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# BEHAVIORAL SENSITIZATION TO ETHANOL IS MODULATED BY ENVIRONMENTAL CONDITIONS, BUT IS NOT ASSOCIATED WITH CROSS-SENSITIZATION TO ALLOPREGNANOLONE OR PENTOBARBITAL IN DBA/2J MICE

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**Abstract—Rationale: The ability of ethanol to facilitate $GABA_A$ receptor-mediated transmission may result in $GABA_A$ receptor alterations during repeated ethanol administration, and lead to dynamic behavioral changes, including sensitization to the locomotor stimulant effect of ethanol. Since alterations in $GABA_A$ receptors are likely to alter sensitivity to GABAergic drugs such as 3α-hydroxy-5α-pregnan-20-one (allopregnanolone) and pentobarbital, we determined whether enhanced sensitivity to ethanol was associated with enhanced sensitivity (cross-sensitization) to these drugs. Two procedures that produced differences in the magnitude of expression of ethanol-induced locomotor sensitization were used.**

**Methods: After habituation to testing procedures for 2 days, female DBA/2J mice were injected with ethanol or saline for 12 days. On the following day, locomotion was recorded after a challenge injection of ethanol (2 g/kg), allopregnanolone (10 or 17 mg/kg), or pentobarbital (10 or 20 mg/kg). Due to evidence that exposure to the test chambers influenced sensitization, in some experiments, mice were exposed to the test apparatus on the day prior to challenge.**

**Results: Exposure to the test apparatus prior to drug challenge attenuated the expression of ethanol sensitization, compared with mice without this pre-exposure. Cross-sensitization was not observed to either allopregnanolone or pentobarbital under any condition; however, some groups of repeated ethanol-treated mice displayed tolerance to the initial stimulant effects of allopregnanolone and pentobarbital.**

**Conclusions: These studies indicate that behavioral sensitization to ethanol is not associated with cross-sensitization to pentobarbital or allopregnanolone, and that the expression of ethanol sensitization is influenced by the relative novelty of the test chamber. In addition, these results do not support a mechanism in which alterations in the neurosteroid or barbiturate modulatory sites of the $GABA_A$ receptor are responsible for the expression of sensitization to the locomotor stimulant effects of ethanol.** 

**Key words: alcohol, neurosteroid, barbiturate, neuroactive steroids, locomotion, GABA.**

Alcoholism is a disease whose development depends on multiple exposures to alcohol (ethanol). The neurobiological adaptations associated with the development of alcoholism have yet to be fully characterized. One broadly defined possibility is that there is an increase in sensitivity to the reinforcing or incentive motivational effects of ethanol upon multiple ethanol exposures (Koob and Le Moal, 1997, 2001; Rodd-Henricks et al., 2001; Schmidt et al., 2000). In some rodents, ethanol stimulates locomotor activity (Dudek et al., 1991; Lister, 1987; Rodd et al., 2004), and behavioral sensitization (an increase in this response) develops following repeated ethanol administration (Correa et al., 2003; Hoshaw and Lewis, 2001; Masur and Boerngen, 1980; Phillips et al., 1995). The locomotor stimulant and reinforcing properties of ethanol and other abused drugs appear to have some neurobiological substrates in common (Amalric and Koob, 1993; Tzschentke and Schmidt, 2000; Wise and Bozarth, 1987), although differences in their regulation are also known to exist. Similarly, studies using sensitized mice have suggested a correlation between behavioral sensitization and the intake of abused drugs (Robinson and Berridge, 1993; Cornish and Kalivas, 2001), including ethanol (Grahame et al., 2000; Lessov et al., 2001a; Phillips et al., 1995). However, the neuroadaptations underlying behavioral sensitization to ethanol have been less studied. Understanding the neurobiological mechanisms of ethanol sensitization may provide useful insights into the adaptations that are critical for the development of dependence in animal models and human alcoholics.

A limited number of studies have investigated the biological processes associated with behavioral sensitization to ethanol; these few studies have indicated that several neural systems are involved. Changes in opioid systems have been suggested to underlie the development of ethanol-induced behavioral sensitization (Camarini et al., 2000b; Miquel et al., 2003), and pharmacological antagonists of *N*-methyl-D-aspartate (NMDA) receptors, a subclass of glutamate receptors, have been reported to atten-

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*Abbreviations:* Allopregnanolone, 3α-hydroxy-5α-pregnan-20-one; ANOVA, analysis of variance; EHS, ethanol–home cage–saline; EMS, ethanol–monitor–saline; MK-801, dizocilpine maleate; NMDA, *N*-methyl-D-aspartate; SHE, saline–home cage–ethanol; SME, saline–monitor–ethanol; THIP, 4,5,6,7-tetrahydroisoxazolo-(5,4,-*C*)pyridim-3-ol.

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uate the development and expression of ethanol-induced behavioral sensitization (Broadbent et al., 2003; Broadbent and Weitemier, 1999; Camarini et al., 2000a; Chester et al., 2001). Recently, brain catalase levels have been proposed as an important factor in ethanol-induced sensitization (Correa et al., 2004). At the molecular level, ethanol-induced behavioral sensitization has been associated with increases in dopamine $D_2$ receptor binding (Souza-Formigoni et al., 1999), although no effect of the dopamine receptor antagonist, haloperidol, on the development of ethanol-induced behavioral sensitization was found (Broadbent et al., 1995). Further, dopamine $D_2$ receptor gene knockout mice displayed enhanced, rather than reduced, ethanol-induced sensitization (Palmer et al., 2003). Finally, results have been mixed when baclofen, a $GABA_B$ receptor agonist, was used to examine the development of ethanol-induced locomotor sensitization (Broadbent and Harless, 1999; Chester and Cunningham, 1999), and 4,5,6,7-tetrahydroisoxazolo-(5,4,-*C*)pyridim-3-ol (THIP), a $GABA_A$ receptor agonist which acts at the GABA binding site, did not affect sensitization (Broadbent and Harless, 1999). To our knowledge, the effects of $GABA_A$ receptor antagonists, or of ligands for other $GABA_A$ modulatory sites, on ethanol-induced behavioral sensitization are unknown.

One problem with pharmacological blockade studies like those just reviewed, is that many drugs reported to block behavioral sensitization also have effects on the acute locomotor response to ethanol. We recently showed that the effect of higher doses of the NMDA receptor antagonist, MK-801, on ethanol-induced sensitization is related to the effect of MK-801 on the acute ethanol response. A lower dose of MK-801, which had no effect on the acute locomotor response to ethanol, actually potentiated the development of sensitization (Meyer and Phillips, 2003). This suggests that the ability of MK-801 and some other drugs to block or attenuate the development of sensitization may be a result of their ability to alter the acute effects of ethanol. Similar arguments have been made for psychostimulant sensitization, pointing out the importance of state-dependency in behavioral sensitization (e.g. Gronig et al., 2004; Stephens et al., 2000). Another method for studying neurochemical determinants of sensitization, cross-sensitization, removes this interpretational confound.

In cross-sensitization studies, behavioral sensitization is induced by exposure to one drug, and then sensitized and non-sensitized (non-drug treated) animals are compared for their behavioral response to a novel drug. Cross-sensitization is evidenced by an enhanced behavioral response to the novel drug in sensitized compared with non-sensitized animals, and infers that the neurobiological mechanisms involved in determining the response to the novel drug have been altered in the sensitized animals. Evidence for cross-sensitization has been obtained between ethanol and cocaine, ethanol and morphine, and ethanol and restraint stress (Itzhak and Martin, 1999; Lessov and Phillips, 2003; Nestby et al., 1997; Roberts et al., 1995), suggesting specific changes in dopamine, opioid and stress-related pathways. In the current studies, due to a large literature supporting a role for $GABA_A$ receptor mediated processes in neuroadaptation to ethanol, such as that associated with tolerance and dependence (reviewed by Chandler et al., 1998; Kumar et al., 2004), we examined cross-sensitization to two $GABA_A$ receptor acting compounds.

Acutely, ethanol has been found to enhance $GABA_A$ receptor function, and its effects are altered by prior ethanol exposure (Allan and Harris, 1987; Buck and Harris, 1990a,b). These effects are dependent on the dose of ethanol used, as well as the subunit composition of the $GABA_A$ receptor (Wallner et al., 2003). In addition, there are several modulatory sites on the $GABA_A$ receptor that can alter its function. One is a proposed binding site for neuroactive steroids, such as 3$\alpha$-hydroxy-5$\alpha$-pregnan-20-one (allopregnanolone), an endogenous metabolite of progesterone (Im et al., 1990; Purdy and Paul, 1999; Ueno et al., 2004). Acute ethanol administration has been found to rapidly increase the concentrations of neuroactive steroids that act as positive allosteric modulators of the $GABA_A$ receptor in the brains of certain strains of rats and mice (Barbaccia et al., 1999; Finn et al., 2004; Gabriel et al., 2004; O'Dell et al., 2004). Thus, one proposed mechanism for the effects of ethanol on GABAergic signaling is the induction of allopregnanolone in the brain (VanDoren et al., 2000). Previous studies have found a genetic association between sensitivity to the acute locomotor effect of ethanol and allopregnanolone (Korpi et al., 2001; Palmer et al., 2002a,b). The role that neuroactive steroids may play in ethanol-induced locomotor sensitization has not been studied.

Another modulatory site on the $GABA_A$ receptor is a barbiturate binding site. Similar to allopregnanolone, there appears to be a genetic association between sensitivity to the acute locomotor stimulant effects of ethanol and pentobarbital (Phillips et al., 1992; Palmer et al., 2002a). Cross-tolerance has been found between ethanol and barbiturates (Bitran and Kalant, 1993; Le et al., 1992), although the role of $GABA_A$ receptor changes has been questioned (Allan et al., 1992; Mihic et al., 1992). This barbiturate binding site has not been investigated for a role in ethanol-induced sensitization. In the present experiments, we hypothesized that ethanol-sensitized mice would display enhanced locomotor stimulant responses to allopregnanolone and pentobarbital.

Behavioral sensitization is a complex phenomenon that is known to depend not only on pharmacology, but also on environmental factors (Badiani et al., 2000; Fraioli et al., 1999; Ohmori et al., 1995; Quadros et al., 2003; Wise et al., 1996), which may or may not involve common neuroadaptive processes. Initial studies presented in this report led us to suspect that the relative novelty of the test chamber substantially influenced the expression of ethanol sensitization. Thus, by manipulating the novelty of the chamber, we examined cross-sensitization using two procedures that produced differences in the expression of behavioral sensitization to ethanol. We hypothesized that cross-sensitization to allopregnanolone and pentobarbital