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Altered amygdalar endocannabinoid signaling in ethanol dependent animals

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Withdrawal in alcoholics is characterized by an acute period of physical discomfort followed by a protracted period of negative emotional symptoms such as increased anxiety and depression. Because this deficit in affective state can be alleviated through continued drinking, this process may constitute a major driving force for continued alcohol consumption. However, the neural mechanisms underlying this negative reinforcement process are not well understood. A growing body of evidence shows that acute ethanol exposure alters brain endocannabinoid levels and that chronic ethanol exposure induces persistent changes in the function of this system. Moreover, recent data suggests that anxiogenic situations activate the endocannabinoid system, which serves to dampen neuronal responses that contribute to anxiety-like behavior. Based on this evidence, it may be hypothesized that alterations in endocannabinoid function contribute to dysregulations of stress responsivity and affective state associated with ethanol dependence. This presentation will focus on recent work evaluating the effects of chronic ethanol exposure and abstinence on endocannabinoid signaling in the rat central nucleus of the amygdala (CeA). We find that endocannabinoid levels in the CeA are substantially reduced in ethanol-dependent animals during acute abstinence, an effect that is corrected by resumed ethanol consumption. In contrast, ethanol consumption does not alter endocannabinoid levels in the CeA of non-dependent rats. The consequence of endocannabinoid manipulations on withdrawal-associated anxiety-like behavior and excessive ethanol self-administration will also be presented. Collectively, our findings suggest that deficient eCB signaling in the CeA may underlie a sensitized anxiety-like phenotype that may contribute to excessive ethanol consumption.

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Involvement of CB1 receptor in the behavioural and biochemical processes underlying ethanol addiction

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There is considerable evidence that the endocannabinoid system plays a significant role in appetitive drive and associated behaviours. Attenuation of the activity of this system would have therapeutic benefit in treating disorders that might have a component of excess appetitive drive or over-activity of the endocannabinoid system, such as ethanol addiction. Studies using CB1 knockout mice have demonstrated that this receptor participates in the control of several behavioural responses including locomotion, anxiety- and depressive-like states and in the behavioural processes underlying ethanol addiction. We used CB1 knockout mice generated in a CD1 background to study several behavioural responses to ethanol and also the neuroadaptations regarding both NMDA and GABAA receptors after chronic ethanol exposure. We compared the results with those obtained in adenosine A2A receptors knockouts since these GPCR are also localized in the reward system and because in contrast to CB1 receptors, A2A receptors are coupled to Gs protein activating adenylate cyclase. In the present work, we have investigated several behavioural responses to ethanol such as sensitivity (locomotion, sedation, hypothermia), tolerance, consumption, reward, withdrawal severity in A2A, CB1 and A2A/CB1 knockout mice that were generated in different genetic backgrounds (CD1, C57BL/6J). Both glutamatergic and gabaergic systems are known for their sensitivity to acute alcohol exposure and adaptation after chronic exposure, thus experiments were designed to investigate the effect of lacking CB1 receptors in the neuroadaptations induced following chronic alcohol exposure on NMDA and GABAA receptors. Our results show that both A2A and CB1 receptors are involved in the sensitivity to the acute effects of ethanol and may play a role in alcohol-drinking behavior. Importantly, our results also stress the importance of including the effect of the genetic background when using knockout mice in the behavioral effects of alcohol. Our results indicate that the CB1 receptor is involved in the behavioural and biochemical processes underlying ethanol addiction.

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Role of CRF and orexin/hypocretin in mediating dopamine neuron activity and ethanol-dependent behaviors

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The VTA dopamine (DA) neurons, their projections to the nucleus accumbens (NAcb), and neurons within the NAcb form the mesolimbic system, a key modulator of drug-dependent behaviors. Increasing evidence suggests that stress-dependent ethanol consumption is modulated by CRF receptors acting on dopaminergic neurons, although the synaptic events and the mechanism underlying this phenomenon are poorly understood. My laboratory has recently demonstrated that CRF, a peptide released during stress, excites dopaminergic neurons by increasing NMDAR-mediated responses. Furthermore, we are interested in better understanding the contribution of lateral hypothalamic (LH) projections to the VTA, which are considered a critical element in motivation and reward circuits activated by drugs of abuse. A recent study from my laboratory suggests that orexin/hypocretin, similarly to CRF, promotes dopaminergic neurotransmission by increasing NMDAR activity and firing rate of DA neurons. Furthermore, we have provided evidence that orexin release in the VTA plays a central role in the development of behavioral sensitization, a behavioral index of drug craving, as well as in mediating cocaine self-administration. While there is evidence that stress increases ethanol-dependent behaviors and that orexin/hypocretin modulates addictive behaviors produced by both cocaine and morphine, the role of these peptides in modulating ethanol-dependent behaviors is unknown. Here, we will present evidence for a mechanistic relationship between CRF and orexin/hypocretin in the VTA, and the role of these two peptides in underlying stress-dependent ethanol drinking via activation of VTA neurons. We believe that both CRF and orexin/hypocretin effects in the VTA, if investigated in more detail, might represent promising therapeutic targets to counteract alcohol abuse.

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Opposing roles of amygdala PKC delta and epsilon on neuropeptide expression, anxiety, and alcohol consumption

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Amygdala CRF promotes anxiety and alcohol consumption, whereas amygdala NPY reduces these behaviors. Mice lacking the novel protein kinase C (PKC) isozyme, PKCε, show reduced anxiety-like behavior and reduced ethanol consumption. Recently we found that mice lacking a different novel PKC, PKCδ, show increased anxiety and ethanol consumption. Here we investigated whether these behaviors are due to altered expression of amygdala CRF or NPY. We found that CRF was reduced in the amygdala of PKCε knockout mice. In cultured amygdala neurons, a selective PKCε activator increased pro-CRF levels; this effect was prevented by knockdown of PKCε through RNA interference. Infusion of CRF into the amygdala of PKCε knockout mice increased anxiety-like behavior to wild type levels. Finally, reducing PKCε levels in the amygdala of wild type mice by RNA interference reduced anxiety-like behavior and decreased ethanol consumption. In contrast, mice lacking PKCδ showed reduced levels of amygdala NPY and knockdown of PKCδ in the amygdala of wild type mice increased anxiety-like behavior, mimicking the phenotype of PKCδ knockout mice. In addition, PKCδ knockout mice that had repeatedly self-administered

alcohol for two weeks showed reduced anxiety-like behavior immediately after a drinking session, suggesting that their increased alcohol consumption is partly motivated by the anxiolytic effect of alcohol. Current studies are determining whether PKCδ regulates NPY expression in amygdala neurons and whether knockdown of amygdala PKCδ increases ethanol consumption. Thus, PKCε and PKCδ may regulate anxiety-like behavior and alcohol consumption through differential actions on amygdala expression of CRF and NPY.

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Orexin neurons in lateral hypothalamus: A role in alcohol and drug abuse

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The orexins (or hypocretins) are neuropeptides recently identified as neurotransmitters in hypothalamic neurons. Although studies show orexin neurons are involved in arousal and sleep, these cells also project to reward-associated brain regions, including the nucleus accumbens and ventral tegmental area (VTA). This indicates a possible role for these neurons in reward function, consistent with previous studies implicating these neurons in feeding. Here, we show that activation of orexin neurons in lateral hypothalamus (LH) is strongly linked to preferences for stimuli associated with drug or food reward. We also recently found that the Orexin1 (Ox1) antagonist SB334867 blocked cue-induced reinstatement of extinguished cocaine self-administration. In addition, we show that local activation of LH orexin neurons reinstates an extinguished conditioned place preference (CPP) for morphine. Moreover, orexin injected into the VTA also reinstated morphine CPP. In addition, we have recently developed a CPP paradigm in which rats show preference for an environment in which they drank 10% ethanol. Studies are aimed at determining whether orexin neurons play a role in this ethanol reward. Recent results reveal that SB334867 attenuates cue-induced reinstatement of ethanol seeking in rats. These data indicate that reward for ethanol and drugs, as well as food, involves the LH orexin system, and reveal a novel role for LH orexin neurons in reward-seeking, drug and alcohol relapse, and addiction.

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Role of stress in the effects of alcohol on the nervous system

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The neurological effects of ethanol consumption are complex, encompassing effects on both the central and peripheral nervous systems. We have used a sensory neuron involved in alcoholic neuropathy, the primary afferent nociceptor, as a model cell to study the effects of alcohol on the nervous system and its modulation by stress. Chronic alcohol consumption induces a painful small-fiber peripheral neuropathy, the severity of which increases during alcohol withdrawal. Ethanol administered to animals, to achieve blood concentrations similar to those observed in humans who chronically abuse alcohol, causes excitatory changes in membrane currents in cultured sensory neurons. Alcohol consumption also potently activates the two major neuroendocrine stress axes, leading to the sustained release of glucocorticoids and catecholamines, which is also exacerbated during alcohol withdrawal, in a model of binge drinking. The presence of adrenergic and glucocorticoid receptors on sensory neurons couples with the persistent increased plasma concentrations of glucocorticoids and catecholamines in alcoholics led us to evaluate their role in alcohol neuropathic pain. We have observed that adrenal medullectomy and administration of a glucocorticoid receptor antagonist, mifepristone, both prevented and reversed painful peripheral neuropathy in alcohol binge-drinking rats. Chronic administration of stress levels of epinephrine to rats that had undergone adrenal medullectomy reconstituted this phenotypes. Attenuation of beta2-adrenergic- or glucocorticoid-receptor in nociceptors also prevented and reversed the pro-nociceptive effects of ethanol. Our results suggest a convergence of the effects of mediators of the hypothalamic-pituitary- and sympathoadrenal-stress axes on sensory neurons in the induction and maintenance of alcohol-induced painful peripheral neuropathy.

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Surprising actions of ethanol on the NMDA receptor in the dorsal striatum

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Drug and alcohol addiction are characterized by compulsive drug-taking and -seeking and these behaviors are increasingly viewed as a maladaptive and persistent habit. Increasing evidence suggests that the dorsal striatum plays a critical role in processes that underlie habit learning. The NMDA receptor is a major mediator of synaptic plasticity, a process that underlies learning, including habit learning. The NMDA receptor is also a major target for ethanol in the brain. Our research, employing a combination of molecular, electrophysiological and behavioral studies, was therefore aimed to determine whether and how ethanol exposure alters the activity of the NMDA receptor in the dorsal striatum. We will present data to suggest that the upregulation of the NR2B-containing NMDA receptors in response to ethanol contributes to the mechanisms underlying of alcohol drinking behavior. Specifically, we observed a long-term facilitation of NMDAR activity in dorsal striatal neurons following ethanol exposure, which was mediated by the activation of Fyn kinase and the phosphorylation of the NR2B subunit. Repeated administration of ethanol in vivo resulted in a long-lasting increase in the contribution of the NR2B subunit to the activity of the channel in this brain region. Finally, we provide evidence to suggest that Fyn and NR2B-containing NMDA receptors in the dorsal striatum contribute to alcohol drinking behavior. This signaling cascade that is activated by ethanol may be an important mechanism leading to the habitual and compulsive ethanol-seeking and -taking which characterize alcoholism.

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NMDA receptors are alcohol sensors in the brain

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N-methyl-D-aspartate (NMDA) receptors are glutamate-activated ion channels essential for normal brain function. Studies conducted by this laboratory and others have shown that alcohol inhibits NMDA receptor function and that both NR2 subunits and NR1 splice variants determine the acute alcohol sensitivity of the receptor. The novel NR3 NMDA subunit has subtle effects on alcohol inhibition of NR1/NR2 receptors. NR3 subunits can also form glycine-activated receptors in combination with NR1 subunits and the alcohol sensitivity of these receptors is relatively modest. The site of action of alcohol on NMDA receptors is unknown although recent studies suggest that amino acids within the TM3 or TM4 domains of NR1 and NR2 subunits may define an alcohol site of action. In neurons, NMDA receptors are expressed at synaptic and extrasynaptic sites and are normally activated only during periods of coordinated pre- and post-synaptic activity. This feature along with their high calcium permeability is critical in mechanisms of synaptic plasticity involved in associative learning and cognition. Despite the somewhat modest effects of alcohol on NMDA receptor activity (less than 50% in most cases), alcohol induces profound changes in networks whose activity requires functional NMDA receptors. For example, in the prefrontal cortex, alcohol disrupts network-dependent persistent activity of deep-layer pyramidal neurons. This effect results from inhibition of NMDA receptors and occurs at concentrations of alcohol that have no effect on AMPA or GABAA receptors. Chronic exposure to alcohol induces compensatory increases in the synaptic localization of NMDA receptors that may lead to maladaptive plasticity associated with chronic alcohol intake. Overall, NMDA receptors are sensitive detectors of brain alcohol.