reward functions outlined above.

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Adaptive properties of DA responsiveness to conventional and drug reinforcers in nucleus accumbens compartments.

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Changes in dopamine (DA) transmission in the shell and core compartments of the nucleus accumbens (NAc) in response to appetitive (palatable food) and aversive stimuli (tail-pinch) were monitored by brain microdialysis. In rats fed ad libitum with standard food, unpredicted consumption of an unusual palatable food (Fonzies) phasically stimulated DA in the shell and, to a lesser extent, in the core. Pre-exposure to food stimuli either conditioned (perforated Fonzies-filled box) or unconditioned (Fonzies-feeding), while sensitized feeding-associated stimulation of DA in the core, inhibited it in the shell. Application of an unconditioned aversive stimulus (tail-pinch) increased DA in the core but not in the shell and sensitized the response to a second application of tail-pinch. Thus, while DA transmission in the core is responsive to generic motivational stimuli, that in the shell is phasically activated by unusual, unpredicted and unconditioned appetitive stimuli. Moreover, while the DA response in the core undergoes one-trial sensitization that in the shell undergoes habituation. These properties are suggestive of a role of DA responsiveness in the shell in acquisition (learning) and in the core in expression (responding) of motivation. Drugs of abuse are homologues to appetitive stimuli in their property of stimulating acutely DA transmission in the shell but differ in their failure to undergo one-trial habituation. Abnormal adaptation of DA transmission in the shell to repeated drug exposure might be the basis for impaired motivational learning and, ultimately, drug addiction.

Interactions between glucocorticoids and dopamine in tooning reward.

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Research on drug abuse has recently focused on understanding the vulnerability to develop addiction that is spontaneously present in certain individuals or induced by stress in others. Experimental studies in laboratory rats have shown that glucocorticoid hormones may be one of the biological factors determining individual vulnerability to develop psychostimulant-intake. Different lines of data support this hypothesis. First, rats spontaneously vulnerable to develop psychostimulant intake (HRs) show a longer lasting stress-induced corticosterone secretion and a higher sensitivity to the behavioral effects of this hormone than animals that are spontaneously resistant to develop this behavior (LRs). Second, administration of corticosterone to LRs increases their predisposition to de develop drug intake. Third, blockade of corticosterone secretion decreases cocaine self-administration and relapse of this behavior after a withdrawal period. Finally, administration of corticosterone reinstates responding for cocaine after extinction of this behavior. The effects glucocorticoids on vulnerability to drugs may be mediated by mesencephalic dopaminergic neurons. These neurons are the main neural substrate of the reinforcing effects of psychostimulant; their activity is spontaneously higher in HR rats and it is increased by acute and repeated exposure to stress. Administration of corticosterone, at doses that give plasma levels of the hormone that are in the stress range, increases extracellular concentrations of dopamine in the nucleus accumbens, the dopaminergic projection most involved in the mediation of the reinforcing effects of psychostimulants. In parallel, suppression of endogenous glucocorticoids by adrenalectomy decreases the basal release of dopamine in the accumbens as well as the release of dopamine induced by drugs of abuse. Interestingly the effects of glucocorticoids seem localized to the shell part of the nucleus accumbens, the region most involved in mediating addictive properties of drugs. Finally, suppression of stress-induced corticosterone will also suppress the higher dopaminergic activity observed in spontaneously predisposed subjects. In conclusion, corticosterone, and mesencephalic dopaminergic neurons seem to be organized in a pathophysiological chain determining vulnerability to drugs.

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ELECTROPHYSIOLOGICAL CHANGES AFFECTING MESOPREFRONTAL, BUT NOT MESOLIMBIC, DOPAMINE NEURONS AFTER CHLORAL HYDRATE ANESTHESIA AND IMMOBILIZATION STRESS: RELEVANCE TO CHRONIC DRUG ADMINISTRATION.

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Midbrain (mesolimbic and mesoprefrontal) dopamine (DA) neurons arising from the ventral tegmentum (VTA) are considered to play a crucial role in processes such as motivation, stress, cognition, addiction and schizophrenia. In particular, the activity of mesolimbic (MAcc) DA neurons after chronic administration of substances of abuse is considered informative in the context of drug addiction, while their acute effects allow identification of the primary site of action. To this regard, addicting drugs such as ethanol, morphine and cannabinoids acutely stimulate the neuronal activity of midbrain DA systems (1; 2; 3; 4) including the MAcc one, but upon suspension from chronic treatment hamper their neuronal functioning suggesting that the MAcc DA system is a major target in the actions of chronically administered addicting drugs. Accordingly, we have previously studied the effect of drug withdrawal on MAcc DA neurons, observing a reduction in their spontaneous electrical activity (5; 6; 7). These findings, at least in the case of ethanol, seem to be causally related to withdrawal, and not due to experimental stressful conditions due to the use of muscle-relaxing agents (8). Since the mesoprefrontal (MPfc) DA system seems to be a major target of stressful stimuli we compared the spontaneous electrical activity of both MAcc and MPfc DA neurons in chloral hydrate-anesthetized (AN) and gallamine-paralyzed (UNAN) rats. Extracellular single unit recordings from the VTA DA neurons coupled with antidromic activation from either the nucleus Accumbens and prefrontal cortex were performed in both the AN and UNAN rats. The spontaneous firing rates of MPfc DA neurons were found to be lower (≅ 44%) in AN as compared with UNAN rats, while no differences were observed between the groups of animals for MAcc DA cells. On the basis of these results, we further investigated the responsiveness to acute chloral hydrate (50-400 mg/kg iv). Chloral hydrate increased the firing rate of both MPfc and MAcc DA cells, but in a different manner. The effect was not only of different magnitude, but also of opposite sign as time elapsed from injection. After a brief initial increment in firing rate neuronal activity was reduced (≅ 40%) to levels comparable to those observed in UNAN rats. When viewed together our results suggest that 1) Higher spontaneous firing rates in the MPfc DA system observed in UNAN rats, as compared with UNAN ones, may be due to the effect of immobilization stress due to experimental conditions. 2) Mesolimbic DA neurons do not appear to be affected by different experimental conditions (AN vs. UNAN). 3) Although initially stimulatory on MPfc DA firing, chloral hydrate reduces (≅ 40%) neuronal firing at longer times (20 min) and this effect appears to be selective for the MPfc DA system. 4) The lack of difference in spontaneous neuronal activity observed in the MAcc DA system between AN vs. UNAN rats suggests a relative insensitivity to manipulation of experimental conditions and indirectly supports the conclusion that hypofunction of this system is causally related to withdrawal of chronically administered drugs of abuse. Finally, the present results suggest a specific role of MPfc DA system in response to immobilization stress, and conversely support the involvement of the MAcc system in drug addiction.

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POSTERS

Extinction of long-term cocaine self-administration alters D_4 dopaminergic receptor binding in the nucleus accumbens of rats

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It is widely accepted that dopaminergic system is one of the most important target of cocaine actions. The aim of the present work has been to test the effect of cocaine self-administration and its withdrawal on D_4 dopaminergic receptor binding sites of the nucleus accumbens. Seventy two littermate male Lewis rats were randomly assigned in triads according to a yoked-box procedure to one of three conditions: a) contingent intravenous self-administration of 1mg/kg/injection of cocaine(CONT) and b) non-contingent injections of either 1mg/kg/injection of cocaine(NONCONT) or c) saline yoked (SALINE) to the intake of the self-administering subject. The self-administering rats were trained to self-administer cocaine under a FR5 schedule of reinforcement during daily 2 hr sessions for at least 4 weeks. After stable baseline levels of drug intake had reached, saline was substituted for drug during 5 days. Following this first extinction period, cocaine self-administration was reinstated for an additional minimum period of 2 weeks and saline was again substituted for cocaine during 0 (last day of intravenous cocaine self-administration), 1, 5 and 10 days (second extinction period). On each one of these extinction days, animal brains in each triad were removed to be procesed for quantitative autoradiography using 1 nM ^{3}H-YM 09151 for labeling D_2-like family of receptors and 800 nM of raclopride to displace D_2 and D_3 binding. Non-specific binding was defined using 10μM of (+)Butaclamol. Binding to D_4 receptors in DAY 0 showed up a statiscally significant decrease compared to DAYS 1, 5 and 10 of extinction in the CONT versus NONCONT and SALINE groups. Binding to D_4 receptors was also lower in NONCONT group compared to SALINE group. These results suggest that changes in D_4 receptor binding in the nucleus accumbens could mediate some of the effects of cocaine withdrawal from long-term cocaine self-administration.(Supported by DGES PM27-0027).

NALTREXONE BLOCKS THE EXPRESSION OF BEHAVIORAL SENSITIZATION TO AMPHETAMINE ON THE DRL 72-SECONDS SCHEDULE OF REINFORCEMENT.

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Introduction: Repeated intermittent treatment with amphetamine (AMPH) sensitizes animals to the locomotor-activating effects of the drug (Wise, Behav. Pharm., 1993, 4, 339-49). Furthermore, rats trained on a Differential-Reinforcement-of-Low-Rate Schedule 72 seconds (DRL 72-s) have been reported to show also behavioral sensitization to AMPH when injected repeatedly and intermittently (Balcells-Olivero et al., Psychopharm.1997, 133, 207-13).

Methods: In the present experiment, two groups of rats where trained on the DRL 72-s schedule until they reached stable baseline performance. Then, one group was treated intermittently (twice a week) with 1.5 mg/Kg AMPH (AMPHGp)(n=16) to induce sensitization to the behavioral effects of the drug while the other group received saline 1 ml/Kg at the corresponding time (SALGp)(n=15). Two separated tests with a challenging dose of AMPH (0.5 mg/Kg) were conducted in both groups.

Results: The group of rats that had been previously injected with repeated AMPH (AMPHGp) showed a sensitized response when challenged with 0.5 mg/Kg of AMPH. When compared with the control group on the DRL 72-s performance, the frequency of reinforces decreased, the rate of responding increased and the inter-response time distribution was shifted to the left ($P<0.05$) in the AMPHGp. When the second test was performed with a 5 mg/Kg naltrexone injection prior to the 0.5 mg/Kg AMPH challenge, no sensitization to AMPH was seen and there were no significant differences on the DRL 72-s performance between groups.

Conclusion: These results show that naltrexone blocks the expression of behavioral sensitization to AMPH in a behavioral schedule of reinforcement. This results may also have further implications for drug addiction treatment (Robinson & Berridge, Brain Res. Review, 1993, 18, 247-91) and explain the anti-craving effects hypothesized for naltrexone (Balcells-Olivero & Vezina, Psychopharm., 1997, 131, 230-8).

Modulation of the responsiveness of mesolimbic dopamine transmission to highly- palatable food and ethanol

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Feeding of an unusual highly palatable food (Fonzies) to rats fed ad libitum with standard food increased extracellular dopamine (DA), monitored by means microdialysis, in the Nucleus Accumbens (NAc). This response undergoes habituation. In undeprived rats feeding of a chocolate solution through an intraoral catheter increased extracellular DA in the NAc in a monophasic manner and this response, like that to Fonzies undergoes habituation in a singol trial. In undeprived rats the administration of an ethanol (10%) solution,or an ethanol (10%) -sucrose (20%) solution, or an ethanol (10%)-chocolate solution through an intraoral catheter increased the extracellular DA in the NAc in two peaks. That are differentially sensitive to habituation. Conventional reinforcers (Fonzies or chocolate) are homologus to drugs of abuse for their property to activate DA transmission in the NAc shell, but differ for their sensitivity to habituation. Non-habituating, i.e. repetitive, stimulation of DA transmission in the extended

Cannabinoid receptor antagonist SR141716A decreases operant ethanol self administration in rats exposed to ethanol-vapor chambers

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Previous reports have established that pharmacological blockade of cannabinoid CB_1 receptors decreased ethanol intake/preference in rodents. The potential role of dependence status on CB_1-mediated blockade of ethanol self-administration has not been addressed. In the present study we examined the effects of the cannabinoid antagonist SR 141716A (0, 0.03, 0.3, and 3 mg/kg) on operant ethanol (10% v/v) self-administration in male Wistar rats that were made ethanol-dependent by chronic (14 days) exposure to ethanol vapor-chambers or exposed to air in identical vapor chambers. Dependent animals responded more for ethanol than did air control nondependent rats. The acute administration of a 3 mg/kg dose of SR141716A almost suppressed ethanol self-administration only in ethanol dependent animals. However, operant responses for food were not affected by the administration of SR141716A. These results further support that cannabinoid CB_1 receptor blockade may have a potential utility for the treatment of alcoholism.

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CD81 expression after acute cocaine treatment in the adult rat brain

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We are investigating the changes in gene expression in the mesolimbic dopaminergic system upon the administration of the stimulant drugs, particularly cocaine, in the adult rat brain. In the present study we used an acute treatment and withdrawal model. Hippocampus, nucleus accumbens, lateral striatum and ventral tegmentum were dissected out 24 hours after the last of four subcutaneous cocaine injections (one injection of 30 mg/kg every two hours) and used for differential display to screen for mRNAs regulated by cocaine. Among over 40 cDNA fragments differentially expressed upon cocaine treatment in one or several of the above regions, CD81 mRNA was found to be over-expressed in accumbal tissue. The CD81 protein, also known as TAPA (target of an anti-proliferative antibody), is found on astrocytes and is up-regulated after neuronal injury. It is a member of the tetraspan family and was intensively studied for its role in the immune system as an important component of tetraspan-integrin complexes on immune cells. To further understand the role of this molecule in cocaine action, CD81-knockout mice were used in place-preference and locomotor activity tests following i.p. cocaine. Results are presented here together with a neurochemical analysis of striatal dopamine levels. In addition, *in situ* hybridisation was performed to study the distribution of CD81 mRNA in the brain following acute and chronic cocaine or amphetamine treatment.

STRESS-INDUCED ALCOHOL BUT NOT COCAINE RELAPSE IS INHIBITED BY CENTRAL ADMINISTRATION OF THE NOVEL ANTIOPIOID PEPTIDE NOCICEPTIN/ORPHANINFQ.

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Medical literature reports that condition of stress increase vulnerability to relapse in abstinent former drug addict individuals. Similarly, preclinical studies showed that stress induced by electrical foot-shock evokes both cocaine- and alcohol-seeking behavior in drug-free rats previously trained to self-administer the drug. It has been proposed that nociceptin/orphanin FQ (Noc/oFQ) a recently isolated neuropeptide that is structurally related to opioid peptides, possess anti-stress properties following intracerebroventricular (ICV) injection. In the present study we investigated whether pretreatment with Noc/oFQ was able to inhibit relapse to alcohol or cocaine induced by stress. For this purpose two groups of rats were trained on a FR-1 schedule of reinforcement to self-administer 10% ethanol or 0.25 mg/inf. cocaine. Following 14 days of acquisition during which the rats self-administered cocaine (baseline: 13.47 ± 0.3 infusions; n=7) or ethanol (baseline: 22.7 ± 2.04, deliveries; n=11) they underwent 14 daily extinction session during which lever presses did not result in the delivery the drug. At completion of the extinction (responses: 3.36 ± 1.03 for ethanol and 6.79 ± 0.73 for cocaine), the two groups of rats were subjected to an intermittent 15 min foot-shock (current intensity 0.5 mA, train length 0.5 sec, variable interval 40 sec), and then the levers were presented to measure alcohol or cocaine seeking-behavior. Foot-shock significantly reinstated lever pressing for both alcohol [19.90 ± 5.1 responses, $F(1,10)= 14.31$ $P < 0.01$] and cocaine [25 ± 5.49 responses, $F(1,6)= 11.35$ $P < 0.05$]. Pretreatment with Noc/oFQ (100-2000 ng/ICV) just before the stress significantly inhibited stress-induced alcohol-seeking behavior [$F(3,10)= 3.50$ $P < 0.05$], but not cocaine relapse ($P > 0.05$). The present result, support the hypothesis that Noc/oFQ system is involved in the regulation of stress mechanisms In addition, because Noc/oFQ prevents only stress-induced relapse to alcohol allows to speculate that different neural mechanisms are involved in cocaine and alcohol-seeking behavior.

Lack of effects of prenatal blockade of CB_1 canabinoid receptors on the response to the dopamine D_2 receptor agonist quinpirole

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Recent reports have established that the endogenous cannabinoid system plays a role in the modulation of dopamine signaling in the brain. Previous works in our laboratory have also shown that perinatal stimulation of the endogenous cannabinoid system with cannabinoid CB-1 receptor agonists induced developmental alterations in dopaminergic neurons in rodents. These alterations include the appearance in the adult animals, perinatally exposed, of a vulnerable phenotype to the reinforcing effects of opioids, as well as in a general disturbance in stress responses. The present study was aimed to evaluate the effects of maternal exposure to the cannabinoid receptor antagonist SR 141716A on the functionality of the dopaminergic system in the pups as adults. Maternal exposure to the cannabinoid antagonist did not affect the behavioral responses to the dopamine D-2 receptor agonist quinpirole in the adult, neither in female nor in male pups. However, prenatal exposure to SR 141716A resulted in a potentiation of the behavioural effects of the cannabinoid receptor agonist HU-210. In these animals exposure to HU-210 resulted in an increase in immobility and catelepsy, indicating the presence of a more sensitive cannabinoid receptor-mediated responses as results of prenatal blockade with SR141716A. The present results indicate that cannabinoid receptor blockade does not profoundly affect the dopaminergic systems but may induce long lasting effects on cannabinoid CB-1 receptors.

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A role for the endogenous cannabinoid system in motivational aspects of food intake

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Exposure to *cannabis* preparations has been associated to alterations in appetite. The existence of specific receptors in the brain for the psychoactive compounds present in marijuana points to the existence of an endogenous cannabinoid tone regulating appetite. The present work explored the effects of drugs acting at the endogenous cannabinoid system on different aspects of food intake. Acute administration of a cannabinoid receptor agonist, HU-210, decreased operant responses for food in food restricted animals. This effect appear at doses which induced immobility in the open field test. The administration of the cannabinoid CB-1 receptor antagonist SR141716A did not affect operant responses for food. These data indicate that the endogenous cannabinoid system does not affect motivational responses for food in food restricted animals. However, this was not the case when the animals were tested for food intake after 24 hr.of food deprivation. Thus, acute administration of the indirect cannabinoid agonist AM404 (an anandamide uptake blocker) induced increased feeding both in food deprived animals, or in animals food deprived but partially satiated before drug administration. Additionaly, the cannabinoid receptor antagonist reversed the stimulatory effect on food intake of AM 404, while it was able of decreasing total food intake by its own. Taken together, these results indicate that the endogenous cannabinoid system plays a role in motivational aspects of food intake, being able of modifying satiety signals produced after eating.

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Reversal of dopamine D_2-receptor responses by an anandamide transport inhibitor

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The endogenous cannabinoid anandamide is released by activation of D_2-family dopamine receptors in rat dorsal striatum, where one of its functions may be to modulate dopamine-induced facilitation of motor activity. Thus inhibitors of anandamide transport, a primary route of anandamide inactivation, might offer a strategy to correct behavioral abnormalities associated with excess dopamine activity. Like clinically used antipsychotic drugs, the anandamide transport inhibitor AM404 attenuated hyperactivity and stereotypies elicited by quinpirole and apomorphine, two dopamine agonists. On its own AM404 significantly reduced movement, but it did not elicit other typical effects of cannabinoid or antipsychotic drugs such as catalepsy. Anatomical studies indicated that AM404-sensitive anandamide transport is localized in brain regions that receive extensive dopaminergic innervation and are critically involved in motor control. Our findings support an important role of anandamide in the regulation of movement, and suggest that anandamide transport might represent a target for novel psychotherapeutic agents.

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Cocaine activation discriminates dopaminergic projections by temporal response: an fMRI study

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We applied a sensitive new functional magnetic resonance imaging technique to identify the pattern and determinants of cocaine-induced brain activation in drug naive rats as compared with rats which had self-administered cocaine for a period of 3 weeks. At doses greater than 0.1 mg/kg iv, cocaine produced robust activation throughout cortex and selectively within dopamine innervated subcortical structures including dorsomedial and ventrolateral striatum, nucleus accumbens, and dorsal thalamus in both sets of animals.. Although the spatial pattern and dose response of cocaine-induced brain activation was similar in both groups, the temporal pattern of response differentiated regions along distinct neuroanatomic boundaries: animals that had previously self-administered cocaine demonstrated a delayed and significantly protracted duration of activation in the terminal fields of dopaminergic projections to the forebrain known to originate in the Ventral Tegmental Area. Pharmacologic specificity was demonstrated by blocking cocaine-induced brain activation with SCH-23390, a selective D1-antagonist, in both sets of animals. Our data demonstrate the utility of fMRI to identify spatio-temporal patterns of cocaine-induced brain activaton, implicate D1 dopaminergic mechanisms, and reveals that previous exposure to cocaine accentuates a delayed time to peak activation and protracted duration of activation of meso-limbic-cortical brain circuits (supported by NIH grants DA-09467, DA-00384, DA-00354).

EXPRESSION OF THE CANNABINOID RECEPTOR CB1 IN DISTINCT NEURONAL SUBPOPULATIONS OF THE ADULT MOUSE FOREBRAIN.

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The recently discovered cannabinoid system is involved in many functions of the mammalian brain, such as control of motor behaviour, learning and memory, cognition and pain perception. To understand physiological and pharmacological mechanisms of cannabinoids' action, it is a prerequisite to determine the CB1-positive neurons at single cell resolution. Based on morphological features, CB1-expressing neurons in hippocampus and other cortical regions have been proposed to be GABAergic. To test this notion, we performed a double ISH study on the murine forebrain, using a probe for CB1 in combination with probes for glutamic acid decarboxylase 65k (GAD 65), for the calcium binding proteins parvalbumin (PV), calretinin (CRT) and calbindin D28k (C28), and for the neuropeptide cholecystokinin (CCK), respectively. Our results revealed that CB1-expressing cells can be divided into distinct neuronal subpopulations. One general criteria is characterized by the levels of CB1 expression. The majority of high CB1-expressing cells are GABAergic neurons belonging partly to the CCK-positive and PV-negative type of interneurons (basket cells), and at lower extent to the C28-positive mid-proximal dendritic inhibitory interneurons. Low CB1-expressing cells are only partly GABAergic. In hippocampus, amygdala and entorhinal cortex area, CB1 mRNA is present at low but significant levels in many non-GABAergic cells that can be considered as projecting CCK-positive principal cells. The high degree of CB1 coexpression with GAD 65 and with CCK opens new perspectives to understand the mechanisms by which cannabinoids exert their modulatory effects in the forebrain.

Effects of cocaine on dopamine uptake in frontal cortex and striatum in NET knock-out mice

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It has been described that the reinforcing effects of cocaine are mainly related to the inhibition of dopamine (DA) reuptake into neurons of the mesolimbic dopamine pathway. However, it is known that DA uptake occurs through the norepinephrine transporter (NET) in frontal cortex.

The effects of cocaine and other monoamine transporter blockers on [^{3}H]-DA uptake into synaptosomes obtained from frontal cortex and striatum were evaluated in wild-type and NET knock-out mice. Cocaine inhibited DA uptake in wild-type mice being approximately 1000-fold more potent in striatum than in frontal cortex. In presence of the selective NET blocker nisoxetine, in a concentration that completely blocks NET, cocaine failed to produce an effect on DA uptake in frontal cortex. Under the same experimental conditions, DA uptake was blocked in a dose-dependent manner in striatum of wild-type mice.

Studies conducted in the NET knock-out mice showed that cocaine did not affect DA uptake in frontal cortex. The cocaine dose-dependent curve was shifted to the right when compared to the one obtained from "wild-type" mice in striatum.

Since these animals self-administered cocaine as well as the wild-type, the data obtained suggest that the ability of cocaine to block DA uptake in the frontal cortex might be not involved in the mechanisms underlying the reinforcing effects of this drug.

D3 RECEPTOR PARTIAL AGONIST REDUCES COCAINE- BUT NOT HEROIN-SEEKING BEHAVIOUR MEASURED USING A SECOND-ORDER SCHEDULE OF REINFORCEMENT.

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Previous studies in our laboratory have established a second-order schedule of intravenous cocaine and heroin reinforcement, as a method for investigating drug-seeking behaviour. In the present study, we have studied the effects of drugs acting primarily at the D3 dopamine receptor on this cue-controlled drug seeking behaviour. In particular, we have evaluated the effects of a dopamine D3 receptor partial agonist (BP 897) , and two dopamine D3 receptor antagonists (U99194A and nafadotride), as well as a combination of BP 897 and nafadotride, on responding for cocaine and heroin under a second-order schedule of reinforcement. Pre-treatment with the dopamine D3 receptor partial agonist BP 897 (0.05, 0.5 1.0 mg/kg) reduced cocaine-seeking behaviour, as responding for cocaine decreased significantly and dose dependently. This effect was long-lasting and highly specific, the drug having no measurable effect on other behavioural measures such as locomotor activity. Moreover, BP 897 at the same doses did not affect cocaine self-administration itself and was not self-administered when substituted for cocaine. Injections of the D3 receptor antagonist U99194A (8, 16, 32 mg/kg) did not affect cocaine-seeking behaviour. By contrast, administration of nafadotride (0.0125, 0.125, 0.25 mg/kg) decreased cocaine-seeking behaviour The lowest dose of this antagonist also blocked the effect of the partial agonist when administered before it Pre-treatment with BP 897 was without effect on heroin-seeking behaviour at any dose.

The results show that the selective D3 receptor partial agonist BP 897 reduced cocaine- but not heroin seeking behaviour, and is itself without primary reinforcing effects. This may suggest that such drugs may have therapeutic potential in the treatment of aspects of cocaine addiction.

Modulation of $GABA_A$ receptor activity by protein kinase C

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$GABA_A$ receptor activity can be regulated by Ser, Threonine and tyrosine phosphorylation. PKC has been implicated in $GABA_A$ receptor regulation, but very little is known about receptor modulation by specific PKC isoforms. We recently developed PKC-ε mutant mice and found that these mutant mice have greater sensitivity to the sedative effects of ethanol, pentobarbital and diazepam than wild type littermates, suggesting altered function of $GABA_A$ receptor (Hodge et al). To examine $GABA_A$ receptor function directly, we measured muscimol-stimulated ^{36}Cl flux in microsacs prepared from cerebral cortex. $GABA_A$ receptors isolated from PKC- ε mutant mice show a greater sensitivity to allosteric modulators of GABA receptors such as benzodiazepines and ethanol. Flunitrazepam (0.1μM) increased muscimol stimulated flux by 109%± 17% in mutant mice and only 47%± 27% in wild type mice ($p<0.05$ %, t-test). Similarly, ethanol increased muscimol-stimulated ^{36}Cl uptake by 92% ± 17% in mutant mice and only 43± 15% in wild type mice ($p<0.05$, t-test). However, the flux stimulated by 1-20 μM Muscimol was no different in wild-type and mutant microsacs. To evaluate the possibility of a developmental alteration in PKC-ε mutant mice, we incubated microsacs from wild-type mice with a PKC-ε specific inhibitory peptide. We found that the sensitivity of $GABA_A$ receptor to benzodiazepines and ethanol was enhanced by 187%± 69 in the presence of this peptide, while a scrambled version of this peptide had no effect. Similarly the inhibitory peptide had no effect on PKC-ε mutant microsacs. These findings suggest that PKC-ε regulates the effects of ethanol and benzodiazepines, and this might be due to modulation of $GABA_A$ receptors by PKC-ε.

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BEHAVIOURAL AND BIOCHEMICAL EVIDENCE FOR REWARDING EFFECTS OF Δ9-TETRAHYDROCANNABINOL IN MICE

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Δ9-tetrahydrocannabinol (THC) is the psychoactive constituent of marijuana, the most widely consumed illicit drug. However, the rewarding properties of THC are difficult to be demonstrated in rodents [1]. In this study, we used different behavioural protocols to evaluate the motivational responses of THC in the place conditioning paradigm. An elevated number of pairings (5 pairings with THC + 5 pairings with vehicle) and a long time of conditioning (45 min per session) have been used in all the assays. In a first experiment, mice were conditioned with vehicle or THC (1 or 5 mg/kg, ip). A place aversion was observed with 5 mg/kg of THC using a standard protocol. Similar results were obtained when the CB-1 receptor antagonist SR 141716A (1 mg/kg, ip) was administered immediately after each THC conditioning period, before to return the mice to the home cage. However, mice receiving a priming THC injection in the home case and conditioned 24 h later showed a significant place preference with 1 mg/kg of THC and not effect with 5 mg/kg of THC. The same responses were obtained by using this procedure in mice receiving SR 141716 after each THC conditioning session. Interestingly, 10 min after THC (1 mg/kg) administration *in vivo* ERK activation was strongly increased in some mouse brain areas such as nucleus accumbens (core and shell) and central amygdala. Moreover, as previously reported[2] this ERK activation is critically involved in zif 268 mRNA regulation since the MEK inhibitor SL327 prevented it. These results reveal that (1) THC produces a clear place preference in mice by using a long period of conditioning and avoiding the possible dysphoric consequences of the first drug exposure (2) MAPkinase signalling pathway in the limbic system could be involved in the instatement of the rewarding effects induced by THC. *Supported by Dr Esteve S.A. Laboratories.*

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Effects of chronic nicotine treatment on brain nitric oxide and nitric oxide synthase

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Nicotine exerts its central actions by impinging on nicotinic acetylcholine receptors and regulating ionic fluxes. These actions, however, exhibit sexual dimorphism in the sense that, in rats, males and females show differences in receptor densities and regulation by chronic nicotine treatment[4,6]. Nicotine- may be modifying events occurring beyond the nicotinic receptor, including the regulation of nitric oxide (NO) synthases (NOS), either by increasing intracellular Ca^{2+} or through the actions of hormones and neurotransmitters involved in its action. Sex differences in brain NO_2+NO_3 levels, stabile metabolites of NO[7], and modulation by gonadectomy[3] have been shown. The present study was undertaken to analyze the effects of chronic nicotine administration on NO production and NOS expression by the measuring of NO_2+NO_3 levels and the cell density of neurones immunoexpressing NOS and the related histochemical activity NADPH-diaphorase (ND) respectively. These effects were analyzed in male and female different brain regions, cortex, corpus striatum and nucleus accumbens.

Two sets of animals (males and females) were injected with nicotine (15 days/once daily; 0.4 mg/kg, sc.); controls were injected with saline. One set of rats were decapitated, their brains dissected and rapidly cut with a vibratome into coronal 200 mm slices. Tissue from striatum, acumbens and cortex was taken using the micropunching technique[5]. NO_2+NO_3 levels from tissue homogenates were measured by Griess reaction after enzymatic reduction[1,2]. Tissue nitrite and nitrate levels expressed as mmol g/wet weight. The other set of animals were fixed by perfusion with Somogyi fixative, the brains removed, postfixed, and cut in a cryostat on 30 mm coronal sections. Free-floating sections were Nissl stained, immunolabelled for neuronal NOS or processed for ND-histochemistry. Assessment of the density of stained neurones was made by referring the number of positive cells to the area of each compartment where they appeared. Statistical analysis was performed to compare nicotine-treated vs. control animals and to compare sexes within each region analyzed.

A multifactorial ANOVA with Sex (male, female), Treatment (naïve, saline or nicotine), and Brain Region (cortex, n.accumbens, c.striatum) as the factors and cell counts as the dependent variable revealed significant main effects of Brain Region [$p<0.0001$] and Treatment [$p<0.0001$]. Saline injections reduced NOS positive neurons and nicotine caused an increase.

Nicotine causes an overall increase in NOS positive neurons. Stress arising from injections reduces the number of NOS positive neurons, and this effect is the most prominent in the female corpus striatum. In preliminary studies, biochemical assays of NO_2+NO_3 in rat brain following nicotine treatment reveal a greater level of NO production compared to the present study, suggesting the involvement of other NO sources different from neurons.

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