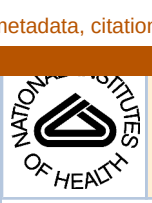

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The Role of Neuroactive Steroids in Ethanol/Stress Interactions Proceedings of Symposium VII at the Volterra Conference on Alcohol and Stress, May 2008

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Abstract

This report summarizes the proceedings of the symposium VII on the role of neuroactive steroids in stress/alcohol interactions. The production of GABAergic neuroactive steroids, including (3α,5α)-3-hydroxypregnan-20-one (3α,5α-THP) and (3α,5α)-3,21-dihydroxypregnan-20-one (3α,5α-THDOC) is a consequence of both acute stress and acute ethanol exposure. Acute, but not chronic ethanol administration elevates brain levels of these steroids and enhances $GABA_A$ receptor activity. Neuroactive steroids modulate acute anticonvulsant effects, sedation, spatial memory impairment, anxiolytic-like, antidepressant-like and reinforcing properties of ethanol in rodents. Furthermore, these steroids participate in the homeostatic regulation of the hypothalamic-pituitary-adrenal (HPA) axis. Therefore, it is not surprising that neuroactive steroids are involved in ethanol/stress interactions. Nevertheless, the interactions are complex and not well understood. This symposium addressed the role of neuroactive steroids in both stress and alcohol responses and their interactions. Professor Giovanni Biggio of the University of Cagliari, Italy presented the effects of juvenile isolation stress on neuroactive steroids, $GABA_A$ receptor expression and ethanol sensitivity. Professor Howard Becker of the Medical University of South Carolina, USA presented evidence for neuroactive steroid involvement in ethanol dependence and drinking behavior. Professor Patrizia Porcu of the University of North Carolina, USA described a potential neuroactive steroid biomarker that may predict heavy drinking in monkeys and mice. These presentations provide a framework for new theories on the nature of ethanol/stress interactions that may be amenable to therapeutic interventions.

Keywords

neuroactive steroids; $GABA_A$ receptors; social isolation; C57BL/6J mice; DBA/2J mice; HPA axis

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Introduction

The production of GABAergic neuroactive steroids, including (3α,5α)-3-hydroxypregnan-20-one (3α,5α-THP) and (3α,5α)-3,21-dihydroxypregnan-20-one (3α,5α-THDOC) is a consequence of both acute stress and acute ethanol exposure in rodents (Barbaccia et al., 1999; Purdy et al., 1991). However, chronic ethanol exposure blunts the elevation of neuroactive steroids observed following acute ethanol challenge (Khisti et al., 2005; Morrow et al., 2001b). Neuroactive steroids modulate acute anticonvulsant effects (VanDoren et al., 2000), sedation (Khisti et al., 2003), spatial memory impairment (Matthews et al., 2002; Morrow et al., 2001b), anxiolytic-like (Hirani et al., 2005) antidepressant-like (Hirani et al., 2002) and reinforcing properties of ethanol (Janak et al., 1998; Morrow et al., 2001b; Sinnott et al., 2002b) in rodents. Similarly, neuroactive steroids contribute to the discriminative stimulus effects of ethanol in rodents and monkeys (Bowen et al., 1999; Grant et al., 2008; Shelton and Grant, 2002), as well as subjective effects of ethanol in humans (Pierucci-Lagha et al., 2005).

GABAergic neuroactive steroids participate in the homeostatic regulation of the hypothalamic-pituitary-adrenal (HPA) axis. The activation of the HPA axis in response to acute stress increases the release of corticotropin releasing factor (CRF) from the hypothalamus that stimulates the release of adrenocorticotropic hormone (ACTH) from the pituitary, which in turn, stimulates the adrenal cortex to release glucocorticoids, neuroactive steroid precursors and GABAergic neuroactive steroids. Glucocorticoids, mainly cortisol in humans and non-human primates, and corticosterone in rodents, provide negative feedback upon the hypothalamus and pituitary. Likewise, GABAergic neuroactive steroids inhibit CRF production, release, ACTH release and subsequent corticosterone levels in rodents (Owens et al., 1992; Patchev et al., 1996; Patchev et al., 1994). The ability of neuroactive steroids to reduce HPA axis activation may play an important role in returning the animal to homeostasis following stressful events. The HPA axis response to stress challenge appears to be critical for mental health since it is dysregulated in various psychiatric disorders including alcoholism (Adinoff et al., 2005a; b; Adinoff et al., 1990; Clarke et al., 2008; Costa et al., 1996; Ehrenreich et al., 1997; Inder et al., 1995; Wand and Dobs, 1991), depression (de Kloet et al., 2005; Nemeroff and Vale, 2005; Nestler et al., 2002; Pariante and Lightman, 2008), and premenstrual dysphoric disorder (Girdler and Klatzkin, 2007). Considering the actions of ethanol to increase neuroactive steroids and the role of neuroactive steroids in stress responses, it is not surprising that neuroactive steroids are involved in ethanol/stress interactions.

The interactions of ethanol and stress are not well understood. This symposium proceeding addresses the role of neuroactive steroids in both stress and alcohol responses and their interactions. Professor Giovanni Biggio of the University of Cagliari, Italy described the effects of social isolation stress on $GABA_A$ receptor function and gene expression, and ethanol effects on neuroactive steroids, as well as adaptations to chronic ethanol exposure. Professor Howard Becker of the Medical University of South Carolina, USA described neuroactive steroid involvement in the drinking behavior of ethanol dependent mice. Professor Patrizia Porcu of the University of North Carolina, USA described a potential neuroactive steroid biomarker that may predict heavy drinking in monkeys and mice. Together, these presentations elaborate current theories on the nature of ethanol/stress interactions that may be amenable to therapeutic intervention for treatment of alcoholism.

Juvenile social isolation stress changes neuroactive steroid levels and ethanol sensitivity

Rats deprived of social contact with other rats at a young age experience a form of prolonged stress that leads to long-lasting alteration in their behavioural profile. This chronic stress paradigm is thought to be anxiogenic for these normally gregarious animals and their abnormal reactivity to environmental stimuli, when reared under this condition, is though to be a product of prolonged stress (Hilakivi et al., 1989; Parker, 1986; Serra et al., 2000; Voikar et al., 2005; Wongwitdecha and Marsden, 1996). Social isolation of rats immediately after weaning is associated with a reduction in the cerebrocortical and plasma concentrations of progesterone and its metabolites 3α,5α-THP and 3α,5α-THDOC (Serra et al., 2000).

The expression of specific $GABA_A$ receptor subunit genes and the consequent subunit composition of the receptor are affected by social isolation. The level of α_4 subunit immunoreactivity was also found to be increased throughout the hippocampus of socially isolated rats compared with group-housed rats (Serra et al., 2006). An increase in transcription of the gene for the α_4 subunit in the hippocampus of isolated rats together with the prolonged decrease in the level of 3α,5α-THP might contribute to the increase in seizure susceptibility observed in isolated rats, as well as to the anxiety-like behaviour apparent in such animals (Serra et al., 2000). Social isolation is also associated with small increases in the amount of δ subunit immunoreactivity in the hippocampus of rats (Serra et al., 2006). The increased expression of both of these α_4 and δ subunits in the hippocampus of isolated rats likely results in the formation of $GABA_A$ receptors that contain both these subunits (Sur et al., 1999). Given that the δ subunit substitutes for the γ_2 subunit and that the latter subunit is essential for synaptic localization of $GABA_A$ receptors (Essrich et al., 1998), receptors containing α_4 and δ subunits would be expected to be extrasynaptic. Accordingly, we found that the amplitude of $GABA_A$ receptor-mediated tonic inhibitory currents in granule cells of the dentate gyrus was markedly greater in hippocampal slices from socially isolated rats than in those from group-housed animals (Serra et al., 2006). The reduction in tonic current noise induced by bath application of the $GABA_A$ receptor antagonist bicuculline was significantly greater in hippocampal slices from isolated rats than in those from group-housed animals (Serra et al., 2006). In addition, the enhancement of tonic current induced by 3α,5α-THP was markedly greater in granule cells of the dentate gyrus from isolated rats than in those from group-housed animals (Serra et al., 2006).

The increased expression of $GABA_A$ receptors containing both α_4 and δ subunits associated with social isolation might also be expected to have important consequences for the ability of ethanol to directly modulate GABAergic inhibitory transmission, given that recombinant $GABA_A$ receptors containing these subunits are suggested to have a high and selective sensitivity to low concentrations of ethanol (Sundstrom-Poromaa et al., 2002), although this issue remains controversial (Borghese and Harris, 2007). We found that the potency of ethanol in increasing the amplitude of miniature inhibitory postsynaptic currents (mIPSCs) is markedly greater in hippocampal slices from isolated rats than in those from group-housed animals. Indeed, whereas ethanol at a concentration of 50 mM significantly increased mIPSCs amplitude in neurons from isolated animals, it proved ineffective in those from group-housed rats (Serra et al., 2006). This action of ethanol is mediated by an increased production of 3α,5α-THP, and was inhibited by finasteride, an inhibitor of 3α,5α-THP synthesis (Azzolina et al., 1997). This conclusion is consistent with our observation that ethanol increases local neuroactive steroid synthesis in the brain independent of the HPA axis (Sanna et al., 2004).

These findings, together with results showing that socially isolated rats are more sensitive to the effects of ethanol on the brain concentrations of 3α,5α-THP (Serra et al., 2003), suggest that chronic stress may induce plastic adaptation of neuronal systems that contribute to a

vulnerability to alcohol abuse. We have therefore recently examined whether chronic voluntary ethanol consumption or ethanol vapours exposure modifies the effects of isolation on neuroactive steroid concentrations, anxious behaviour, $GABA_A$ receptor gene expression and function and neuronal plasticity.

Immediately after weaning (PN21), isolated rats had 24-h access to increasing concentrations of ethanol or water for four weeks (two bottle choice, ethanol: increasing concentrations 2.5-10% w/v per week, or were exposed to ethanol vapour for 12 h/day for eight days (ethanol concentration: 50 ml/h; air flow: 10 l/min; air pressure: 10 psi). Four experimental groups were used: isolated control, isolated ethanol, group housed control, group housed ethanol. Animals that had 24-hr access to ethanol were sacrificed between 9 and 10 a.m. Blood alcohol levels (BALs) in control animals were undetectable; rats exposed to ethanol vapor were sacrificed immediately after their removal from the chambers (10.00 a.m.), BALs: 205.5 ± 40.2 mg/dl.

Voluntary ethanol consumption by both group-housed and isolated rats slightly increased as the concentration of the ethanol solution increased in the first three weeks, and doubled during the 4^{th} week when the ethanol concentration was 10%. During the 1^{st} and 2^{nd} week, the amount of ethanol consumed by isolated animals was less than that consumed by group-housed rats: Week 1 (2.5% EtOH) grouped: 0.45±0.06, isolated: 0.26±0.04 g/kg/day, $p<0.05$; Week 2 (5% EtOH) grouped: 0.67±0.07, isolated 0.42±0.04 g/kg/day, $p<0.05$. At Week 4, (10% EtOH) there was no effect of rearing condition on ethanol intake (1.57±0.1 and 1.89±0.39 g/kg/day, in isolated and grouped, respectively). Daily intake and ethanol preference (i.e. the ratio of ethanol intake to total fluid intake) was related to kilogram of body mass. Although, the mean intake of ethanol did not significantly differ between the two groups (isolated, 13.7±1.1; grouped, 17.3±1.6 g/kg), isolated rats showed a significant decrease in ethanol preference compared to group-housed animals (13.9±1.1% vs. 21.1±2.1%, respectively, $p<0.01$).

Similar to previous results (Janis et al., 1998), we found that voluntary ethanol consumption induced a significant decrease in the cerebrocortical level of 3α,5α-THP both in social isolated and in group-housed rats (-47% and -44% vs respective water drinking control). On the contrary, ethanol vapour exposure resulted in an increase in the brain concentration of 3α,5α-THP with the percentage increases markedly greater in isolated rats than in group-housed animals (+211% and 91% vs respective air exposed control). Since at the time of the sacrifice, blood ethanol concentration was 205 mg/dl, intermittent vapor exposure for 8 days did not produce tolerance to the ability of ethanol to increase cortical 3α,5α-THP levels. Moreover, in agreement with an earlier study (Serra et al., 2003), this result shows that socially isolated rats are more sensitive to the effects of ethanol on the brain concentrations of 3α,5α-THP.

Both chronic ethanol treatments had no effect on α_4 subunit levels in the hippocampus of group-housed and socially isolated animals, whilst they induced an increase in immunoreactivity for the δ subunit of the $GABA_A$ receptor throughout the hippocampus of socially isolated and group-housed rats respective to each control (socially isolated and group-housed water drinking and air-exposed animals).

The increase in the expression of the δ subunit of the $GABA_A$ receptor induced by voluntary ethanol consumption correlates with the increase in $GABA_A$ receptor mediated tonic inhibitory currents in granule cells of the dentate gyrus. Moreover, the responsiveness of synaptic $GABA_A$ receptors to ethanol is reduced in the hippocampus of socially isolated animals with free access to ethanol, as demonstrated by the observation that 50 mM ethanol increased the amplitude of $GABA_A$ receptor mediated mIPSCs in CA1 pyramidal neurons of control isolated rats, but not in those of isolated rats that had free access to ethanol.

These results support the idea that the GABAergic neuroactive steroids play a crucial role in the capability of specific brain areas to express selective $GABA_A$ receptor subunits in response

to environmental changes. Alteration in this allostatic response may be an important factor that increases vulnerability to drug abuse and mental disorders.

Neuroactive steroid 3α,5α-THP involvement in ethanol dependence and drinking behavior in C57BL/6J mice

Prolonged excessive ethanol consumption can lead to the development of dependence. Ethanol dependence represents the culmination of a complex and dynamic process that has been shown to involve a host of neuroadaptive changes that are integral to brain reward and stress systems (for reviews see (Hansson et al., 2008; Heilig and Koob, 2007; Koob and Le Moal, 2008; Vengeliene et al., 2008)). Among many biological and environmental factors that play a role in governing drinking behavior, stress has been generally viewed as a significant factor in influencing ethanol drinking and, in particular, relapse (Brady and Sonne, 1999; Pohorecky, 1990; 1991; Sillaber and Henniger, 2004). Additionally, chronic ethanol exposure and withdrawal experience has been shown to profoundly alter brain stress systems, including HPA axis dysregulation and activation of CRF (neuroendocrine-independent) pathways (Heilig and Koob, 2007; Rivier, 2000; Wand, 2000). Resultant changes in extra-hypothalamic CRF activity as well as circulating levels of glucocorticoids may have ramifications for ethanol's motivational properties through interaction with brain reward circuitry (Fahlke et al., 1996; Piazza and Le Moal, 1997). HPA axis activation also is known to impact both circulating and brain levels of neuroactive steroids (e.g., 3α,5α-THP), which are potent $GABA_A$ receptor positive modulators that are known to interact with ethanol.

A large body of evidence indicates that neuroactive steroids modulate a variety of ethanol effects, including anticonvulsant, anxiolytic, ataxic/sedative, cognitive impairing effects, and the discriminative stimulus and reinforcing effects of ethanol (Finn et al., 2004a; Khisti et al., 2002; Morrow et al., 2001a). Further, acute ethanol administration has been shown to increase brain and plasma concentrations of neuroactive steroids in a dose-, time- and sex-dependent manner in rodents (Barbaccia et al., 1999; Finn et al., 2004a; Khisti et al., 2003; Morrow et al., 1999; O'Dell et al., 2004a; VanDoren et al., 2000), with somewhat similar findings reported in adolescent humans (Torres and Ortega, 2003; 2004). Similarly, exposure to various stressors increases brain and plasma concentrations of neuroactive steroids in both rodents and humans to levels sufficient to enhance $GABA_A$ receptor function and produce corresponding behavioral effects (Barbaccia et al., 1996; Droogleever Fortuyn et al., 2004; Purdy et al., 1991). In rodents, chronic ethanol exposure results in decreased brain and plasma neuroactive steroid levels while at the same time enhancing sensitivity to some (e.g., anticonvulsant), but not all (e.g., ataxia) effects of neuroactive steroids (Cagetti et al., 2004; Devaud et al., 1996; Janis et al., 1998). The magnitude and duration of this effect appears to be influenced by genotype and sex as well as the extent of chronic ethanol exposure (Alele and Devaud, 2007; Finn et al., 2004a; Finn et al., 2000). Additionally, neuroactive steroids have been shown to possess reinforcing properties (Finn et al., 1997a; Sinnott et al., 2002b) and to increase (Janak et al., 1998; Nie and Janak, 2003; Sinnott et al., 2002a) or decrease (Morrow et al., 2001b; O'Dell et al., 2005) ethanol self-administration, depending on dose and whether the subjects are dependent upon ethanol. Thus, alterations in neuroactive steroids following chronic ethanol exposure and withdrawal may contribute to enhanced propensity to drink in dependent subjects.

Since alcoholism is a chronic relapsing disease, many ethanol dependent individuals experience multiple bouts of heavy drinking followed by intervening periods of abstinence (withdrawal). Numerous studies using animal models have demonstrated increased ethanol responding and/or drinking in dependent compared to non-dependent subjects. For example, a history of ethanol dependence and repeated withdrawal experiences has been reported to increase voluntary ethanol consumption in mice (Becker and Lopez, 2004; Dhaher et al., 2008; Finn et al., 2007; Lopez and Becker, 2005) and rats (Rimondini et al., 2002; Sommer et

al., 2008). Likewise, studies using operant procedures have demonstrated increased ethanol self-administration in mice (Chu et al., 2007; Lopez et al., 2008) and rats (O'Dell et al., 2004b; Roberts et al., 1996; Roberts et al., 2000) with a history of repeated chronic ethanol exposure and withdrawal experience. Given that chronic ethanol exposure and withdrawal experience represent potent stressors and neuroactive steroids are responsive to a variety of stressful stimuli (including ethanol exposure/withdrawal), dependence-related perturbations in brain neuroactive steroid activity may have significant implications regarding motivation for ethanol self-administration behavior. The present study examined whether stress associated with repeated cycles of chronic ethanol exposure and withdrawal produces changes in brain 3α,5α-THP levels that relate to enhanced ethanol drinking in a mouse model of ethanol dependence and relapse drinking.

Adult male C57BL/6J mice were first trained to drink 15% (v/v) ethanol in a two-bottle choice (with water as the alternative fluid) limited access (2 hr/day) procedure. Mice were never food or water deprived during the experiment. After stable baseline daily intake was established, mice were then exposed to either chronic intermittent ethanol vapor (EtOH) or air (CTL) in inhalation chambers (16 hr/day for four days). At 72 hr following chronic intermittent ethanol (or air) exposure, all mice were given the opportunity to again drink ethanol in the limited access paradigm for five consecutive days. This procedure was repeated for a second cycle, and EtOH and CTL mice were sacrificed either immediately upon final withdrawal (HR-0), or at later times following withdrawal (HR-8 and HR-72), or after four days access to ethanol (Day-5). Brains were rapidly removed and cortex was dissected and shipped frozen to Prof. A. L. Morrow (UNC-Chapel Hill) for later determination of 3α,5α-THP levels using an established radioimmunoassay (RIA) procedure. At time of sacrifice, trunk blood samples were collected for measuring plasma corticosterone (CORT) levels by RIA. A separate group of EtOH-naïve mice were included for baseline cortical 3α,5α-THP and plasma CORT determinations.

Results indicated that EtOH mice consumed significantly more ethanol (3.1 ± 0.1 g/kg) compared to CTL mice (2.6 ± 0.1 g/kg) during test sessions. Additionally, ethanol consumption was significantly increased in EtOH mice above their own baseline level of intake (2.5 ± 0.1 g/kg), whereas ethanol consumption remained relatively stable in CTL mice throughout the study when compared to their own baseline intake (2.7 ± 0.1 g/kg). These results demonstrating enhanced voluntary ethanol consumption in dependent mice compared to non-dependent mice are in agreement with our previous findings using this same model of dependence and relapse drinking (Becker and Lopez, 2004; Lopez and Becker, 2005). These results are also in agreement with previous reports of elevated voluntary ethanol intake in ethanol dependent mice (Dhaher et al., 2008; Finn et al., 2007) and rats (Rimondini et al., 2002; Sommer et al., 2008).

Plasma CORT levels (collapsed across the different sacrifice time points) did not significantly differ between CTL mice (9.18 ± 0.62 ug/dl) and EtOH-naïve mice (8.63 ± 1.15 μg/dl). Immediately following chronic intermittent ethanol exposure (HR-0), plasma CORT levels were elevated above control levels (12.41 ± 0.10 ug/dl). At peak withdrawal (HR-8), plasma CORT levels were significantly higher in EtOH mice (23.07 ± 1.04 μg/dl), with levels returning to baseline control levels at 72 hr post-withdrawal (6.97 ± 1.26 ug/dl). Additionally, elevated ethanol drinking over four days did not change CORT levels in EtOH mice (Day-5: 7.21 ± 1.0 ug/dl). Overall, these results are congruent with findings indicating that chronic ethanol exposure and withdrawal activate the HPA axis, as measured by elevated circulating levels of glucocorticoids (Rivier, 2000; Wand, 2000).

3α,5α-THP concentrations in cortex were unchanged in EtOH mice immediately following chronic intermittent ethanol exposure (1.77 ± 0.13 ng/g) compared to pooled control levels (1.85 ± 0.10 ng/g) and the EtOH-naïve group (1.86 ± 0.11 ng/g). During peak withdrawal

(HR-8), 3α,5α-THP levels were slightly higher (2.05 ± 0.09 ng/g), but did not significantly differ from control levels. However, at the 72-hr time point, cortical 3α,5α-THP levels were significantly elevated above control levels (2.47 ± 0.16 ng/g). 3α,5α-THP levels in cortex returned to control baseline levels after four days of voluntary ethanol drinking in the model (1.75 ± 0.12 ng/g). This pattern of change in brain 3α,5α-THP levels following chronic ethanol exposure in our model differs from other reports, but this is not surprising given a number of significant methodological differences among the studies. For example, a small but significant decrease in cortical 3α,5α-THP levels was reported in rats chronically exposed to ethanol in a liquid diet (Janis et al., 1998). However, brain 3α,5α-THP levels returned to control levels at peak withdrawal (Janis et al., 1998). Similarly, no change in plasma 3α,5α-THP levels at peak withdrawal was reported in another study involving rats fed ethanol in a liquid diet (Devaud et al., 1996). In the present study, we found no significant change in cortical 3α,5α-THP levels compared to controls either immediately upon withdrawal or at peak withdrawal from chronic intermittent ethanol vapor exposure. However, brain 3α,5α-THP levels were significantly elevated at 72 hours post-withdrawal. To our knowledge, this is the first report of a change in brain 3α,5α-THP levels at a later stage during ethanol withdrawal. After four days of limited access drinking, cortical 3α,5α-THP levels returned to control levels. Ethanol intake under a limited access schedule has been reported to increase brain 3α,5α-THP levels in C57BL/6J mice (Finn et al., 2004b). In the present study, brain tissue was collected 24 hours (not immediately) following the last limited access drinking session. Further, it can not be determined from the present study whether return to control levels of the neuroactive steroid was due to prior ethanol intake in the dependent mice.

Overall, these results indicate that repeated cycles of chronic intermittent ethanol exposure activate the HPA axis, with elevated plasma CORT levels registered at peak withdrawal. Repeated cycles of chronic intermittent ethanol exposure also produced significant increases in 3α,5α-THP concentrations in cortex at 72 hours post-withdrawal relative to controls. Interestingly, this increase in cortical 3α,5α-THP levels in EtOH mice coincides with the time point when these animals typically demonstrate enhanced voluntary ethanol drinking (compared to CTL mice). Further, while greater ethanol consumption in EtOH mice compared to CTL mice over four days following the final withdrawal did not alter plasma CORT levels, this drinking experience was associated with 3α,5α-THP levels in cortex that approximated control levels. Whether elevated ethanol intake in dependent mice contributed to normalization of cortical 3α,5α-THP levels is unclear at present. Ongoing studies in our laboratory are aimed at addressing this question. As previously noted, the effects of chronic ethanol exposure and withdrawal on neuroactive steroids in brain are complex (Cagetti et al., 2004; Devaud et al., 1996; Finn et al., 2004a; Finn et al., 2000; Janis et al., 1998). Studies will need to examine the effects of chronic intermittent ethanol exposure on 3α,5α-THP levels in other brain regions, as well as evaluate the relationship of such changes to resultant HPA axis activation. Additionally, future studies will need to determine the extent to which changes in brain 3α,5α-THP levels influence excessive ethanol drinking in this model of ethanol dependence and relapse drinking.

Identification of a potential neuroactive steroid biomarker of alcohol consumption in monkeys and mice

Animal models of ethanol dependence show adaptations in HPA axis function and stimulated neuroactive steroid concentrations. Chronic ethanol consumption in rodents decreases corticosterone responses to stress (Spencer and McEwen, 1990) and blunts challenge-induced elevations of plasma and brain levels of 3α,5α-THP and deoxycorticosterone (Khisti et al., 2005; Morrow et al., 2001b). Similar alterations in the HPA axis are observed in cynomolgus monkeys after long-term ethanol self-administration (Porcu et al., 2006b) and in actively drinking or alcohol dependent human subjects (Adinoff et al., 2005a; b; Wand and Dobs, 1991).

We have recently demonstrated that plasma deoxycorticosterone and pregnenolone levels in ethanol naïve cynomolgus monkeys are differentially regulated by various challenges to the HPA axis (Porcu et al., 2006a; Porcu et al., 2006c). Plasma deoxycorticosterone levels are sensitive to hypothalamic and pituitary activation of the axis and to negative feedback mechanisms assessed by the dexamethasone test. Thus, administration of naloxone at the doses of 125 and 375 μg/kg, increased plasma deoxycorticosterone levels up to 86 and 97%, respectively. This is consistent with data showing an activation of the HPA axis and increased cortisol and ACTH levels in humans and non-human primates (Jackson et al., 1995; Wand et al., 1998; Williams et al., 2003). CRF (1 μg/kg) increased plasma deoxycorticosterone levels up to 111% while dexamethasone (130 μg/kg) decreased deoxycorticosterone levels by 42%, in agreement with a suppression of HPA axis activity. In contrast, administration of ACTH (10 ng/kg) 4-6 hours after 0.5 mg/kg dexamethasone had no effect on plasma deoxycorticosterone levels, suggesting that deoxycorticosterone synthesis is independent of ACTH stimulation of the adrenals.

Pregnenolone levels in the same cynomolgus monkey subjects were differentially regulated from deoxycorticosterone. Naloxone administration (125 and 375 μg/kg) increased plasma pregnenolone up to 222 and 216%, respectively. In contrast, CRF (1 μg/kg) and dexamethasone (130 μg/kg) had no effect on pregnenolone levels, while ACTH (10 ng/kg), 4-6 hours after 0.5 mg/kg dexamethasone, decreased plasma pregnenolone levels by 43%. CRF and ACTH administration decreased the ratio of plasma pregnenolone/deoxycorticosterone suggesting increased metabolism of pregnenolone into deoxycorticosterone or other neuroactive steroids (Porcu et al., 2006c). Thus, circulating pregnenolone levels are subject to complex regulation involving factors other than direct HPA axis modulation.

We also found a specific neuroactive steroid response to HPA axis challenge in ethanol naïve monkeys that was predictive of subsequent alcohol consumption. Dexamethasone suppression of plasma deoxycorticosterone levels in ethanol-naïve monkeys was negatively correlated with daily average ethanol consumption over the subsequent twelve months of alcohol self-administration (Pearson's $r = -0.78$, $p=0.006$). That is, the highest alcohol drinking was found in the monkeys that showed the lowest suppression of deoxycorticosterone levels in response to dexamethasone (Porcu et al., 2006a). In contrast, no other deoxycorticosterone responses to HPA axis stimulation in ethanol naïve monkeys were correlated with subsequent voluntary drinking. Thus, the dexamethasone suppression of plasma deoxycorticosterone levels may have the potential to become a trait marker of risk for high alcohol consumption in cynomolgus monkeys. More studies are needed to further explore this finding.

The relationship between dexamethasone suppression of deoxycorticosterone and subsequent voluntary alcohol consumption has not yet been explored in other species. Therefore, we investigated if dexamethasone suppression of deoxycorticosterone would predict alcohol consumption in two strains of mice that have been widely used in drinking behavior models, the C57BL/6J and DBA/2J mice.

C57BL/6J and DBA/2J mice are well characterized in terms of drinking phenotype. When using the two-bottle choice paradigm, C57BL/6J mice avidly drink alcohol, while DBA/2J mice avoid it (Belknap et al., 1977; Phillips et al., 1994). However, both strains will self-administer similar amounts of ethanol via the intravenous route (Grahame and Cunningham, 1997). These two strains also differ in many alcohol-related behavioral traits such as ethanol-induced locomotor activity and ethanol withdrawal severity (Phillips and Crabbe, 1991). DBA/2J mice have more severe handling-induced convulsions after withdrawal from both acute (Roberts et al., 1992) and chronic (Crabbe et al., 1983) ethanol. Furthermore, these mice show differences in the behavioral response to neuroactive steroids. C57BL/6J mice are more sensitive to the anxiolytic, locomotor stimulant, and anticonvulsant effects of 3α,5α-THP

compared to the DBA/2J mice (Finn et al., 1997b). We hypothesized that dexamethasone suppression of plasma and/or brain deoxycorticosterone levels may be correlated with consumption and/or preference for alcohol measured using the two-bottle choice paradigm.

Male C57BL/6J and DBA/2J mice (eight weeks old) were purchased from The Jackson Laboratories (Bar Harbor, ME, USA). After arrival at the animal facility, they were allowed to acclimate for one week. They were housed four/six per cage under 12h light, 12h dark cycle (light on from 0700 to 1900 h) and at a constant temperature of 22 ± 2°C and relative humidity of 65%. They had free access to water and standard laboratory food at all times.

Mice were injected with dexamethasone sodium salt (0.075, 0.1 or 0.13 mg/kg, subcutaneously) or saline at 8:00 am (beginning of the light cycle). They were sacrificed six hours later by decapitation. This method has been adapted from previous published papers that have shown a suppressed corticosterone response in C57BL/6J mice after dexamethasone (Bartolomucci et al., 2004; Groenink et al., 2002). Deoxycorticosterone responses to dexamethasone were measured in plasma and cerebral cortical samples by radioimmunoassay as previously described (Khisti et al., 2005; Porcu et al., 2006a).

C57BL/6J and DBA/2J mice did not differ in basal deoxycorticosterone levels measured after saline administration. Average (ng/ml) ± SEM for plasma deoxycorticosterone was 4.65 ± 0.39 (n=14) and 4.18 ± 0.59 (n=13) in C57BL/6J and DBA/2J mice, respectively; unpaired t-test $p = 0.50$. Average (ng/g) ± SEM for cerebral cortex deoxycorticosterone levels was 3.37 ± 0.46 (n=13) and 2.47 ± 0.46 (n=12) in C57BL/6J and DBA/2J mice, respectively; unpaired t-test $p = 0.18$.

Following dexamethasone administration, plasma deoxycorticosterone levels in DBA/2J mice were decreased by 61, 55 and 58%, at 0.075, 0.1 and 0.13 mg/kg, respectively ($p<0.001$). In contrast, plasma deoxycorticosterone levels in C57BL/6J mice were not altered by administration of dexamethasone 0.075 and 0.1 mg/kg; only the dose of 0.13 mg/kg induced a significant decrease (-62%, $p<0.05$).

Similar to plasma, cerebral cortical deoxycorticosterone levels in DBA/2J mice were decreased by 68, 85 and 82%, at 0.075, 0.1 and 0.13 mg/kg, respectively ($p<0.001$). In contrast, cerebral cortical deoxycorticosterone levels in C57BL/6J mice were decreased by about 30% following administration of dexamethasone 0.075 and 0.1 mg/kg, but the effect was not statistically significant; only the dose of 0.13 mg/kg induced a significant decrease (-80%, $p<0.05$).

These results suggest that C57BL/6J and DBA/2J mice differ in their sensitivity to dexamethasone suppression of deoxycorticosterone levels in a manner that relates to their drinking behavior under the two-bottle choice paradigm. Similar to monkeys, the high alcohol drinking and high alcohol preferring C57BL/6J mice show a weaker dexamethasone suppression of plasma and brain deoxycorticosterone levels relative to DBA/2J mice. Studies are under way to evaluate dexamethasone suppression of deoxycorticosterone in relation to alcohol consumption, preference, and alcohol-induced place preference across the BXD recombinant inbred strains of mice.

The BXD recombinant inbred mice were generated using C57BL/6J and DBA/2J as parental strains. Several parameters related to ethanol behavior have already been characterized across some of the BXD lines. The BXD mice show a wide pattern of ethanol consumption that spans between the two parental strains (Phillips et al., 1994). Data on blood ethanol concentration (Crabbe, 1998; Grisel et al., 2002), ethanol withdrawal handling-induced convulsions (Buck et al., 1997), ethanol-induced place preference (Cunningham, 1995), acute locomotor activity (Phillips et al., 1995) and ethanol-induced loss of righting reflex (Rodriguez et al., 1995) are also available.

Future studies will provide an analysis on the role of deoxycorticosterone and HPA axis suppression across different strains of mice, in relation to alcohol-related behaviors. These studies will contribute to species comparisons of neuroactive steroid responses. Furthermore, these studies may have the potential to identify neuroendocrine and genetic biomarkers for alcohol preference and consumption in mice that can potentially persist across different species.

Symposium Conclusions

GABAergic neuroactive steroid levels are elevated by acute stress or ethanol administration. Such responses to stress challenges are altered in opposing ways following to chronic social isolation stress and chronic ethanol exposure in rats. Alcohol dependence is characterized by increased drinking which can be associated with blunted neuroactive steroid responses to stress/ethanol challenges as observed in ethanol dependent rats. However, socially isolated rats exhibit elevated neuroactive steroid responses to ethanol challenge as well as increased ethanol preference. Following repeated ethanol withdrawals, relapse drinking in mice is associated with elevations in basal levels of the GABAergic neuroactive steroid, 3α,5α-THP. Finally, the ability of dexamethasone to suppress deoxycorticosterone in ethanol naïve cynomolgus monkeys accounts for more than half the variance in subsequent voluntary alcohol drinking. The studies presented in this symposium clearly indicate a role for GABAergic neuroactive steroids in stress/alcohol interactions. Future studies are needed to determine cause and effect relationships that may influence ethanol drinking in animal models of ethanol dependence and relapse drinking.

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References

Adinoff B, Krebaum SR, Chandler PA, Ye W, Brown MB, Williams MJ. Dissection of hypothalamic-pituitary-adrenal axis pathology in 1-month-abstinent alcohol-dependent men, part 1: adrenocortical and pituitary glucocorticoid responsiveness. Alcohol Clin Exp Res 2005a;29:517–527. [PubMed: 15834216]

Adinoff B, Krebaum SR, Chandler PA, Ye W, Brown MB, Williams MJ. Dissection of hypothalamic-pituitary-adrenal axis pathology in 1-month-abstinent alcohol-dependent men, part 2: response to ovine corticotropin-releasing factor and naloxone. Alcohol Clin Exp Res 2005b;29:528–537. [PubMed: 15834217]

Adinoff B, Martin PR, Bone GH, Eckardt MJ, Roehrich L, George DT, Moss HB, Eskay R, Linnoila M, Gold PW. Hypothalamic-pituitary-adrenal axis functioning and cerebrospinal fluid corticotropin releasing hormone and corticotropin levels in alcoholics after recent and long-term abstinence. Arch Gen Psychiatry 1990;47:325–330. [PubMed: 2157379]

Alele PE, Devaud LL. Sex differences in steroid modulation of ethanol withdrawal in male and female rats. J Pharmacol Exp Ther 2007;320:427–436. [PubMed: 17021261]

Azzolina B, Ellsworth K, Andersson S, Geissler W, Bull HG, Harris GS. Inhibition of rat alpha-reductases by finasteride: evidence for isozyme differences in the mechanism of inhibition. J Steroid Biochem Mol Biol 1997;61:55–64. [PubMed: 9328210]

Barbaccia ML, Affricano D, Trabucchi M, Purdy RH, Colombo G, Agabio R, Gessa GL. Ethanol markedly increases “GABAergic” neurosteroids in alcohol-preferring rats. Eur J Pharmacol 1999;384:R1–R2. [PubMed: 10611449]

Barbaccia ML, Roscetti G, Bolacchi F, Concas A, Mostallino MC, Purdy RH, Biggio G. Stress-induced increase in brain neuroactive steroids: antagonism by abecarnil. Pharmacol Biochem Behav 1996;54:205–210. [PubMed: 8728559]

Bartolomucci A, Pederzani T, Sacerdote P, Panerai AE, Parmigiani S, Palanza P. Behavioral and physiological characterization of male mice under chronic psychosocial stress. Psychoneuroendocrinology 2004;29:899–910. [PubMed: 15177705]

Becker HC, Lopez MF. Increased ethanol drinking after repeated chronic ethanol exposure and withdrawal experience in C57BL/6 mice. Alcohol Clin Exp Res 2004;28:1829–1838. [PubMed: 15608599]

Belknap JK, Belknap ND, Berg JH, Coleman R. Preabsorptive vs. postabsorptive control of ethanol intake in C57BL/6J and DBA/2J mice. Behav Genet 1977;7:413–425. [PubMed: 603475]

Borghese CM, Harris AR. Studies of ethanol actions on recombinant δ-containing γ-aminobutyric acid type A receptors yield contradictory results. Alcohol 2007;41:155–162. [PubMed: 17521845]

Bowen CA, Purdy RH, Grant KA. Ethanol-like discriminative stimulus effects of endogenous neuroactive steroids: effect of ethanol training dose and dosing procedure. J Pharmacol Exp Ther 1999;289:405–411. [PubMed: 10087031]

Brady KT, Sonne SC. The role of stress in alcohol use, alcoholism treatment, and relapse. Alcohol Res Health 1999;23:263–271. [PubMed: 10890823]

Buck KJ, Metten P, Belknap JK, Crabbe JC. Quantitative trait loci involved in genetic predisposition to acute alcohol withdrawal in mice. J Neurosci 1997;17:3946–3955. [PubMed: 9133412]

Cagetti E, Pinna G, Guidotti A, Baicy K, Olsen RW. Chronic intermittent ethanol (CIE) administration in rats decreases levels of neurosteroids in hippocampus, accompanied by altered behavioral responses to neurosteroids and memory function. Neuropharmacology 2004;46:570–579. [PubMed: 14975681]

Chu K, Koob GF, Cole M, Zorrilla EP, Roberts AJ. Dependence-induced increases in ethanol self-administration in mice are blocked by the CRF1 receptor antagonist antalarmin and by CRF1 receptor knockout. Pharmacol Biochem Behav 2007;86:813–821. [PubMed: 17482248]

Clarke TK, Treutlein J, Zimmermann US, Kiefer F, Skowronek MH, Rietschel M, Mann K, Schumann G. HPA-axis activity in alcoholism: examples for a gene-environment interaction. Addict Biol 2008;13:1–14. [PubMed: 17910738]

Costa A, Bono G, Martignoni E, Merlo P, Sances G, Nappi G. An assessment of hypothalamo-pituitary-adrenal axis functioning in non-depressed, early abstinent alcoholics. Psychoneuroendocrinology 1996;21:263–275. [PubMed: 8817725]

Crabbe JC. Provisional mapping of quantitative trait loci for chronic ethanol withdrawal severity in BXD recombinant inbred mice. J Pharmacol Exp Ther 1998;286:263–271. [PubMed: 9655868]

Crabbe JC Jr, Young ER, Kosobud A. Genetic correlations with ethanol withdrawal severity. Pharmacol Biochem Behav 1983;18(Suppl 1):541–547. [PubMed: 6685310]

Cunningham CL. Localization of genes influencing ethanol-induced conditioned place preference and locomotor activity in BXD recombinant inbred mice. Psychopharmacology 1995;120:28–41. [PubMed: 7480533]

de Kloet ER, Joels M, Holsboer F. Stress and the brain: from adaptation to disease. Nat Rev Neurosci 2005;6:463–475. [PubMed: 15891777]

Devaud LL, Purdy RH, Finn DA, Morrow AL. Sensitization of γ-aminobutyric $acid_A$ receptors to neuroactive steroids in rats during ethanol withdrawal. J Pharmacol Exp Ther 1996;278:510–517. [PubMed: 8768698]

Dhaher R, Finn D, Snelling C, Hitzemann R. Lesions of the extended amygdala in C57BL/6J mice do not block the intermittent ethanol vapor-induced increase in ethanol consumption. Alcohol Clin Exp Res 2008;32:197–208. [PubMed: 18162080]

Droogleever Fortuyn HA, van Broekhoven F, Span PN, Backstrom T, Zitman FG, Verkes RJ. Effects of PhD examination stress on allopregnanolone and cortisol plasma levels and peripheral benzodiazepine receptor density. Psychoneuroendocrinology 2004;29:1341–1344. [PubMed: 15288714]

Ehrenreich H, Schuck J, Stender N, Pilz J, Gefeller O, Schilling L, Poser W, Kaw S. Endocrine and hemodynamic effects of stress versus systemic CRF in alcoholics during early and medium term abstinence. Alcohol Clin Exp Res 1997;21:1285–1293. [PubMed: 9347091]

Essrich C, Lorez M, Benson JA, Fritschy JM, Luscher B. Postsynaptic clustering of major $GABA_A$ receptor subtypes requires the γ2 subunit and gephyrin. Nat Neurosci 1998;1:563–571. [PubMed: 10196563]

Fahlke C, Hard E, Hansen S. Facilitation of ethanol consumption by intracerebroventricular infusions of corticosterone. Psychopharmacology 1996;127:133–139. [PubMed: 8888379]

Finn DA, Ford MM, Wiren KM, Roselli CE, Crabbe JC. The role of pregnane neurosteroids in ethanol withdrawal: behavioral genetic approaches. Pharmacol Ther 2004a;101:91–112. [PubMed: 14761701]

Finn DA, Gallaher EJ, Crabbe JC. Differential change in neuroactive steroid sensitivity during ethanol withdrawal. J Pharmacol Exp Ther 2000;292:394–405. [PubMed: 10604976]

Finn DA, Phillips TJ, Okorn DM, Chester JA, Cunningham CL. Rewarding effect of the neuroactive steroid 3α-hydroxy-5α-pregnan-20-one in mice. Pharmacol Biochem Behav 1997a;56:261–264. [PubMed: 9050083]

Finn DA, Roberts AJ, Lotrich F, Gallaher EJ. Genetic differences in behavioral sensitivity to a neuroactive steroid. J Pharmacol Exp Ther 1997b;280:820–828. [PubMed: 9023296]

Finn DA, Sinnott RS, Ford MM, Long SL, Tanchuck MA, Phillips TJ. Sex differences in the effect of ethanol injection and consumption on brain allopregnanolone levels in C57BL/6 mice. Neuroscience 2004b;123:813–819. [PubMed: 14751275]

Finn DA, Snelling C, Fretwell AM, Tanchuck MA, Underwood L, Cole M, Crabbe JC, Roberts AJ. Increased drinking during withdrawal from intermittent ethanol exposure is blocked by the CRF receptor antagonist D-Phe-CRF(12-41). Alcohol Clin Exp Res 2007;31:939–949. [PubMed: 17403068]

Girdler SS, Klatzkin R. Neurosteroids in the context of stress: implications for depressive disorders. Pharmacol Ther 2007;116:125–139. [PubMed: 17597217]

Grahame NJ, Cunningham CL. Intravenous ethanol self-administration in C57BL/6J and DBA/2J mice. Alcohol Clin Exp Res 1997;21:56–62. [PubMed: 9046373]

Grant KA, Helms CM, Rogers LS, Purdy RH. Neuroactive steroid stereospecificity of ethanol-like discriminative stimulus effects in monkeys. J Pharmacol Exp Ther 2008;326:354–361. [PubMed: 18436788]

Grisel JE, Metten P, Wenger CD, Merrill CM, Crabbe JC. Mapping of quantitative trait loci underlying ethanol metabolism in BXD recombinant inbred mouse strains. Alcohol Clin Exp Res 2002;26:610–616. [PubMed: 12045468]

Groenink L, Dirks A, Verdouw PM, Schipholt M, Veening JG, van der Gugten J, Olivier B. HPA axis dysregulation in mice overexpressing corticotropin releasing hormone. Biol Psychiatry 2002;51:875–881. [PubMed: 12022960]

Hansson AC, Rimondini R, Neznanova O, Sommer WH, Heilig M. Neuroplasticity in brain reward circuitry following a history of ethanol dependence. Eur J Neurosci 2008;27:1912–1922. [PubMed: 18412612]

Heilig M, Koob GF. A key role for corticotropin-releasing factor in alcohol dependence. Trends Neurosci 2007;30:399–406. [PubMed: 17629579]

Hilakivi LA, Ota M, Lister RG. Effect of isolation on brain monoamines and the behavior of mice in tests of exploration, locomotion, anxiety and behavioral ‘despair’. Pharmacol Biochem Behav 1989;33:371–374. [PubMed: 2479036]

Hirani K, Khisti RT, Chopde CT. Behavioral action of ethanol in Porsolt’s forced swim test: modulation by 3α-hydroxy-5α-pregnan-20-one. Neuropharmacology 2002;43:1339–1350. [PubMed: 12527484]

Hirani K, Sharma AN, Jain NS, Ugale RR, Chopde CT. Evaluation of GABAergic neuroactive steroid 3α-hydroxy-5α-pregnane-20-one as a neurobiological substrate for the anti-anxiety effect of ethanol in rats. Psychopharmacology 2005;180:267–278. [PubMed: 15719223]

Inder WJ, Joyce PR, Ellis MJ, Evans MJ, Livesey JH, Donald RA. The effects of alcoholism on the hypothalamic-pituitary-adrenal axis: interaction with endogenous opioid peptides. Clin Endocrinol (Oxf) 1995;43:283–290. [PubMed: 7586596]

Jackson RV, Grice JE, Hockings GI, Torpy DJ. Naloxone-induced ACTH release: mechanism of action in humans. J Pharmacol Exp Ther 1995;285:518–526.

Janak PH, Redfern JEM, Samson HH. The reinforcing effects of ethanol are altered by the endogenous neurosteroid, allopregnanolone. Alcohol Clin Exp Res 1998;22:1106–1112. [PubMed: 9726282]

Janis GC, Devaud LL, Mitsuyama H, Morrow AL. Effects of chronic ethanol consumption and withdrawal on the neuroactive steroid 3α-hydroxy-5α-pregnan-20-one in male and female rats. Alcohol Clin Exp Res 1998;22:2055–2061. [PubMed: 9884151]

Khisti RT, Boyd KN, Kumar S, Morrow AL. Systemic ethanol administration elevates deoxycorticosterone levels and chronic ethanol exposure attenuates this response. Brain Res 2005;1049:104–111. [PubMed: 15936001]

Khisti RT, Penland SN, VanDoren MJ, Grobin AC, Morrow AL. GABAergic neurosteroid modulation of ethanol actions. World J Biol Psychiatry 2002;3:87–95. [PubMed: 12479081]

Khisti RT, VanDoren MJ, O'Buckley TK, Morrow AL. Neuroactive steroid 3α-hydroxy-5α-pregnan-20-one modulates ethanol-induced loss of righting reflex in rats. Brain Res 2003;980:255–265. [PubMed: 12867266]

Koob GF, Le Moal M. Addiction and the brain antireward system. Annu Rev Psychol 2008;59:29–53. [PubMed: 18154498]

Lopez MF, Anderson RI, Becker HC. Repeated cycles of chronic intermittent ethanol exposure increase both self-administration and the reinforcing value of ethanol in C57BL/6J mice. Alcohol Clin Exp Res 2008;32:163A.

Lopez MF, Becker HC. Effect of pattern and number of chronic ethanol exposures on subsequent voluntary ethanol intake in C57BL/6J mice. Psychopharmacology 2005;181:688–696. [PubMed: 16001125]

Matthews DB, Morrow AL, Tokunaga S, McDaniel JR. Acute ethanol administration and acute allopregnanolone administration impair spatial memory in the Morris water task. Alcohol Clin Exp Res 2002;26:1747–1751. [PubMed: 12436065]

Morrow AL, Janis GC, VanDoren MJ, Matthews DB, Samson HH, Janak PH, Grant KA. Neurosteroids mediate pharmacological effects of ethanol: A new mechanism of ethanol action? Alcohol Clin Exp Res 1999;23:1933–1940. [PubMed: 10630613]

Morrow, AL.; VanDoren, MJ.; Fleming, R.; Penland, S. Ethanol and neurosteroid interactions in the brain. In: Biggio, G.; Purdy, RH., editors. Int Rev Neurobiol. San Diego: Academic Press; 2001a. p. 349-377.

Morrow AL, VanDoren MJ, Penland SN, Matthews DB. The role of GABAergic neuroactive steroids in ethanol action, tolerance and dependence. Brain Res Brain Res Rev 2001b;37:98–109. [PubMed: 11744078]

Nemeroff CB, Vale WW. The neurobiology of depression: inroads to treatment and new drug discovery. J Clin Psychiatry 2005;66(Suppl 7):5–13. [PubMed: 16124836]

Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM. Neurobiology of depression. Neuron 2002;34:13–25. [PubMed: 11931738]

Nie H, Janak PH. Comparison of reinstatement of ethanol- and sucrose-seeking by conditioned stimuli and priming injections of allopregnanolone after extinction in rats. Psychopharmacology 2003;168:222–228. [PubMed: 12719962]

O'Dell LE, Alomary AA, Vallee M, Koob GF, Fitzgerald RL, Purdy RH. Ethanol-induced increases in neuroactive steroids in the rat brain and plasma are absent in adrenalectomized and gonadectomized rats. Eur J Pharmacol 2004a;484:241–247.

O'Dell LE, Purdy RH, Covey DF, Richardson HN, Roberto M, Koob GF. Epipregnanolone and a novel synthetic neuroactive steroid reduce alcohol self-administration in rats. Pharmacol Biochem Behav 2005;81:543–550. [PubMed: 15950269]

O'Dell LE, Roberts AJ, Smith RT, Koob GF. Enhanced alcohol self-administration after intermittent versus continuous alcohol vapor exposure. Alcohol Clin Exp Res 2004b;28:1676–1682.

Owens MJ, Ritchie JC, Nemeroff CB. 5α-Pregnane-3α,21-diol-20-one (THDOC) attenuates mild stress-induced increases in plasma corticosterone via a non-glucocorticoid mechanism: comparison with alprazolam. Brain Res 1992;573:353–355. [PubMed: 1504771]

Pariante CM, Lightman SL. The HPA axis in major depression: classical theories and new developments. Trends Neurosci 2008;31:464–468. [PubMed: 18675469]

Parker V, Morinan A. The socially-isolated rat as a model for anxiety. Neuropharmacology 1986;25:663–664.

Patchev VK, Hassan AHS, Holsboer F, Almeida OFX. The neurosteroid tetrahydroprogesterone attenuates the endocrine response to stress and exerts glucocorticoid-like effects on vasopressin gene transcription in the rat hypothalamus. Neuropsychopharmacology 1996;15:533–540. [PubMed: 8946427]

Patchev VK, Shoaib M, Holsboer F, Almeida OFX. The neurosteroid tetrahydroprogesterone counteracts corticotropin-releasing hormone-induced anxiety and alters the release and gene expression of corticotropin-releasing hormone in the rat hypothalamus. Neuroscience 1994;62:265–271. [PubMed: 7816204]

Phillips, TJ.; Crabbe, JC. Behavioral studies of genetic differences in alcohol action. In: Crabbe, JC.; Harris, RA., editors. The Genetic Basis of Alcohol and Drug Actions. New York: Plenum Press; 1991. p. 25-104.

Phillips TJ, Crabbe JC, Metten P, Belknap JK. Localization of genes affecting alcohol drinking in mice. Alcohol Clin Exp Res 1994;18:931–941. [PubMed: 7978106]

Phillips TJ, Huson M, Gwiazdon C, Burkhart-Kasch S, Shen EH. Effects of acute and repeated ethanol exposures on the locomotor activity of BXD recombinent inbred mice. Alcohol Clin Exp Res 1995;19:269–278. [PubMed: 7625557]

Piazza PV, Le Moal M. Glucocorticoids as a biological substrate of reward: physiological and pathophysiological implications. Brain Res Brain Res Rev 1997;25:359–372. [PubMed: 9495563]

Pierucci-Lagha A, Covault J, Feinn R, Nellissery M, Hernandez-Avila C, Oncken C, Morrow AL, Kranzler HR. GABRA2 alleles moderate the subjective effects of alcohol, which are attenuated by finasteride. Neuropsychopharmacology 2005;30:1193–1203. [PubMed: 15702134]

Pohorecky LA. Interaction of ethanol and stress: research with experimental animals--an update. Alcohol Alcohol 1990;25:263–276. [PubMed: 1973897]

Pohorecky LA. Stress and alcohol interaction: an update of human research. Alcohol Clin Exp Res 1991;15:438–459. [PubMed: 1898494]

Porcu P, Grant KA, Green HL, Rogers LS, Morrow AL. Hypothalamic-pituitary-adrenal axis and ethanol modulation of deoxycorticosterone levels in cynomolgus monkeys. Psychopharmacology 2006a; 186:293–301. [PubMed: 16133132]

Porcu, P.; Grant, KA.; Morrow, AL. 2006 Neuroscience Meeting Planner. Atlanta, GA: Society for Neuroscience; 2006b. Long-term voluntary alcohol consumption increases plasma deoxycorticosterone levels and alters hypothalamic-pituitary-adrenal axis modulation in cynomolgus monkeys. Online Program No 192.3

Porcu P, Rogers LSM, Morrow AL, Grant KA. Plasma pregnenolone levels in cynomolgus monkeys following pharmacological challenges of the hypothalamic-pituitary-adrenal axis. Pharmacol Biochem Behav 2006c;84:618–627. [PubMed: 16790266]

Purdy RH, Morrow AL, Moore PH Jr, Paul SM. Stress-induced elevations of gamma-aminobutyric acid type A receptor-active steroids in the rat brain. Proc Natl Acad Sci USA 1991;88:4553–4557. [PubMed: 1852011]

Rimondini R, Arlinde C, Sommer W, Heilig M. Long-lasting increase in voluntary ethanol consumption and transcriptional regulation in the rat brain after intermittent exposure to alcohol. FASEB J 2002;16:27–35. [PubMed: 11772933]

Rivier, C. Effects of alcohol on the neuroendocrine system. In: Noronha, A.; Eckardt, M.; Warren, K., editors. Review of NIAAA's Neuroscienceand Behavioral Research Portfolio. Bethesda, MD: National Institute on Alcohol Abuse and Alcoholism; 2000. p. 61-81.

Roberts AJ, Cole M, Koob GF. Intra-amygdala muscimol decreases operant ethanol self-administration in dependent rats. Alcohol Clin Exp Res 1996;20:1289–1298. [PubMed: 8904984]

Roberts AJ, Crabbe JC, Keith LD. Genetic differences in hypothalamic-pituitary-adrenal axis responsiveness to acute ethanol and acute ethanol withdrawal. Brain Res 1992;579:296–302. [PubMed: 1320985]

Roberts AJ, Heyser CJ, Cole M, Griffin P, Koob GF. Excessive ethanol drinking following a history of ethanol dependence: animal model of allostasis. Neuropsychopharmacology 2000;22:581–594. [PubMed: 10788758]

Rodriguez LA, Plomin R, Blizard DA, Jones BC, McClearn GE. Alcohol acceptance, preference, and sensitivity in mice. II. Quantitative trait loci mapping analysis using BXD recombinant inbred strains. Alcohol Clin Exp Res 1995;19:367–373. [PubMed: 7625571]

Sanna E, Talani G, Busonero F, Pisu MG, Purdy RH, Serra M, Biggio G. Brain steroidogenesis mediates ethanol modulation of $GABA_A$ receptor activity in rat hippocampus. J Neurosci 2004;24:6521–6530. [PubMed: 15269263]

Serra M, Mostallino MC, Talani G, Pisu MG, Carta M, Mura ML, Floris I, Maciocco E, Sanna E, Biggio G. Social isolation-induced increase in α4 and δ subunit gene expression is associated with a greater efficacy of ethanol on steroidogenesis and $GABA_A$ receptor function. J Neurochem 2006;98:122–133. [PubMed: 16805802]

Serra M, Pisu MG, Floris I, Cara V, Purdy RH, Biggio G. Social isolation-induced increase in the sensitivity of rats to the steroidogenic effect of ethanol. J Neurochem 2003;85:257–263. [PubMed: 12641747]

Serra M, Pisu MG, Littera M, Papi G, Sanna E, Tuveri F, Usala L, Purdy RH, Biggio G. Social isolation-induced decreases in both the abundance of neuroactive steroids and $GABA_A$ receptor function in rat brain. J Neurochem 2000;75:732–740. [PubMed: 10899949]

Shelton KL, Grant KA. Discriminative stimulus effects of ethanol in C57BL/6J and DBA/2J inbred mice. Alcohol Clin Exp Res 2002;26:747–757. [PubMed: 12068241]

Sillaber I, Henniger MS. Stress and alcohol drinking. Ann Med 2004;36:596–605. [PubMed: 15768831]

Sinnott RS, Mark GP, Finn DA. Reinforcing effects of the neurosteroid allopregnanolone in rats. Pharmacol Biochem Behav 2002a;72:923–929. [PubMed: 12062582]

Sinnott RS, Phillips TJ, Finn DA. Alteration of voluntary ethanol and saccharin consumption by the neurosteroid allopregnanolone in mice. Psychopharmacology 2002b;162:438–447. [PubMed: 12172699]

Sommer WH, Rimondini R, Hansson AC, Hipskind PA, Gehlert DR, Barr CS, Heilig MA. Upregulation of voluntary alcohol intake, behavioral sensitivity to stress, and amygdala crhr1 expression following a history of dependence. Biol Psychiatry 2008;63:139–145. [PubMed: 17585886]

Spencer RL, McEwen BS. Adaptation of the hypothalamic-pituitary-adrenal axis to chronic ethanol stress. Neuroendocrinology 1990;52:481–489. [PubMed: 2126355]

Sundstrom-Poromaa I, Smith DH, Gong QH, Sabado TN, Li X, Light A, Wiedmann M, Williams K, Smith S. Hormonally regulated $\alpha_4\beta_2\delta$ $GABA_A$ receptors are a target for alcohol. Nat Neurosci 2002;5:721–722. [PubMed: 12118257]

Sur C, Farrar SJ, Kerby J, Whiting PJ, Atack JR, McKernan RM. Preferential coassembly of α4 and δ subunits of the γ-aminobutyric $acid_A$ receptor in rat thalamus. Mol Pharmacol 1999;56:110–115. [PubMed: 10385690]

Torres JM, Ortega E. Alcohol intoxication increases allopregnanolone levels in female adolescent humans. Neuropsychopharmacology 2003;28:1207–1209. [PubMed: 12700685]

Torres JM, Ortega E. Alcohol intoxication increases allopregnanolone levels in male adolescent humans. Psychopharmacology 2004;172:352–355. [PubMed: 14647956]

VanDoren MJ, Matthews DB, Janis GC, Grobin AC, Devaud LL, Morrow AL. Neuroactive steroid 3α-hydroxy-5α-pregnan-20-one modulates electrophysiological and behavioral actions of ethanol. J Neurosci 2000;20:1982–1989. [PubMed: 10684899]

Vengeliene V, Bilbao A, Molander A, Spanagel R. Neuropharmacology of alcohol addiction. Br J Pharmacol 2008;154:299–315. [PubMed: 18311194]

Voikar V, Polus A, Vasar E, Rauvala H. Long-term individual housing in C57BL/6J and DBA/2 mice: assessment of behavioral consequences. Genes Brain Behav 2005;4:240–252. [PubMed: 15924556]

Wand, G. Hypothalamic-pituitary-adrenal axis: Changes and risk for alcoholism. In: Noronha, A.; Eckardt, M.; Warren, K., editors. Review of NIAAA's neuroscience and behavioral research portfolio: NIAAA Research Monograph 34. Bethesda, MD: National Institute on Alcohol Abuse and Alcoholism; 2000. p. 397-415.

Wand GS, Dobs AS. Alterations in the hypothalamic-pituitary-adrenal axis in actively drinking alcoholics. J Clin Endocrinol Metab 1991;72:1290–1295. [PubMed: 2026749]

Wand GS, Mangold D, El Deiry S, McCaul ME, Hoover D. Family history of alcoholism and hypothalamic opiodergic activity. Arch Gen Psychiatry 1998;55:1114–1119. [PubMed: 9862555]

Williams KL, Ko MC, Rice KC, Woods JH. Effect of opioid receptor antagonists on hypothalamic-pituitary-adrenal activity in rhesus monkeys. Psychoneuroendocrinology 2003;28:513–528. [PubMed: 12689609]

Wongwitdecha N, Marsden CA. Social isolation increases aggressive behaviour and alters the effects of diazepam in the rat social interaction test. Behav Brain Res 1996;75:27–32. [PubMed: 8800657]

Abbreviations

GABA	γ-aminobutyric acid
3α,5α-THP	(3α5α)-3-hydroxypregnan-20-one
3α,5α-THDOC	(3α5α)-3,21-dihydroxypregnan-20-one
HPA	hypothalamic-pituitary-adrenal
CNS	central nervous system
CRF	corticotropin releasing factor
ACTH	adrenocorticotropic hormone