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Divergent neuroactive steroid responses to stress and ethanol in rat and mouse strains: Relevance for human studies

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Abstract

Rationale—Neuroactive steroids are endogenous or synthetic steroids that rapidly alter neuronal excitability via membrane receptors, primarily $GABA_A$ receptors. Neuroactive steroids regulate many physiological processes including hypothalamic-pituitary-adrenal (HPA) axis function, ovarian cycle, pregnancy, aging, and reward. Moreover, alterations in neuroactive steroid synthesis are implicated in several neuropsychiatric disorders.

Objectives—This review will summarize the pharmacological properties and physiological regulation of neuroactive steroids, with a particular focus on divergent neuroactive steroid responses to stress and ethanol in rats, mice and humans.

Results—GABAergic neuroactive steroids exert a homeostatic regulation of the HPA axis in rats and humans, whereby the increase in neuroactive steroid levels following acute stress counteracts HPA axis hyperactivity and restores homeostasis. In contrast, in C57BL/6J mice, acute stress decreases neurosteroidogenesis and neuroactive steroids exert paradoxical excitatory effects upon the HPA axis. Rats, mice and humans also differ in the neuroactive steroid responses to ethanol. Genetic variation in neurosteroidogenesis may explain the different neuroactive steroid responses to stress or ethanol.

Conclusions—Rats and mouse strains show divergent effects of stress and ethanol on neuroactive steroids in both plasma and brain. The study of genetic variation in the various processes that determine neuroactive steroids levels as well as their effects on cell signaling may underlie these differences and may play a relevant role for the potential therapeutic benefits of neuroactive steroids.

Keywords

Neuroactive steroids; allopregnanolone; stress; HPA axis; ethanol; rat; C57BL/6J mice; DBA/2J mice

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Introduction

Neuroactive steroids are endogenous or synthetic steroids that rapidly alter neuronal excitability via membrane receptors. These steroids are derived from cholesterol, which is converted to pregnenolone that is further metabolized into several steroid hormones that include glucocorticoids, estrogens, progesterone, dehydroepiandrosterone (DHEA) as well as their metabolites. The 3α,5α- and 3α,5β-reduced metabolites of progesterone, deoxycorticosterone (DOC), DHEA and testosterone are positive modulators of γ-aminobutyric acid type A ($GABA_A$) receptors. Among these steroids, the progesterone metabolite (3α,5α)-3-hydroxypregnan-20-one (3α,5α-THP or allopregnanolone) and the DOC metabolite (3α,5α)-3,21-dihydroxypregnan-20-one (3α,5α-THDOC or allotetrahydrodeoxy-corticosterone) are the most potent $GABA_A$ receptor modulators and the most studied neuroactive steroids.

This review will summarize the current literature on GABAergic neuroactive steroids responses to stress and ethanol in rat and mouse strains as well as humans. We highlight recent work on genetic variation in neuroactive steroid responses in mouse strains that may be particularly relevant for translational studies, since this genetic variation models the genetic diversity of the human population.

Pharmacological properties of neuroactive steroids

Systemic administration of GABAergic neuroactive steroids exerts a variety of pharmacological responses including anxiolytic, antidepressant, anticonvulsant, sedative, anesthetic, and analgesic effects in animal models and human studies (Belelli et al. 1989; Bitran et al. 1991; Hogskilde et al. 1987; Kavaliers and Wiebe 1987; Khisti et al. 2000), that are consistent with their GABAergic actions. However, there are also reports of paradoxical excitatory actions of GABAergic neuroactive steroids that have received less attention in the literature. For example, pregnanolone (3α,5β-THP) and alphaxalone were both shown to produce dose dependent myoclonic seizures in outbred Bantin and Kingman male mice (File and Simmonds 1988), pregnanolone induced seizures in male C57BL/6J mice (Shannon et al. 2005a), 3α,5α-THP enhanced the frequency and amplitude of postural tremor in $GABA_A$ receptor α1 subunit knockout mice (Osterman et al. 2005), and 3α,5α-THP enhanced aggression in outbred Swiss mice in various experimental settings (Fish et al. 2001; Miczek et al. 2003). The molecular mechanisms of these excitatory behavioral actions are unclear, but may involve disinhibition of neuronal circuits via $GABA_A$ receptors, alterations in membrane chloride gradient that result in depolarization by endogenous GABA or novel actions of these steroids. 3α,5α-THP also activates feminine sexual behavior of rodents (Frye et al. 1998), which is thought to involve dopamine transmission as well as pregnane xenobiotic receptors for steroidogenesis (see Walf et al., this issue). Moreover, GABAergic neuroactive steroids, like various drugs of abuse, exhibit rewarding properties in DBA/2J mice and Long-Evans rats (Finn et al. 1997a; Sinnott et al. 2002) and enhance brain stimulation reward in C57BL/6J mice, much like cocaine (Fish et al., this issue). 3α,5α-THP and other GABAergic neuroactive steroids exhibit biphasic modulation of ethanol intake in various rodent models of ethanol self-administration (Besheer et al. 2010; Finn et al. 2008; Ford et al. 2007; Ford et al. 2005; Janak et al. 1998; Morrow et al. 2001), suggesting

complex actions on neuronal circuitry mediating these behaviors. The complexity of these behavioral responses in rodents points to the possibility that genetic variation may play an important role in neuroactive steroid actions across species.

Mechanisms and sites of action

The 3α,5α- and 3α,5β-reduced metabolites of progesterone, DOC, DHEA and testosterone exert inhibitory actions that are mediated by synaptic and extrasynaptic $GABA_A$ receptors. These neuroactive steroids interact with synaptic $GABA_A$ receptors to produce phasic inhibition via specific binding sites on the TM1 region of the α subunits that allosterically modulate binding to GABA and benzodiazepine recognition sites (Hosie et al. 2006). $GABA_A$ receptor function is enhanced by potentiation of GABA-mediated Cl^- conductance as well as direct stimulation of Cl^- conductance (Harrison et al. 1987; Majewska et al. 1986; Morrow et al. 1987). $GABA_A$ receptors appear to have multiple neurosteroid recognition sites that likely reflect distinct recognition sites on $GABA_A$ receptor subtypes (Morrow et al. 1990). Neuroactive steroids modulate both synaptic and extrasynaptic $GABA_A$ receptors with lower potency at synaptic receptors that contain γ2 subunits and higher potency at extrasynaptic receptors that contain subunits and mediate tonic inhibition (Belelli and Lambert 2005; Carver and Reddy 2013). The excitatory neuroactive steroids include the sulfated derivatives of pregnenolone and DHEA as well as the 3α,5α- and 3α,5β-reduced metabolites of cortisol. The excitatory actions of sulfated steroids are partially mediated by direct interactions with N-methyl-D-aspartate (NMDA) receptors, where they act as weak agonists (micromolar potency) (Park-Chung et al. 1997; Wu et al. 1991), as well as by a blocking action on $GABA_A$ receptors (Akk et al. 2001). Pregnenolone sulfate also potently increases glutamate release (Meyer et al. 2002) and inhibits GABA release (Mtchedlishvili and Kapur 2003), effects that are blocked by sigma-1 receptor antagonists. Finally, pregnenolone sulfate enhances the trafficking of NMDA receptors, resulting in greater excitatory drive (Kostakis et al. 2013). In addition, 3α,5α- and 3α,5β-reduced cortisol have antagonist properties at both GABA and neurosteroid recognition sites of $GABA_A$ receptors and these compounds are the most abundant metabolites of cortisol in human urine (Penland and Morrow 2004).

It is not clear how endogenous neuroactive steroids influence neurotransmission in the brain. The available data suggests that elevations of 3α,5α-THP within a cell reduce the excitability of that particular cell (Akk et al. 2007; Saalmann et al. 2006; Tokuda et al. 2011; Tokuda et al. 2010). 3α,5α-THP may access the neuroactive steroid transmembrane binding sites on $GABA_A$ receptors via intracellular (i.e. presynaptic) lateral diffusion through the cell membrane (Akk et al. 2007) or by a paracrine or autocrine mechanism (Herd et al. 2007), as no active release mechanism has been identified. There is also evidence for extracellular pockets of neuroactive steroid storage that allow for rapid and repeated access to extracellular binding sites on neurons (Akk et al. 2007; Akk et al. 2005). Figure 1 summarizes these putative mechanisms and sites of action of GABAergic neuroactive steroids.

Physiological regulation of neuroactive steroid levels in plasma and brain

Neuroactive steroid concentrations vary across physiological conditions mostly associated with reproduction and development such as puberty, ovarian cycling, pregnancy or aging. Brain levels of 3α,5α-THP are elevated in the embryonic Sprague-Dawley rat, decrease immediately before birth, remain low throughout early life (Grobin and Morrow 2001) and increase immediately before puberty in Sprague-Dawley rats (Grobin and Morrow 2001), C57BL/6J mice (Shen et al. 2007) and humans (Fadalti et al. 1999). Circulating 3α,5α-THP levels decline during menopause in women (Bixo et al. 1997). A similar decline has been observed in several rat brain areas and it was hypothesized it might contribute to cognitive impairment in aged Sprague-Dawley rats (George et al. 2010).

Interestingly, alterations in neuroactive steroid levels that occur *in vivo* under some physiological conditions are associated with changes in $GABA_A$ receptor function and expression. These data are essential to understand the behavioral sequelae of changes in levels of these steroids. This work is reviewed in several other papers in this special issue and we refer the reader to those contributions for a complete review of neuroactive steroid regulation of $GABA_A$ receptor gene expression (see, MacKenzie and Maguire, this issue).

GABAergic neuroactive steroids concentrations vary throughout the ovarian cycle in both rodents and humans. 3α,5α-THP and progesterone levels vary throughout the estrus cycle in brain and plasma of HsdOla:Tuck-Ordinary mice (Corpechot et al. 1997). In female C57BL/6J mice, the diestrus phase is accompanied by elevated levels of progesterone and 3α,5α-THP, and a subsequent increase in tonic inhibition and decreased seizure susceptibility and anxiety (Maguire et al. 2005). Moreover, $GABA_A$ receptor plasticity throughout the ovarian cycle is accompanied to changes in sensitivity to exogenous 3α,5α-THP; administration of 3α,5α-THP potentiates tonic inhibition and exerts a protective action against hippocampus kindling epileptogenesis during the diestrus phase in female C57BL/6-129SV hybrid mice (Wu et al. 2013). Increased circulating levels of 3α,5α-THP have been reported during the luteal phase of the menstrual cycle in women (Wang et al. 1996), and fluctuations in neuroactive steroid concentrations across the menstrual cycle correlate with symptoms of premenstrual dysphoric disorder (Girdler et al. 2001; Wang et al. 1996). Interestingly, treatment with hormonal contraceptives decreases plasma neuroactive steroids and prevents the increase in 3α,5α-THP during the luteal phase in women (Follesa et al. 2002; Rapkin et al. 2006). The same treatment also dramatically decreased brain 3α,5α-THP and progesterone concentrations, altered $GABA_A$ receptor subunit expression and induced anxiety-like behavior in female Sprague-Dawley rats (Follesa et al. 2002; Porcu et al. 2012).

Neuroactive steroid concentrations increase dramatically during pregnancy in both rats and women (Concas et al. 1998; Gilbert Evans et al. 2005). Levels of progesterone and 3α,5α-THP decrease immediately before parturition and return to baseline levels two days after parturition in Sprague-Dawley rats (Concas et al. 1998). These abrupt changes in steroid concentrations may contribute to post-partum depressive symptoms.

GABAergic neuroactive steroids and stress/HPA axis regulation

The hypothalamic-pituitary-adrenal (HPA) axis is regulated by numerous neurotransmitter systems and by negative feedback of steroid hormones. Activation of the HPA axis in response to acute stress increases the release of corticotrophin releasing hormone (CRH) from the hypothalamus that stimulates the release of adrenocorticotropic hormone (ACTH) from the pituitary, which, in turn, stimulates the adrenal cortex to release glucocorticoids (cortisol in humans and corticosterone in rodents) as well as the GABAergic neuroactive steroids. The ability of these steroids to modulate HPA axis activation may play an important role in stress response, homeostasis and allostasis. In contrast, chronic stress leads to dysregulation of the HPA axis, a feature observed in several psychiatric and neurologic disorders, which are also associated with alterations in neuroactive steroid levels in plasma, cerebrospinal fluid or brain (Girdler and Klatzkin 2007; Morrow et al. 2006; Uzunova et al. 1998). The following sections will describe the effects of neuroactive steroids on the stress/HPA axis response in rats, mice and human subjects (see also Table 1 for a summary).

Homeostatic regulation of the HPA axis in rats

Acute swim stress increases brain and plasma concentrations of neuroactive steroids in Sprague- Dawley rats (Purdy et al. 1991). Likewise, other paradigms of acute stress also increase brain and plasma levels of GABAergic neuroactive steroids and their precursors in male Sprague-Dawley rats (Barbaccia et al. 1994; Barbaccia et al. 1996; Barbaccia et al. 1997; Vallee et al. 2000). The increase in neuroactive steroids is mediated by HPA activation as adrenalectomized rats show no circulating 3α,5α-THP and 3α,5α-THDOC levels, although brain levels of 3α,5α-THP are still detectable (Purdy et al. 1991). Thus, activation of the HPA axis ultimately leads to a release of glucocorticoids and neuroactive steroids from the adrenals. Glucocorticoids provide negative feedback upon the hypothalamus and pituitary. Likewise, GABAergic neuroactive steroids inhibit CRH production, release, ACTH release and subsequent corticosterone levels following stress in rats. 3α,5α-THP exerts a regulatory effect on HPA axis activity by reducing the neuroendocrine response to stress. While 3α,5α-THP per se appears not to be involved in basal CRH release, it counteracts the anxiety behavior induced by CRH administration in Wistar rats (Patchev et al. 1994). Moreover, 3α,5α-THP or 3α,5α-THDOC administration before stress attenuates the stress-induced increase in ACTH and corticosterone in Wistar rats (Owens et al. 1992; Patchev et al. 1996). In agreement, intracerebroventricular administration of 3α,5α-THP antiserum to male and female Wistar rats potentiated the corticosterone response to stress in prepubertal and adult rats, without affecting basal plasma corticosterone levels (Guo et al. 1995).

Under normal basal conditions, systemic administration of 3α,5α-THP to adult male non-stressed Sprague-Dawley rats increases hypothalamic CRH content as well as serum ACTH and corticosterone (Naert et al. 2007), supporting a regulatory role for this neuroactive steroid in HPA function, whereby 3α,5α-THP increases hormone levels in basal conditions and decreases them in stress-induced perturbations in order to restore homeostasis. Systemic administration of pregnenolone, DHEA and their sulfate metabolites also increased hypothalamic CRH and serum ACTH and corticosterone (Naert et al. 2007). All these

effects were rapid and likely mediated by a direct action of neuroactive steroids on GABA and glutamate neurotransmission in the hypothalamus that regulate HPA axis activation. Indeed, the hypothalamus contains the enzymes to locally synthesize 3α,5α-THP and is an area particularly rich in 3α,5α-THP immunoreactivity (Saalmann et al. 2007).

Acute stress rapidly decreases GABAergic transmission in male Sprague-Dawley rats (Biggio et al. 2007), which leads to HPA axis activation. The elevations in neuroactive steroids levels that occur approximately 30 minutes after acute stress may represent a homeostatic mechanism to restore GABAergic inhibition upon the paraventricular nucleus of the hypothalamus, thus acting as a brake upon HPA axis activity.

GABAergic neuroactive steroid regulation of HPA axis function may differ across lifespan. In early development, administration of 3α,5α-THDOC to Wistar rat pups, exposed to repeated maternal separation, attenuated vocalizations and abolished the anxiety-like behavior observed in these rats during adulthood (Patchev et al. 1997). Moreover, pretreatment of Wistar rat pups with 3α,5α-THP or 3α,5α-THDOC exerts long-term protection against HPA responses to stress (Mitev et al. 2003; Patchev et al. 1997), suggesting that neuroactive steroids may protect the developing brain against adverse challenges.

A heightened HPA axis response to stress with prolonged glucocorticoid release has been reported in adolescent Sprague-Dawley rats (Lupien et al. 2009). However it is not clear whether alterations in neuroactive steroids may contribute to these changes in HPA responsivity since data is not available in adolescent animals and the effects of neuroactive steroids on the HPA axis appear to differ with genetic background.

Intracerebroventricular administration of 3α,5α-THP antiserum potentiated the stress response in prepubertal and adult Wistar rats, but not aged rats suggesting that the modulatory effects of 3α,5α- THP on the HPA response to stress are lost during aging (Guo et al. 1995). However, another study reported that aged male Sprague-Dawley rats show increased sensitivity to stress with greater elevations in cerebral cortical 3α,5α-THP and 3α, 5α-THDOC levels following acute stress exposure, compared to adult rats (Barbaccia et al. 1998). These results may differ due to different stress paradigms or to the different rat strains.

Chronic stress exposure induces adaptations in HPA axis function that lead to hyper-responsiveness of the axis to stressful stimuli and to altered neuroactive steroid responses. Thus, adult male Sprague-Dawley rats subjected to social isolation, an animal model of anxiety disorders that generates chronic stress, show decreased basal levels of 3α,5α-THP and 3α,5α-THDOC in brain and plasma, but a greater neuroactive steroid response to a stress challenge, compared to group-housed rats (Serra et al. 2000). Alterations in HPA axis responsiveness and neurosteroidogenesis have also been found in adult male offspring of socially isolated Sprague-Dawley rats (Pisu et al. 2013) and in juvenile offspring of Long-Evans rats subjected to chronic gestational restraint stress (Paris and Frye 2011).

Orthodromic regulation of the HPA axis in C57BL/6J mice

In contrast to the findings in rats, acute swim stress decreases plasma concentrations of 3α, 5α- THP, measured by gas chromatography/mass spectrometry, 3α-hydroxysteroid dehydrogenase (3α-HSD) mRNA levels in the adrenal glands and 3α,5α-THP immunoreactivity in various brain regions of male C57BL/6J mice (Maldonado-Devincci et al., this issue). Furthermore, recent studies have highlighted a novel action of GABAergic neuroactive steroids in HPA axis regulation. In C57BL/6J male mice, these steroids promote the physiological response to stress. Under basal conditions, local administration of 3α,5α-THDOC into the paraventricular nucleus decreased circulating levels of corticosterone, consistent with the homeostatic regulation of the HPA axis by GABAergic neuroactive steroids. However, under stress conditions, administration of 3α,5α-THDOC, 30 minutes before restraint stress, increased corticosterone levels, an effect abolished by finasteride pretreatment, suggesting that neurosteroidogenesis plays a critical role in HPA axis response to stress. Moreover, finasteride treatment before restraint stress decreases anxiety in the elevated plus maze test, suggesting that neurosteroidogenesis is required to mediate the anxiety-like behavior induced by stress in these mice (Sarkar et al. 2011). Under basal conditions, 3α,5α-THDOC decreases the spontaneous firing of CRH neurons; in contrast, following restraint stress, 3α,5α-THDOC increases the firing rate of CRH neurons. These excitatory actions of neuroactive steroids are mediated by $GABA_A$ subunit-containing receptors, since that they are absent in subunit knock-out mice. Moreover, following stress, GABAergic transmission on CRH neurons switches from inhibitory to excitatory, due to a dephosphorylation and downregulation of the K^+/Cl^- co-transporter KCC2, which may represent a novel target for stress and neuroactive steroid responses (Sarkar et al. 2011).

Paradoxical excitatory effects of neuroactive steroids have also been reported in mice during puberty. Thus, administration of 3α,5β-THP to adolescent C57BL/6J female mice increased anxiety following restraint stress; this effect appears to be mediated by inhibition of the tonic GABAergic currents through α4β2δ $GABA_A$ receptors in the hippocampus (Shen et al. 2007). It is unknown if these excitatory actions of neuroactive steroids extend to other mouse strains, rats or humans.

The effects of HPA axis adaptations to chronic stress on neuroactive steroid actions are largely unexplored in mice. Similar to the above mentioned studies in rats, social isolation decreases 3α,5α-THP content and 5α-reductase expression in frontal cortex, hippocampus, amygdala and olfactory bulb of adult male Swiss-Webster mice (Dong et al. 2001; Pibiri et al. 2008), suggesting a potential dysregulation at the HPA level. Moreover, it has recently been shown that poor maternal care, a model of early life adversity, blunts the 3α,5α-THP-induced suppression of neuronal firing in CRH neurons in young pre-pubertal C57BL/6J-129SvSvJ mice (Gunn et al. 2013). Whether these changes are present in other mouse strains or persist in adult mice, remains to be determined.

Neuroactive steroid regulation of the HPA axis in mice may be more complex and not yet totally unraveled. As already mentioned, GABAergic neuroactive steroids have anticonvulsant properties in both rats (Reddy and Rogawski 2002) and mice [Swiss-Webster (Belelli et al. 1989; Gasior et al. 1997), NIH Swiss (Kokate et al. 1999; Kokate et al. 1994; Kokate et al. 1998; Reddy and Rogawski 2002) and C3HJ strains (Pericic et al. 2007)].

Interestingly, acute swim stress also exerts anticonvulsant actions. In male Sprague-Dawley rats, swim stress increases the seizure threshold along with circulating 3α,5α-THDOC levels; administration of the 5α-reductase inhibitor finasteride prevents the stress-induced increase in 3α,5α-THDOC levels and actually decreases the seizure threshold below that in non stressed rats (Reddy and Rogawski 2002). Acute swim stress also has anticonvulsant effects in CBA and C3HJ mice (Pericic et al. 2007; Pericic et al. 2000). Levels of GABAergic neuroactive steroids have not been measured in these mice, but they may likely play a role. Indeed, prior administration of finasteride does not alter seizure threshold in CBA mice (Pericic et al. 2000) and administration of 3α,5α-THP prior to swim stress potentiates its anticonvulsant effect (Pericic et al. 2007). Thus, acute swim stress might influence seizure susceptibility in both rats and mice through interactions with the GABAergic neuroactive steroids.

GABAergic neuroactive steroids and HPA axis in humans

HPA axis function can be tested in humans by measuring the hormonal response to pharmacological challenges with CRH, ACTH or dexamethasone. Thus, administration of CRH and ACTH to humans (mixed population of males and females) increased serum 3α, 5α-THP, progesterone and DHEA concentrations, while administration of dexamethasone decreased serum levels of these steroids (Genazzani et al. 1998), suggesting a homeostatic regulation of the HPA axis that contributes to neuroactive steroid synthesis. In agreement with these results, we found that concentrations of DOC, the progesterone metabolite and precursor of the GABAergic neuroactive steroid 3α,5α-THDOC, were regulated by the HPA axis at hypothalamic, pituitary and adrenal levels in healthy men. Naloxone, CRH and ACTH challenges increased serum DOC levels, while dexamethasone challenge suppressed them. In contrast, concentrations of the excitatory neuroactive steroid pregnenolone sulfate were regulated by the adrenals but not the hypothalamus or pituitary; in fact, naloxone and CRH challenges failed to affect pregnenolone sulfate levels in healthy men, while ACTH and dexamethasone challenges increased and decreased such levels, respectively (Porcu et al. 2008).

Acute stress activates the HPA axis and increases serum 3α,5α-THP concentrations in human subjects (Droogleever Fortuyn et al. 2004; Girdler et al. 2001), similar to what has been shown in rats. However, others have also reported no change in serum 3α,5α-THP levels after acute stress, albeit different stress paradigms and time points were examined (Altemus et al. 2001; Childs and de Wit 2009; Childs et al. 2010; Girdler et al. 2006). Most of the studies in humans have focused on dysregulation of the HPA axis associated to several psychiatric and neurologic conditions. For instance, a blunted HPA axis function and altered neuroactive steroid levels are reported in premenstrual dysphoric disorder (PMDD), although the literature is controversial, with reports of increases, decreases, and no change in 3α,5α-THP plasma levels (Girdler and Klatzkin 2007). Following mental stress, 3α,5α-THP levels increased in control subjects, as expected, but decreased in PMDD women and this lack of 3α,5α-THP responsiveness to stress was related to the greater baseline 3α,5α-THP concentrations found in these subjects (Girdler et al. 2001). Moreover, women with a history of depression, regardless of PMDD symptoms, had reduced basal neuroactive steroid levels

and a blunted 3α,5α-THP response to stress (Klatzkin et al. 2006). This work is reviewed in detail in another contribution to this Special Issue (Crowley and Girdler, this issue).

Decreased 3α,5α-THP levels have been found in cerebrospinal fluid of women with posttraumatic stress disorder (Rasmusson et al. 2006) and chronic stress exposure may also affect neuroactive steroid sensitivity. A recent study has shown that women with work related psychosocial stress have similar baseline concentrations of 3α,5α-THP compared to controls, but greater sensitivity to exogenous 3α,5α-THP. In fact, 3α,5α-THP administration decreased the saccadic eye velocity to a greater extent in patients compared to controls (Backstrom et al. 2013).

Alterations in HPA axis responsiveness are also found in alcoholism during drinking and abstinence (Morrow and Porcu 2009). Basal levels of 3α,5α-THP and 3α,5α-THDOC are reduced during ethanol withdrawal in humans (Romeo et al. 1996), when circulating cortisol levels are elevated. Their responses to stress have not been studied under these conditions, but are likely to be impacted since both human and animal studies show that neuroactive steroid responses to stress or HPA axis activation are blunted when ACTH or glucocorticoid responses are blunted (Morrow and Porcu 2009). Furthermore, abstinent alcoholics show a blunted pregnenolone sulfate response to adrenal stimulation and a delayed DOC response to CRH challenge, supporting the idea that blunting of the HPA axis also impacts the neuroactive steroid responses to stress in alcoholics (Porcu et al. 2008).

All this experimental evidence emphasizes the important link between HPA axis function and neuroactive steroid levels in the maintenance of homeostasis and healthy brain function.

GABAergic neuroactive steroids and ethanol interactions

The effects of ethanol involve various GABAergic mechanisms that contribute to many of its behavioral effects, including anxiolytic, anticonvulsant, sedative-hypnotic, cognitive-impairing and motor incoordinating actions (see Kumar et al. 2009, for review). These mechanisms are thought to include direct and indirect effects of ethanol on $GABA_A$ receptors as well as effects on GABA release and the synthesis and availability of endogenous neuroactive steroids. This work was primarily based on studies in rat models, but certain effects of ethanol differ across various rat and mouse strains. Here, we focus on the ethanol regulation of GABAergic neuroactive steroid levels in rats as well as across mouse strains and demonstrate how genetic variation in neuroactive steroid responses can be linked to variation in the behavioral actions of ethanol.

Ethanol increases GABAergic neuroactive steroids in rats

Like stress, systemic administration of moderate ethanol doses (1-2.5 g/kg) increases plasma, cerebrocortical and hippocampal levels of 3α,5α-THP and 3α,5α-THDOC in Sprague-Dawley rats (Morrow et al. 1999; Morrow et al. 1998; Porcu et al. 2010; VanDoren et al. 2000) and Sardinian alcohol-preferring rats (Barbaccia et al. 1999). Recent studies using immunohistochemistry to determine ethanol-induced changes in cellular 3α,5α-THP across brain show both regional and cell population specificity in this response in Wistar rats (Cook et al. 2014a). Ethanol increased 3α,5α-THP immunoreactivity in the medial

prefrontal cortex, the hippocampal CA1 pyramidal cell layer, the polymorph cell layer of the dentate gyrus, the bed nucleus of the stria terminalis, and the paraventricular nucleus of the hypothalamus. In contrast, ethanol administration significantly reduced 3α,5α-THP immunoreactivity in the nucleus accumbens and the central nucleus of the amygdala. No changes were observed in the ventral tegmental area, dorsomedial striatum, granule cell layer of the dentate gyrus, or the lateral or basolateral amygdala (Cook et al. 2014a). Therefore, ethanol produces divergent brain region and cell-type specific changes in 3α,5α-THP concentrations.

The ethanol-induced increase in plasma neuroactive steroids is mediated by the HPA axis, since it is no longer observed in hypophysectomized and adrenalectomized Sprague-Dawley (Boyd et al. 2010; Khisti et al. 2003; Porcu et al. 2004) or Wistar (O'Dell et al. 2004) rats. However, studies of ethanol effects using immunohistochemistry reveal that ethanol-induced elevations of 3α,5α-THP are independent of adrenal activation in the CA1 pyramidal cell layer, dentate gyrus polymorphic layer, bed nucleus of the stria terminalis, and paraventricular nucleus of the hypothalamus of Wistar rats. Furthermore, ethanol produced decreases in 3α,5α-THP labeling in the nucleus accumbens shore and central nucleus of the amygdala that also occurred independent of adrenal activation. However, in the medial prefrontal cortex ethanol increased 3α,5α-THP immunoreactivity after sham surgery, but there was no change of 3α,5α-THP after adrenalectomy. These data indicate ethanol dynamically regulates local 3α,5α-THP levels in several subcortical regions, but the adrenal glands contribute to 3α,5α-THP elevations in the medial prefrontal cortex (Cook et al. 2014b).

These data in the hippocampal formation agree with the previous *in vitro* studies showing that ethanol induced local brain synthesis of 3α,5α-THP. For example, it was first shown that incubation with ethanol (50 or 100 mM) can increase 3α,5α-THP levels (measured by radioimmunoassay) and GABAergic transmission in hippocampal minces from intact Sprague-Dawley rats (Sanna et al. 2004) and those that had undergone adrenalectomy/ gonadectomy (Follesa et al. 2006). More recently, it was shown that ethanol increases cellular 3α,5α-THP in CA1 pyramidal cells in the slice preparation from juvenile Sprague-Dawley rats (Tokuda et al. 2011). Clearly, ethanol's ability to stimulate brain synthesis of 3α,5α-THP in the hippocampal formation is isolated to specific cellular populations, since we did not previously observe ethanol-induced changes of 3α,5α-THP in the granule cell layer of the dentate gyrus (Cook et al. 2014a). The CA1 pyramidal cells, and the polymorphic and granule cell layers of the dentate gyrus all exhibit dense 3α,5α-THP staining. Therefore, the presence of this very specific effect of ethanol on cellular 3α,5α-THP in the hippocampus is intriguing and may underlie neuron specific responses to ethanol in the hippocampal formation.

Ethanol-induced elevations in GABAergic steroids reach physiologically relevant concentrations that are capable of enhancing GABAergic transmission (Barbaccia et al. 1999; Morrow et al. 1999; Morrow et al. 1998; VanDoren et al. 2000). A large body of evidence from multiple laboratories suggests that ethanol-induced elevations of GABAergic neuroactive steroids contribute to many behavioral effects of ethanol in rodents. These steroids have been shown to modulate ethanol's anticonvulsant effects (VanDoren et al.

2000), sedation (Khisti et al. 2003), impairment of spatial memory (Matthews et al. 2002; Morrow et al. 2001), anxiolytic-like (Hirani et al. 2005) and antidepressant-like (Hirani et al. 2002) actions. Each of these behavioral responses is prevented by pretreatment with the neuroactive steroid biosynthesis inhibitor finasteride and/or by prior adrenalectomy. The hypnotic effect of ethanol is partially blocked by adrenalectomy. Importantly, administration of 5α-dihydroprogesterone, the immediate precursor of 3α,5α-THP, to adrenalectomized rats restores effects of ethanol, showing that brain synthesis of neuroactive steroids modulates effects of ethanol (Khisti et al. 2003). However, neuroactive steroids do not appear to influence the motor incoordinating effects of ethanol, since neither finasteride administration nor adrenalectomy diminish these actions (Khisti et al. 2004). Taken together, these studies suggest that elevations in neuroactive steroids influence many of the GABAergic effects of ethanol *in vivo* and contribute to sensitivity to behavioral effects of ethanol. We have suggested that ethanol-induced elevations of GABAergic neuroactive steroids protect against the risk for ethanol dependence (Morrow 2007; Morrow et al. 2006). Diminished elevations of GABAergic neuroactive steroids following ethanol exposure would result in reduced sensitivity to the anxiolytic, sedative, anticonvulsant, cognitive-impairing and discriminative stimulus properties of ethanol. Reduced sensitivity to ethanol is associated with greater risk for the development of alcoholism in individuals with genetic vulnerability to alcoholism (Schuckit 1994; 2009). Further studies are needed to test this hypothesis.

Strain dependent effects of ethanol on GABAergic neuroactive steroids in mice

Studies regarding the effects of ethanol on neuroactive steroids in mice have yielded different results depending on which strains have been used. Among the most studied mouse strains are C57BL/6J and DBA/2J. We recently reported that C57BL/6J mice have higher basal plasma neuroactive steroid levels compared to DBA/2J mice and that plasma 3α,5α-THP levels are decreased in C57BL/6J mice and not altered in DBA/2J mice following injection of 2 g/kg ethanol (Porcu et al. 2010). Moreover, acute ethanol administration failed to alter cerebrocortical and hippocampal levels of 3α,5α-THP and progesterone in naïve male C57BL/6J and DBA/2J mice (Porcu et al. 2014). Other studies have reported that injection of 2 g/kg ethanol increased whole brain 3α,5α-THP levels in male DBA/2J (Gabriel et al. 2004) but not C57BL/6J mice (Finn et al. 2004b), while orally consumed ethanol increases whole brain 3α,5α-THP levels in male C57BL/6J mice (Finn et al. 2004b).

These two strains differ 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 DBA/2J mice (Finn et al. 1997b). However, similar discriminative stimulus effects of its 5 isomer pregnanolone were reported for both strains (Shannon et al. 2005a; Shannon et al. 2005b). C57BL/6J and DBA/2J mice are also two of the most widely used inbred strains in addiction research because they show different sensitivity to several drugs of abuse, including ethanol (Belknap et al. 1993a; Belknap et al. 1993b). For instance, DBA/2J mice have more severe handling-induced convulsions after withdrawal from acute (Roberts et al. 1992) and chronic (Crabbe et al. 1983) ethanol, have greater acute functional tolerance (Gallaher et al. 1996), greater locomotor activity (Crabbe et al. 1983), and greater hypnosis (Linsenbardt et al. 2009) compared to C57BL/6J mice. These strains also differ in

ethanol preference and consumption. In the two-bottle choice paradigm, C57BL/6J mice drink alcohol, while DBA/2J mice avoid it (Belknap et al. 1977; Phillips et al. 1994). However, when allowed to self-administer ethanol intravenously, these two strains do not differ in the amount of ethanol self-administered, suggesting that ethanol's reinforcing properties are similar in both strains (Grahame and Cunningham 1997). It has been suggested that endogenous neuroactive steroids may play a role in ethanol sensitivity in these strains. In fact, 3α,5α-THP modulates ethanol intake (Ford et al. 2007; Ford et al. 2005), reinstates ethanol seeking behavior in C57BL/6J mice (Finn et al. 2008) and affects ethanol withdrawal severity (Finn et al. 2004a; Gililland and Finn 2007).

Effects of ethanol on GABAergic neuroactive steroids in humans

The role of neuroactive steroids in alcohol action in humans is relatively unexplored and inconsistent. Studies in humans have shown that male and female adolescents seen in the emergency room for alcohol intoxication had elevated plasma levels of the neuroactive steroid 3α,5α-THP (Torres and Ortega 2003; 2004). Although the peak ethanol concentrations were not determined in these studies, it is likely that high ethanol concentrations were achieved since the subjects were taken to the emergency room for treatment of alcohol intoxication. In contrast, laboratory administration of low or moderate doses of ethanol does not alter plasma levels of 3α,5α-THP and other GABAergic neuroactive steroids (Holdstock et al. 2006; Porcu et al. 2010) or may decrease 3α,5α-THP levels (Nyberg et al. 2005; Pierucci-Lagha et al. 2006). The basis of these conflicting results are unknown, but may involve pharmacologically different ethanol doses, different analytic methods to measure neuroactive steroids or environmental factors that influence neuroactive steroid synthesis in humans. Indeed, the same dose of ethanol consumed in the human laboratory studies above (~80 mg/dl) produced no effect in rats when administered systemically (Porcu et al. 2010), suggesting that dose might be a key factor in the difference between rat and human studies.

Despite these results, other studies suggest the importance of GABAergic neuroactive steroids in ethanol's actions in humans. In fact, various subjective effects of ethanol, measured during the rising phase of the blood alcohol curve, are diminished by prior administration of the neuroactive steroid biosynthesis inhibitor finasteride (Pierucci-Lagha et al. 2005). Finasteride prevents the formation of the 3α,5α-reduced metabolites of progesterone, DOC, DHEA and testosterone, thus blocking 3α,5α-THP synthesis (Azzolina et al. 1997). Indeed, finasteride pretreatment blocked subjective effects of alcohol using three different scales to measure the activating, sedating, anesthetic and peripheral dynamic aspects of alcohol actions. The ability of finasteride to reduce the subjective effects of alcohol was not observed in individuals carrying the $GABA_A$ 2 subunit polymorphism associated with alcoholism, and individuals carrying this polymorphism had reduced sensitivity to alcohol as well as finasteride (Pierucci-Lagha et al. 2005). Covault and collaborators (this issue) further show that inhibition of 5α-reductase with dutasteride also reduced the sedative and anesthetic effects of alcohol in men classified as moderate-heavy drinkers. Interestingly, this study, which was conducted in a larger population, did not find any association of dutasteride effects with the $GABA_A$ α2 subunit polymorphism. Indeed all these observations are consistent with the idea that GABAergic neuroactive steroid

responses contribute to ethanol sensitivity in humans and may be a risk factor for alcoholism.

Covault and collaborators (this issue) also report that dutasteride administration reduced subsequent alcohol consumption in those subjects classified as heavy drinkers. In agreement, it has recently been shown that male subjects, who took finasteride for treatment of male pattern hair loss, reported a decrease in alcohol consumption, which was greater in those subjects who consumed the most alcohol. These subjects also reported increased anxiety, tiredness, dizziness, and in few cases even intoxication, after alcohol exposure, all factors that contributed to their alcohol dislike (Irwig 2013. Taken together, these results further support the hypothesis that neuroactive steroids mediate subjective effects of alcohol in humans.

Studies on GABAergic neuroactive steroids in alcohol dependence have shown that 3α,5α-THP and 3α,5α-THDOC levels are decreased in serum of alcoholics during the peak of alcohol withdrawal and return to normal levels upon recovery (Romeo et al. 1996). Likewise, abstinent alcoholics exhibit diminished progesterone levels as well as a lowered ratio of progesterone to pregnenolone (Hill et al. 2005).

Some recent studies have investigated the role of genetic polymorphisms in alcohol dependence with a focus on neuroactive steroids. In alcohol dependent subjects, naltrexone administration, compared to placebo, increased 3α,5α-THP serum levels among individuals with the Asp40 allele of the OPRM1 gene (AG/GG) but not among those carrying the Asn40 allele (Ray et al. 2010). In this population, no correlation was found between basal 3α,5α-THP levels and average alcohol use frequency, quantity or “Alcohol Use Disorders Identification Test (AUDIT)” scores. Moreover, basal 3α,5α-THP levels were not influenced by smoking status, sex, ethnicity, family history of alcoholism or OPRM1 status. These results highlight a potential contribution of neuroactive steroids to the pharmacological effects of naltrexone in alcohol dependent subjects that lack the OPRM1 polymorphism associated with therapeutic efficacy to naltrexone (Ray et al. 2010). Future studies will be required to further examine these relationships.

Risk of alcohol dependence was found to be associated with polymorphic variation in the enzymes 5α-reductase and 3α-HSD implicated in the conversion of progesterone and DOC to their neuroactive metabolites 3α,5α-THP and 3α,5α-THDOC. The minor C-allele for SRD5A1 exon 1 SNP rs248793, the gene encoding for 5α-reductase, and the minor G-allele for AKR1C3*2 exon 1 SNP rs12529, the gene encoding for 3α-HSD, were found to be more frequent in control subjects compared to alcohol dependent subjects. These results suggest that the minor allele for 5α-reductase type I and 3α-HSD type 2 genes may have a protective effect against alcohol dependence and further provide indirect evidence that neuroactive steroids may play a role in alcohol effects in humans (Milivojevic et al. 2011).

Genetic variation in neuroactive steroid levels across BXD recombinant inbred strains

Individual differences in vulnerability to alcoholism have a genetic component (Schuckit 2009). Studies in rodents indicate a shared genetic sensitivity to ethanol, anxiety and stress/HPA axis response (Boehm et al. 2002; Crabbe et al. 1999). We have identified the genetic regulation of basal levels of the neuroactive steroid DOC across the C57BL/6J (B6) ×DBA/2J (D2) (BXD) recombinant inbred mouse strains, a reference population to study networks of phenotypes and their modulation by gene variants (Gora-Maslak et al. 1991; Williams et al. 2001). DOC is an endogenous neuroactive steroid, synthesized from progesterone mainly in the adrenal glands but also in the brain; it is the precursor of the GABAergic neuroactive steroid 3α,5α-THDOC and of the glucocorticoid corticosterone. DOC levels are elevated in rat brain and mouse plasma following acute ethanol administration (Khisti et al. 2005; Porcu et al. 2010), are regulated by hypothalamic and pituitary activation of the HPA axis in both cynomolgus monkeys and humans and this regulation is altered following ethanol dependence (Porcu et al. 2006; Porcu et al. 2008). Across the BXD mouse population we found significant genetic variation in basal DOC levels. Cerebral cortical DOC levels ranged between 1.4 and 12.2 ng/g, resulting in a 8.7-fold genetic variation and heritability of 0.37. Basal plasma DOC levels ranged between 2.8 and 12.1 ng/ml, resulting in a 4.3-fold genetic variation and heritability of 0.32. We mapped variation in basal DOC levels using genetic and bioinformatics tools in GeneNetwork (www.genenetwork.org), a public repository of genetic and phenotypic data as well as a resource for multivariate genetic analysis of complex traits in genetic reference populations (Chesler et al. 2005; Wang et al. 2003). We identified quantitative trait loci (QTLs) on chromosomes 4 and 14, for basal DOC levels in cerebral cortex and plasma, respectively (Porcu et al. 2011). Moreover, variation in basal DOC levels in both plasma and cerebral cortex was linked to several ethanol and anxiety phenotypes previously characterized across the BXD strains by several groups and whose data is available in GeneNetwork. Thus, basal DOC levels are positively correlated with increased ethanol-induced sedation, ethanol-induced ataxia, and ethanol-induced corticosterone levels. These correlations are of particular importance because studies in rats and humans have suggested that neuroactive steroids may play a role in alcohol sensitivity (Morrow et al. 2006). The finding that mouse strains with higher basal DOC levels show a greater ethanol sensitivity is consistent with the hypothesis that elevated GABAergic neuroactive steroids may protect against the risk for alcohol dependence (Morrow and Porcu 2009; Morrow et al. 2006). Diminished elevations of neuroactive steroids following ethanol exposure would result in reduced sensitivity to the anxiolytic, sedative, anticonvulsant, cognitive-impairing, and discriminative stimulus properties of ethanol (Morrow et al. 2006). Reduced sensitivity to ethanol is associated with greater risk for the development of alcoholism in individuals with genetic vulnerability to alcoholism (Schuckit 1994; Wilhelmsen et al. 2003). Variation in basal DOC levels was also correlated to some anxiety phenotypes and seizure susceptibility, in agreement with the fact that neuroactive steroids exert anxiolytic and anticonvulsant properties (Belelli et al. 1989; Bitran et al. 1991; Reddy and Rogawski 2002) and that their levels are altered in several psychiatric diseases involving stress and anxiety (Eser et al. 2006; Girdler et al. 2001).

Given that many steroids, including DOC, function as gene regulatory molecules by acting on nuclear receptors (McEwen 1991), we used bioinformatics analysis to examine correlations between cerebral cortical DOC levels and whole brain gene expression profiles across the BXD strains. The genetic network analysis revealed the involvement of multiple genes regulating inflammation, vesicle trafficking and nuclear receptor signaling, supporting previous evidence for a role of neuroactive steroids in inflammation, neurotransmitter release and synaptic function (Belelli and Lambert 2005; Brinton 2013; Carver and Reddy 2013; Meyer et al. 2002; Mtchedlishvili and Kapur 2003; Schumacher et al. 2007).

We hypothesized that basal and ethanol-induced 3α,5α-THP levels may differ across BXD strains and may contribute to a network of alcohol-related phenotypes. Thus, based on the genetic variation in basal DOC levels (Porcu et al. 2011), we collected preliminary data on selected BXD strains that were chosen to capture the range of variation in neuroactive steroid content. We measured 3α,5α-THP levels in the cerebral cortex of these mice, 1 hour after acute ethanol administration (2 g/kg, i.p.) or saline (basal levels). We found that basal cerebral cortical 3α,5α-THP levels across strains ranged between 1.81 and 3.72 ng/g, equivalent to a 2.0-fold genetic variation [$F(9,79) = 6.27$, $p < 0.0001$] and heritability of 0.40 (Figure 2a). The ethanol-induced changes in cerebral cortical 3α,5α-THP levels ranged between +4% and +63% (Figure 2b).

We further examined genetic correlations with behavioral phenotypes previously determined in the BXD strains and available in GeneNetwork. Both basal and ethanol-induced cerebral cortical 3α,5α-THP levels were correlated with some ethanol phenotypes. Basal 3α,5α-THP levels were negatively correlated with consumption of 10% ethanol at 2 hours [Spearman $r= -0.82$, $p=0.02$, $n=7$, GeneNetwork ID 12733, (Cook et al., unpublished), Figure 3a], that is those strains with low basal 3α,5α-THP levels consumed more alcohol. Interestingly, the ethanol-induced changes in 3α,5α-THP levels were negatively correlated with ethanol consumption both of 3% [Spearman $r=-0.82$, $p=0.02$, $n=7$, GeneNetwork ID 10474, (Phillips et al. 1994), Figure 3b] and 10% [Spearman $r=-0.82$, $p=0.02$, $n=7$, GeneNetwork ID 10582, (Rodriguez et al. 1994), Figure 3c]. Those strains with increased 3α,5α- THP levels in response to acute ethanol consumed less alcohol. This finding, albeit observed in a small number of strains, supports the hypothesis that neuroactive steroid responses to ethanol may be associated with excessive alcohol consumption. Future studies are required to expand and validate these preliminary results.

Genetic variability in the neuroactive steroid response to ethanol has also been shown for corticosterone. DBA/2J mice display greater plasma ACTH and corticosterone responses to acute ethanol administration compared to C57BL/6J mice, suggesting that the HPA axis in DBA/2J mice is more responsive to ethanol (Roberts et al. 1992). In agreement, the increase in cerebral cortical corticosterone levels, induced by acute ethanol, was greater in DBA/2J mice compared to C57BL/6J mice (Porcu et al. 2014). The effects of acute ethanol administration on plasma corticosterone levels have been examined across the BXD strains: an effect of strain on plasma corticosterone was found 1 hour following ethanol administration, but not following saline injection; the genetic analysis suggested that a single gene may account for most of the genetic variability in the corticosterone response to ethanol at this time point (Roberts et al. 1995). Moreover, the corticosterone response to

ethanol was negatively correlated with consumption of 3% ethanol (Roberts et al. 1995), suggesting that those strains with an increased stress response to acute ethanol consumed less alcohol, similar to our preliminary observation on the ethanol-induced 3α,5α-THP responses.

Thus, genetic variation may contribute to the differences in the neuroactive steroid responses to stress or ethanol challenges. These studies have mainly been conducted in mouse strains, but it is likely that genetic variation could also explain the different neuroactive steroid sensitivity observed in rats and humans, compared to C57BL/6J mice.

Summary and Conclusions

Rats and mouse strains clearly show divergent effects of stress and ethanol on GABAergic neuroactive steroids in both plasma and brain. The molecular mechanisms that underlie these differences are not known and have received very little attention by the field. Endocrine differences between rodents and humans, including the ovarian cycle or the predominant type of neuroactive steroid must be taken into account. Moreover, the amount and chronicity of alcohol exposure that underlies addiction to alcohol also differs between rodent and human studies. How then do we choose an appropriate animal model to better understand the potential role of these neuroactive steroids in various physiological processes in humans? At first glance, it may appear that GABAergic neuroactive steroid responses to stress in humans are more similar to rats than C57BL/6J mice. This may be true with respect to the populations of humans that have been included in laboratory studies. However, it is important to recognize that many individuals are excluded from human studies due to various neuropsychiatric diagnoses or even age restrictions. C57BL/6J mice are widely used in biomedical research, particularly due to their selection for transgenic and knock-out strategies. However, these mice exhibit phenotypes such as excessive drinking, aggression and other traits that would likely result in exclusion from laboratory studies in human populations. Perhaps C57BL/6J mice model some human populations while common laboratory rats better model other human populations. One approach to address these issues lies in the study of genetic variation in the various processes that determine neuroactive steroids levels as well as their effects on cell signaling. However, translation from mouse to human applications of GABAergic neuroactive steroids clearly presents major hurdles that will require further research.

Neuroactive steroids may have great potential for therapeutic development. It will be important however to capitalize on human genetic diversity with respect to neuroactive steroid levels and responses to stress in humans, moving closer to a personalized medicine approach in the field. We must consider human genetic diversity in this field, in order to be successful in our attempts to utilize neuroactive steroids for therapeutic benefits.

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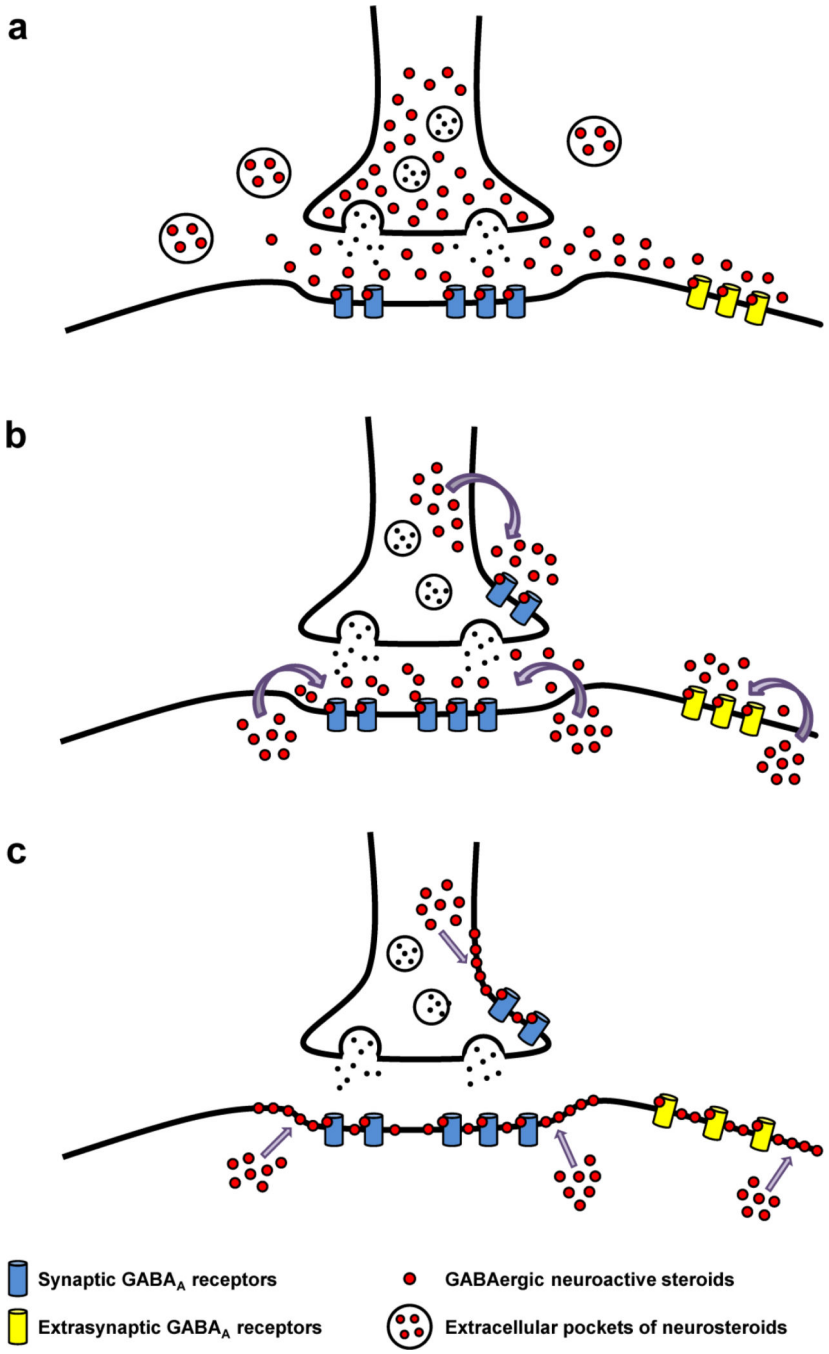

Figure 1.

Schematic representation of the putative mechanisms and sites of action of GABAergic neuroactive steroids. Neuroactive steroids, such as 3α,5α-THP and 3α,5α-THDOC, exert inhibitory actions mediated by synaptic and extrasynaptic $GABA_A$ receptors. No active release mechanism has been identified for endogenous neuroactive steroids. They may access the neuroactive steroid transmembrane binding sites via paracrine (a) or autocrine (b) mechanisms or by intracellular lateral diffusion through the cell membrane (c). There is also evidence for extracellular pockets of neuroactive steroid storage that allow for rapid and

repeated access to extracellular binding sites on neurons. These effects are described and referenced in the text.

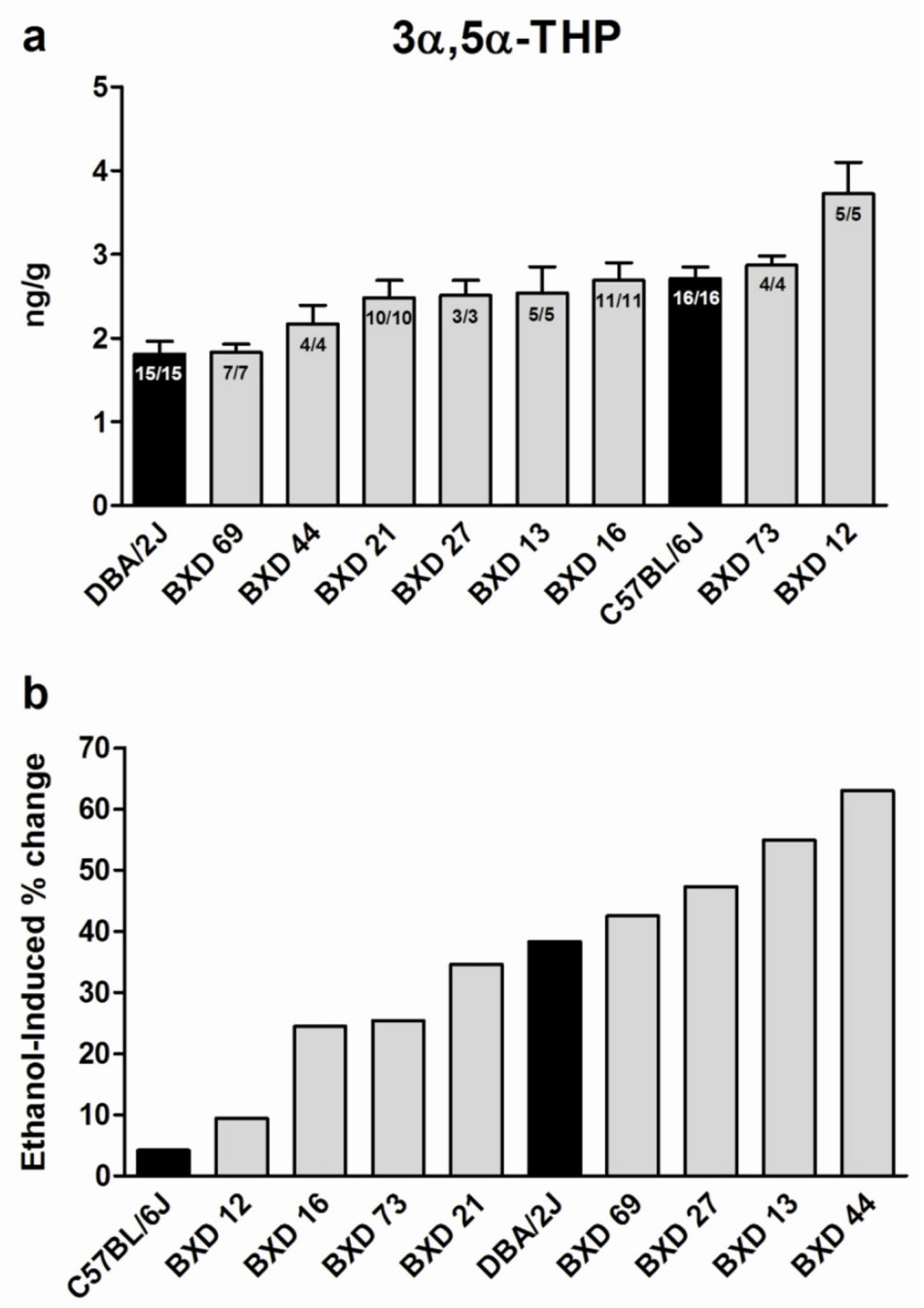

Figure 2.

Variation in basal and ethanol-induced 3α,5α-THP across selected BXD strains. Group-housed male mice (8 to 12 weeks old) were injected with saline (basal levels) or ethanol (2 g/kg, i.p.) at 9:00 am and were euthanized 1 hour later. 3α,5α-THP levels were measured in the cerebral cortex by radioimmunoassay. (a) Data are expressed as ng/g of tissue and are means ± SEM of values from 3-16 mice per strain. (b) Data are expressed as percent change of the average 3α,5α-THP levels in the ethanol-treated group vs. the average 3α,5α-THP levels in the respective saline-treated group (n = 3-16/group/strain).

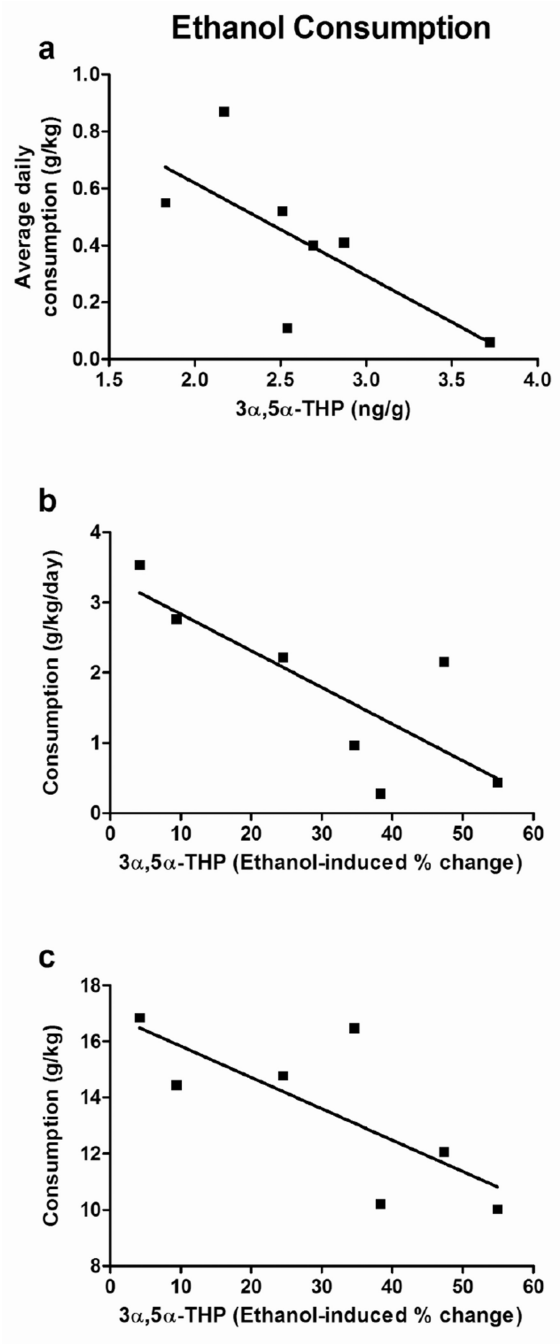

Figure 3.

Genetic correlations between basal or ethanol-induced 3α,5α-THP levels and behavioral phenotypes for ethanol consumption previously characterized in the BXD strains. Group-housed male mice (8 to 12 weeks old) were injected with saline (basal levels) or ethanol (2 g/kg, i.p.) at 9:00 am and were euthanized 1 hour later. 3α,5α-THP levels were measured in the cerebral cortex by radioimmunoassay. Data are expressed as ng/g of tissue (basal levels) or as percent change of the average 3α,5α-THP levels in the ethanol-treated group vs. the average 3α,5α-THP levels in the respective saline-treated group (n = 3-16/group/strain).

Behavioral data for ethanol consumption has been collected by independent labs and has been obtained from GeneNetwork (www.genenetwork.org). (a) Consumption of 10% ethanol at 2 hours, Spearman's $r=-0.82$, p=0.02, n=7, GeneNetwork ID 12733 (Cook et al., unpublished). (b) Consumption of 3% ethanol, Spearman's $r=-0.82$, p=0.02, n=7, GeneNetwork ID 10474 (Phillips et al. 1994). (c) Consumption of 10% ethanol, Spearman's $r=-0.82$, p=0.02, n=7, GeneNetwork ID 10582 (Rodriguez et al. 1994).

Table 1

Summary of the neuroactive steroids effects on the stress/HPA axis response in adult rats, mice and human subjects.

Species	Neuroactive steroid levels	Neuroactive steroid administration
Rat		
Acute stress	↑ basal levels (Barbaccia et al. 1994; Barbaccia et al. 1996; Barbaccia et al. 1997; Purdy et al. 1991; Vallee et al. 2000)	↓ levels of CRH, ACTH and corticosterone (Owens et al. 1992; Patchev et al. 1996; Patchev et al. 1994)
Chronic stress	↓ basal levels (Serra et al. 2000)	N.A.
	↑response to a stress challenge (Serra et al. 2000)	
Mouse		
Acute stress	↓ basal levels (Maldonado-Devincci et al. this issue)	↑ levels of corticosterone (Sarkar et al. 2011)
Chronic stress	↓ basal levels (Dong et al. 2001; Pibiri et al. 2008)	N.A.
Human		
Acute stress/HPA challenges	↑ basal levels (Droogleever Fortuyn et al. 2004; Genazzani et al. 1998; Girdler et al. 2001; Porcu et al. 2008)	N.A.
	-- basal levels (Altemus et al. 2001; Childs and de Wit 2009; Childs et al. 2010; Girdler et al. 2006)	
Chronic stress	↑ basal levels (Girdler et al. 2001)	↑ sensitivity to 3α, 5α-THP (Backstrom et al. 2013)
	-- basal levels (Rapkin et al. 1997; Schmidt et al. 1994; Wang et al. 1996)	
	↓ basal levels (Klatzkin et al. 2006; Monteleone et al. 2000; Rasmusson et al. 2006)	
	↓ response to a stress challenge (Klatzkin et al. 2006)	

These effects are described and referenced in the text.

↑ = increase;

↓ = decrease;

-- = unchanged;

N.A. = not assayed.