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Review

Behavioral sensitization to ethanol: Neural basis and factors that influence its acquisition and expression

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ABSTRACT

Ethanol-induced behavioral sensitization (EBS) was first described in 1980, approximately 10 years after the phenomenon was described for psychostimulants. Ethanol acts on γ-aminobutyric acid (GABA) and glutamate receptors as an allosteric agonist and antagonist, respectively, but it also affects many other molecular targets. The multiplicity of factors involved in the behavioral and neurochemical effects of ethanol and the ensuing complexity may explain much of the apparent disparate results, found across different labs, regarding ethanol-induced behavioral sensitization. Although the mesocorticolimbic dopamine system plays an important role in EBS, we provide evidence of the involvement of other neurotransmitter systems, mainly the glutamatergic, GABAergic, and opioidergic systems. This review also analyses the neural underpinnings (*e.g.*, induction of cellular transcription factors such as cyclic adenosine monophosphate response element binding protein and growth factors, such as the brain-derived neurotrophic factor) and other factors that influence the phenomenon, including age, sex, dose, and protocols of drug administration. One of the reasons that make EBS an attractive phenomenon is the assumption, firmly based on empirical evidence, that EBS and addiction-related processes have common molecular and neural basis. Therefore, EBS has been used as a model of addiction processes. We discuss the association between different measures of ethanol-induced reward and EBS. Parallels between the pharmacological basis of EBS and acute motor effects of ethanol are also discussed.

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1. Introduction

Subjects diagnosed with drug dependence exhibit a compulsive pattern of drug-taking behavior. They spend most of their time seeking the drug, using it, and recovering from its effects (Feltenstein and See, 2008). Not everyone who initiates drug consumption, however, progresses to drug abuse or dependence (Schramm-Sapyta et al., 2009). With regard to ethanol, approximately 11.5% of drinkers worldwide drink heavily weekly (World Health Organization, 2011). Therefore, it is important to assess the factors, alone and combined, that can discriminate subjects who are at risk from subjects who can maintain controlled drinking behavior despite regular contact with the drug. An important and still unanswered question is what are the processes that are involved in the transition from voluntary use to addiction. This review will focus on one of these putative processes: ethanol-induced behavioral sensitization (EBS), a phenomenon primarily expressed at the behavioral level after exposure to chronic, often intermittent, exposure to ethanol. A significant part of the review, however, will be devoted to the neural underpinnings of EBS. The Review is guided by the hypothesis that one of the reasons that make EBS an attractive phenomenon is the assumption that EBS and addiction-related processes have common molecular and neural basis. Empirical evidence supporting this phenomenon will critically discussed throughout the present work.

Before discussing the intricacies of EBS, it is noteworthy tracing back seminal studies that cemented the relevance of studying biological changes that accompany the development of addiction. Studies by Schulteis et al. (1995) and Rossetti et al. (1992) indicated that ethanol withdrawal was associated with an increase in intracranial self-stimulation (ICSS) reward thresholds and a 30% decrease in dopamine output in the ventral striatum. ICSS is a behavioral assay in which animals learn to electrically self-stimulate areas of the brain associated with reward. An increase in the intensity of the stimulation that is required to support the animal's response is taken as an index of depression or, more specifically, anhedonia (Fish et al., 2014). The administration of *N*-methyl-D-aspartate (NMDA) receptor antagonists reversed the dopaminergic deficit in the study by Rossetti et al. (1992), a result that was consistent with later work that suggested that persistent impairment in NMDA receptor-dependent long-term depression could mediate the transition to addiction (Kasanetz et al., 2010).

Another perspective on the processes that are involved in the transition from voluntary use to addiction focuses on detecting individual differences at the behavioral, cellular, and genetic levels that may predispose individuals to problematic drinking. This "marker" perspective has traditionally focused on genetic factors, but the search for a single gene or even a small number of genes that are predictive of alcohol abuse liability has been difficult. Perhaps the most successful finding was the differential probability of alcoholism in subjects who exhibited genetic alterations in the functioning of aldehyde dehydrogenase (ALDH; Garver et al., 2001). This enzyme catalyzes the oxidation of acetaldehyde, the primary metabolite of ethanol. The accumulation of this metabolite in peripheral blood is associated with facial flushing, autonomic activation, and other aversive reactions that seem to protect subjects from continued alcohol use (Inoue et al., 1980). These findings fueled the development of promising preclinical genetic therapies. Ocaranza et al. (2008) observed a long-lasting reduction of ethanol drinking (from ~1.2 g/kg/day to ~0.6 g/kg/day for up to 35 days) in Wistar rats that were injected with a viral vector that carried an anti-*Aldh2* antisense gene that reduced the activity of liver aldehyde dehydrogenase by 85%. Rivera-Meza et al. (2012) utilized dual expression gene transfer to simultaneously increase the activity of liver aldehyde dehydrogenase (ADH; the enzyme that breaks down alcohol into acetaldehyde) and decrease the activity of ALDH. This treatment induced a four-fold increase in arterial acetaldehyde levels, which was associated with a 60% reduction of ethanol consumption. Interestingly, co-administration of the acetaldehyde dehydrogenase inhibitor disulfiram blocked the development of EBS (Kim and Souza-Formigoni, 2010).

An important phenomenon that has been suggested to be associated with neuroadaptations after chronic drug use is behavioral sensitization. In drug-related studies, sensitization usually refers to the enhancement of locomotor activity following chronic drug administration (Masur and Boerngen, 1980; Post, 1980). More specifically, EBS refers to the progressive and long-lasting increase in the motor-activating effect of ethanol that results from repeated, often intermittent, drug administration (Masur and Boerngen, 1980; Post, 1980; Post and Weiss, 1988).

Behavioral sensitization has been related to the transition from drug use to addiction and is postulated to reflect sensitized neural circuits that are responsible for regulating the incentive salience of stimuli, leading the individual to a pathological state of wanting the drug (Robinson and Berridge, 1993). Sensitization to morphine has been associated with greater morphine-induced conditioned place preference (CPP; Shippenberg and Rea, 1997), and repeated exposure to amphetamine facilitates amphetamine self-administration (Piazza et al., 1990). Repeated treatment with a given drug (*e.g.*, tetrahydrocannabinol and cocaine) can also enhance subsequent locomotor activity in response to another drug (*e.g.*, amphetamine; Cortright et al., 2011; Liu et al., 2007), especially in vulnerable populations. Adolescent rats that were given a very brief exposure to nicotine, but not their counterparts that were given vehicle, subsequently exhibited cocaine-induced behavioral sensitization (McQuown et al., 2009). This cross-sensitization between different drugs of abuse suggests a common mechanism that underlies the development of behavioral sensitization and a likely way by which exposure to one drug increases the vulnerability to problematic engagement with another drug. Another finding that validates behavioral sensitization as a model of the transition to addiction is that it can be observed even 12 months after the termination of repeated amphetamine administration (Paulson et al., 1991). This striking persistence suggests that neuroadaptations that are induced by repeated drug treatment can be permanent and result in relapse to drug self-administration when appropriate conditions arise (*e.g.*, re-exposure to drug-associated cues). The relationship between sensitization and relapse, however, is still under scrutiny (Lenoir and Ahmed, 2007; Steketee and Kalivas, 2011). One possibility is that both phenomena are regulated by a third mechanism, such as Pavlovian associations between drug-mediated effects and environmental stimuli.

The present review focuses on behavioral sensitization as a paradigm for analyzing the determinants and consequences of ethanol exposure, beginning with a brief historical account of the discovery of EBS (Masur and Boerngen, 1980) and the resurgence in interest following the highly influential incentive sensitization theory of addiction by Robinson and Berridge (1993, 2001, 2003, 2004, 2008). An emphasis is placed on highlighting the challenges of studying EBS and discrepancies and consistencies across the literature that may help researchers who are interested in this phenomenon design their experiments and determine the optimal experimental strategies to test their hypotheses. One of the main aims of this article is to critically review the relationship between EBS and more conventional measures of ethanol reinforcement and between behavioral sensitization and ethanol drinking. The objective it to establish whether the development of behavioral sensitization to ethanol can be considered a proxy for the increased predisposition to ingest this drug.

Other important issues are also covered, including a detailed discussion of transmitter systems that underlie EBS, the differential sensitivity to EBS that is exhibited by mice *vs.* rats, and age-related

differences between younger and older, mature rats in terms of ethanol-induced acute stimulant effects. The acute stimulant effect of ethanol has been largely used as a proxy for ethanol-induced reinforcement (*e.g.*, Pautassi et al., 2011c), mainly in rats because of their insensitivity to EBS. The relationship between EBS and neural correlates (*i.e.*, the expression of transcription factors or immediate or delayed genes) of intermittent ethanol exposure are also analyzed.

1.1. Brief historical overview

Behavioral sensitization was first described for classic psychostimulant drugs (amphetamines and cocaine) and morphine (Wallach and Gershon, 1971; Shuster et al., 1975). In 1980, repeated ethanol was also shown to induce sensitization to its locomotor stimulant effect (Masur and Boerngen, 1980). In this seminal study, mice received daily ethanol treatment (0.0, 1.0, 2.5, or 3.5 g/kg; i.p.) for 30 days, without a challenge. Each motor assessment was performed during 60 min, and mice were introduced into the experimental arena immediately after the ethanol or saline injection. The experiment also lacked a group of control mice given saline throughout training and then challenged with ethanol at test (*i.e.*, to subtract the sensitization effect from the acute drug effect) and did not measure the contribution of environmental cues. Despite these drawbacks, the study provided the tenets of EBS that have yet to be challenged. The 2.5 g/kg dose induced very modest motor activation at the beginning of training in female mice. By day 30, repeated treatment with this dose induced a three-fold increase in activity compared with animals that were given vehicle. Moreover, after 60 days of training, the effect of the highest 3.5 g/kg dose turned from robust motor depression to moderate, albeit significant, motor activation. These data are depicted in Fig. 1. Notably, either by serendipity or perhaps guided by early reports on sex-related differences following psychostimulant exposure (Brass and Glick, 1981), this initial description of EBS focused on female mice (*i.e.*, two of the three experiments that were reported in the paper were conducted in females), which usually show greater sensitization.

Since the publication of the study by Masur and Boerngen (1980), there was a protracted, decade-long hiatus during which very few studies on EBS were published. A review article by Melgaard (1983) briefly mentioned sensitization of the dopaminergic system after repeated ethanol exposure, and other studies (*i.e.*, Liljequist et al., 1981) indicated that dopamine receptor antagonists blocked the acute motor stimulant effect of ethanol. Another valuable study (Lister, 1987) found that ethanol pretreatment (albeit only one exposure) potentiated the motor-stimulating effects of 2.4 g/kg ethanol in mice but only when pretreatment and testing were conducted in the same context. This early work hinted at the now widely accepted notion that contextual cues modulate the expression of EBS. This relative lack of interest in EBS was likely caused by the emergence and widespread use of preparations based on principles of classical conditioning, such as CPP and conditioned taste aversion (CTA), to analyze ethanol-induced reward and its neural underpinnings. This steady state was significantly affected by two publications that separately highlighted the theoretical relevance of drug-induced sensitization to understand the psychological processes that underlie the transition to addiction and the utility of this approach to detect individuals that are at risk for problematic ethanol use.

Renewed interest in behavioral sensitization occurred in the early 1990s when Robinson and Berridge (1993) proposed the incentive sensitization theory of addiction, which was based on early experimental evidence (Berridge et al., 1989) that indicated a dissociation between motivated, appetitive behavior and the ability to hedonically react to stimuli. According to these tenets, repeated drug exposure may make the drug more appealing in terms of seeking behavior, even when the euphoric, post-ingestive effects of the drug lessen after chronic drug exposure. The authors used the term "incentive salience" to describe higher "drug wanting" that develops after repeated drug exposure. Repeated drug treatment endows environmental stimuli with incentive properties, promoting approach behavior toward these stimuli and enhancing the likelihood of drug consumption. This process places susceptible individuals (*e.g.*, those who exhibit poor impulse or executive control) in a pathological motivational state in which they are more likely to take drugs. The greater incentive salience of drug-associated stimuli is related to critical neuroadaptations that render brain reward systems hypersensitive to drugs. Drug "liking" can be dissociated from drug "wanting"; once these neural reward systems are sensitized, compulsive patterns of drug-seeking behavior arise. This theory was subsequently updated (Berridge and Robinson, 2003; Berridge et al., 2009; Robinson and Berridge, 2001, 2008) and emphasized a learned (Pavlovian) component in the acquisition of drug-induced sensitization. Under this framework, associative learning interacts with the neural sensitization of dopaminergic pathways (Ding et al., 2009) and strengthens seeking behaviors toward drug-related cues or contexts. This could explain why context-conditioned behavioral sensitization is more reliable than unconditioned sensitization (Badiani et al., 2000; Crombag et al., 1996), although context-independent sensitization has been observed (Faria et al., 2008; McDougall et al., 2011; Mattingly et al., 2000; Partridge and Schenk, 1999). Consistent with this notion, glutamate-related plasticity in the ventral tegmental area has been implicated in both the development of behavioral sensitization to cocaine (Luo et al., 2010) and acquisition of the conditioning of contextual cues that are predictive of cocaine administration (Harris and Aston-Jones, 2003). Other research provided independent evidence that supported this theory. An elegant study by Wyvell and Berridge (2001) assessed the effects of amphetamine sensitization on conditioned responding to an auditory cue that was paired with the availability of sucrose pellets. As expected, the cue enhanced sucrose seeking, but this increase was significantly higher in amphetamine-sensitized subjects. Specifically, subjects that were sensitized to amphetamine exhibited a two-fold increase in responding to the sucrose-related cue. This study provided fairly convincing evidence of one of the most important tenets of the incentive sensitization theory of addiction, namely the notion that sensitization results in greater attribution of incentive value to reward-related stimuli.

Although largely a theoretical account of how repeated exposure to unconditioned stimuli can dissociate different components of reward, further support for the incentive sensitization theory was provided by studies with humans. One of these studies helped revive interest in ethanol-induced sensitization in the early 1990s. Newlin and Thomson (1991); also see Newlin and Thomson (1999) exposed subjects to three moderate dose of ethanol (0.5 g/kg) and a placebo challenge over a 2-week period. Measurements of finger-pulse amplitude (*i.e.*, an index of ethanol-induced effects) indicated the development of sensitization in a subset of participants who were genetically at risk for developing alcohol-related problems, indicating that sensitization may be a useful marker of addiction vulnerability in humans. This approach continues to be used, showing that "wanting" and "liking" make unique, non-overlapping contributions to ethanol intake in high-risk drinkers, and the contribution of "liking" decreases after prolonged drinking (Ostafin et al., 2010). This theory, however, has also faced some discrepant findings. Heavy drinkers were tested in a single target implicit association task that assessed implicit wanting and liking. These drinkers had higher scores than light drinkers and alcohol-dependent subjects on both the wanting and liking measures (Tibboel et al., 2015).

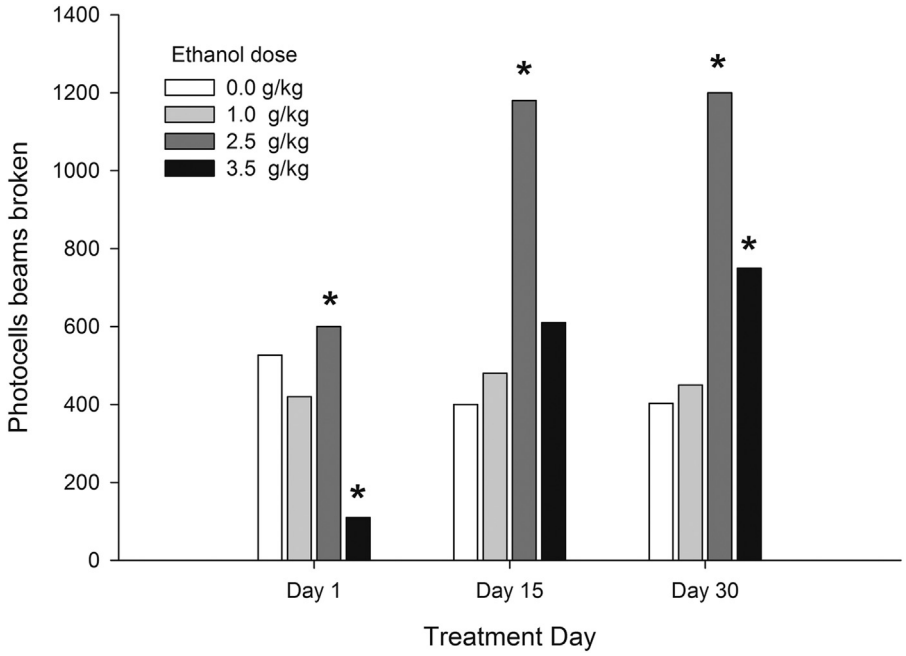

Fig. 1. Mean activity counts (*i.e.*, beam-breaks) in female mice, 60 min after the 1st, 15th, and 30th administration of ethanol (0.0, 1.0, 2.5, and 3.5 g/kg). Animals underwent daily ethanol treatment for 30 days, without a challenge. Each motor assessment was performed during 60 min, and mice were introduced into the experimental arena immediately after the ethanol (or vehicle) injection. The asterisk indicates a significant difference between a given group and the 0.0 g/kg control group. The figure was adapted from Masur and Boerngen (1980), with permission from Elsevier. Please note that the lack of indicators of within-group variability (*e.g.*, vertical bars to indicate standard error of the mean) is due to them being absent in the original, published figure.

After 1990, the amount of literature on EBS suddenly increased, propelled by parallel advancements in understanding the dopaminergic mechanisms that support amphetamine-induced behavioral sensitization (Kalivas and Duffy, 1987; Kalivas and Weber, 1988). The participation of the dopaminergic system on ethanol-induced sensitization was demonstrated by the use of dopamine receptor antagonists (Broadbent et al., 1995; Camarini et al., 2011), the assessment of dopamine binding (Souza-Formigoni et al., 1999), and manipulations of dopamine receptor genes (Harrison and Nobrega, 2009). Subsequent work indicated the involvement of other transmitters and molecules in EBS (see Section 3).

In summary, three successive stages in the study of EBS can be identified. The initial phase begins with the discovery of the phenomenon by Masur and Boerngen (1980), which sparked only a handful of follow-up studies (*e.g.*, Lister, 1987). An intermediate phase provided evidence of EBS in humans (Newlin and Thomson, 1991) and linked EBS to the broad motivational dynamics occurring during the course of addiction (Robinson and Berridge, 1993). This second phase ignited a plethora of contemporary studies (*i.e.*, third phase) that focus on the neural correlates of EBS.

2. Basic concepts and protocols for ethanol-induced behavioral sensitization

The definition of behavioral sensitization seems pretty simple and straightforward: increased motor response following chronic drug administration. Yet this basic plan can, and has been, translated to complex and varied designs. Subtle variations in the schedule (intermittent *vs.* continuous), dose of administration or moment when treatments – other than the target drug – are applied can generate significantly different outcomes. This chapter will review the different protocols available for testing ethanol-induced behavioral sensitization.

Behavioral sensitization is characterized by two phases: induction (or development) and expression. Induction is a temporary sequence of molecular and cellular events that occur in the ventral tegmental area (VTA) of the midbrain that leads to long-term changes in neuronal function. Expression involves lasting neural changes that arise as a consequence of induction and mediate the sensitized behavioral response. Expression is usually, although not always, tested a few days (usually 1 week; Pastor and Aragon, 2006) after the termination of induction in a "challenge" test, in which animals are given either the same or a different dose of alcohol than the one that was used for induction.

Sensitization to ethanol depends on the animal species, strain, and line. Table 1 presents studies that analyzed EBS in rats and mice (male and female) and information on different protocols for EBS. Further information on the protocols that have been employed to assess EBS is also presented in Table 2, which shows data on neural basis of EBS.

As indicated, the challenge for EBS can be conducted with either the same or a different dose as that used during the induction. Moreover, there are two important variations of the protocol for EBS: with and without a challenge after an incubation period. In Masur and Boerngen (1980), for instance, animals underwent daily ethanol treatment, without a challenge. More often, however, is the use of repeated and intermittent (*e.g.*, every other day) ethanol administration (Broadbent and Weitemier, 1999). This treatment generally produces robust EBS in mice (Broadbent et al., 1995; Camarini and Hodge, 2004) but not in rats (Masur et al., 1986). However, there are rare exceptions to this. EBS was observed in rats that were classified as high-responders to novelty prior to the induction of EBS (Hoshaw and Lewis, 2001). Moreover, there is substantial variability in the susceptibility to EBS between mouse strains. Most inbred strains (*e.g.*, DBA/2J; Phillips et al., 1994) are highly susceptible to EBS, but others (*e.g.*, C57BL/6J) are not (Belknap et al., 1993; Camarini and Hodge, 2004; Crabbe et al., 1982; Phillips et al., 1994). Outbred strains may present considerable individual variability or show no sensitization (Quadros et al., 2002b). The lower interindividual variability of inbred mouse strains may contribute to their greater susceptibility to EBS. It has been suggested that experiments that use inbred strains are better equipped to detect subtle

Table 1
Summary of representative studies from Section 2.

Reference	Ethanol dose, length and schedule of induction	Challenge day/locomotor test session	Rat/Mice/Other specie; and strain	Outcome	Additional Details
Broadbent and Harless (1999)	2.0 g/kg, 4 sessions, every 48-h	2.0 g/kg ethanol, 5-min session	DBA/2J mice, male	Sensitized motor response	GABA(B), but not GABA(A) agonists, prevented the development of ethanol-induced behavioral sensitization (EBS)
Camarini and Hodge (2004)	1.0 or 2.0 g/kg, 4 sessions, every 48-h	2.0 g/kg ethanol, 15-min session	DBA/2J and C57BL/6J mice	Sensitized motor response in DBA/2J, but not in C57BL/6J, mice	Challenge (2.0 g/kg ethanol) was conducted before and after ethanol self-administration
Camarini et al. (1995)	2.0 g/kg, 20 daily sessions	10 mg/kg CBZ + 2.0 g/kg ethanol or vehicle + 2.0 g/kg ethanol, 60-min session	Swiss mice, male	Sensitized motor response	Carbamazepine (CBZ), administered between induction and expression of EBS, potentiated EBS
Hoshaw and Lewis (2001)	1.0 g/kg, 15 daily sessions	0.25 g/kg ethanol, 30-min session	Sprague-Dawley rats, male	Sensitized motor response during the first 10 min after ethanol challenge, in rats classified as high-responders to novelty	Prior to induction of EBS, rats were classified as high or low responders to novelty
Kawakami et al. (2007)	2.2 g/kg, 5 sessions, every 48-h	saline and 2.0 g/kg ethanol, 48-h apart, 15-min session	Swiss mice, male and female	Sensitized motor response in males but not in females	Maternal separation during infancy facilitated the development of EBS in females.
Lessov and Phillips (1998)	2.0 g/kg ethanol (acute); 2.5 g/kg ethanol, 10 daily sessions	2.0 g/kg ethanol, 10-min session	Heterogenous stock of female mice	Sensitized motor response	Sensitization lasted for up to 29 days after discontinuation of daily ethanol administration
Masur and Boerngen (1980)	1.0, 2.0, 2.5 or 3.5 g/kg ethanol, 30 or 60 daily sessions	NO	Swiss mice, female	Sensitized motor response after 2.0, 2.5 and 3.5, but not 1.0 g/kg	No tolerance to EBS was observed after 60 days of daily treatment
Melon and Boehm (2011)	2.0 g/kg (acute); 2.5, 3.0 or 3.5 g/kg ethanol, 10 daily sessions	2.0 g/kg, 15-min session	DBA/2J and C57BL/6J mice, male and female, adults and adolescents	Sensitized motor response in DBA/2J and C57BL/6J mice	EBS was greater in adult DBA/2J than in adult C57BL/6J mice. Adolescent DBA/2J exhibited reduced EBS compared to adults
Pastor and Aragon (2006)	2.5 g/kg, 6 sessions, every 48 h	2.5 g/kg ethanol and saline, 24-h apart, 20-min session, but only the last 15-min were considered	Swiss mice, male	Sensitized motor response	Inactivation of all mu opioid receptor subtypes was required to prevent the development of EBS

effects, particularly dose-response relationships, and less prone to incur in type II errors (Festing, 2010). Expression of EBS in outbred strains may be confounded by the natural variability of heterogeneous stocks, in which animals sensitive to EBS are conflated with those unresponsive to it.

The inbred DBA/2J mouse strain can express EBS after only four trials (Broadbent and Harless, 1999; Cunningham and Noble, 1992), although these animals are classified as an alcohol-avoiding strain (Belknap et al., 1993; Camarini and Hodge, 2004). Studies that have used DBA/2J mice mainly administered the drug 4–12 times every other day (Broadbent and Weitemier, 1999), although daily drug treatment (Melón and Boehm, 2011), intermittent administration, or twice-daily injections (Crabbe et al., 1982; Phillips et al., 1994) have also been used.

In contrast to DBA/2J mice, C57BL/6J mice are more resistant to EBS but exhibit high alcohol preference. Although they can show some degree of sensitization after pre-exposure to self-administration or vapor chambers, the magnitude of sensitization is lower compared with DBA/2J mice (Lessov et al., 2001a,b; Zapata et al., 2006). These mouse strains also present differences in the perception of alcohol's preabsorptive (*i.e.*, odor, taste) *vs.* postabsoptive (*i.e.*, pharmacological) properties (Belknap et al., 1977; Blednov et al., 2008), monoamine levels (Kapasova and Szumlinski, 2008; Boone et al., 1997), and γ-aminobutyric acid (GABA), serotonin, and glutamate levels (Boone et al., 1997) in the limbic brain regions after ethanol treatment, which may partially account for different ethanol-induced locomotor responses and ethanol self-administration between these two strains.

A greater variety of schedules and protocols have been used in outbred mice. In outbred Swiss mice, ethanol doses of 1.5–3.0 g/kg effectively induced behavioral sensitization following repeated administration (Carrara-Nascimento et al., 2011; Didone et al., 2008; Masur and Boerngen, 1980). Female Swiss mice that were pretreated with 4.0 g/kg ethanol also exhibited sensitization when challenged with 2.5 g/kg ethanol. An elegant study by Didone et al. (2008) evaluated the effects of different doses of ethanol (1.0–3.0 g/kg) on the expression of sensitization in mice that were previously sensitized to 2.5 g/kg ethanol, demonstrating that the

Table 2
Summary of representative studies from Section 3.

Reference	Ethanol dose, length and schedule of development of EBS	Ethanol dose for the test day*	Rat/Mice/Other specie; and strain	Main Outcome and Additional Details
Dopamine				
Abrahao et al. (2011)	2.2 g/kg, every other day, 5 injections	2.2 g/kg	Swiss mice, male	SCH-23390, a D1 receptor antagonist, administered i.p. or intra-accumbens, blocked the expression of ethanol-induced behavioral sensitization (EBS).
Broadbent et al. (1995)	2.0 g/kg, 4 sessions, every 48-h	2.0 g/kg	DBA/2J mice, male	Haloperidol blocked the acute stimulant effect of ethanol, but did not prevent the development of EBS
Camarini et al. (2011)	2.0 g/kg, 21 daily sessions	2.0 g/kg	Swiss mice, male	SCH-23390 (D1 antagonist) and Sulpiride (D2 antagonist) systemically blocked the development of EBS; only SCH-23390 blocked the expression of EBS
Harrison and Nobrega (2009)	2.2 g/kg, 7 sessions, spaced 3-4 days apart	1.8 g/kg	DBA/2J mice, male	The D3R antagonist, U99194A, blocked the induction but not the expression of EBS. The D3 agonist PD128907 attenuated the expression of EBS.
Quadros et al. (2005)	2.2 g/kg, 21 daily sessions	Expression was not evaluated	Swiss mice, male	D4 binding in the posterior caudate-putamen and the olfactory tubercle was greater in animals given repeated ethanol treatment than in vehicle-treated controls, regardless development of EBS. Only mice that developed EBS (i.e., "sensitized" mice) presented higher D4 binding than controls in the lateral septal nucleus
Souza-Formigoni et al. (1999)	2.4 g/kg, 21 daily sessions	Expression was not evaluated	Swiss mice, male	"Sensitized" mice showed D2 upregulation in the anterior caudate-putamen nucleus, when compared to vehicle-treated controls or "non-sensitized" mice
GABA and Glutamate				
Broadbent and Harless (1999)	2.0 g/kg, 4 sessions, every 48-h	2.0 g/kg	DBA/2J mice, male	Baclofen [GABA(B) agonist], but not THIP [GABA(A) agonist] blocked the development of EBS. Pretreatment with Baclofen prevented the EBS, in the absence of the antagonist
Broadbent et al. (2003)	2.0 g/kg, 4 sessions, every 48-h	2.0 g/kg	DBA/2J mice, male	The non-selective NMDA antagonist MK-801, DNQX (AMPA and Kainate receptor antagonist) and GYKI 52466 (AMPA antagonist), but not ifenprodil, (NR2B antagonist) blocked the expression of EBS.
Camarini et al. (2000a)	2.0 g/kg, 21 daily sessions	2.0 g/kg	Swiss mice, male	Mice treated concomitantly with the NMDA antagonist MK-801 and ethanol did not developed EBS. Pretreatment with MK-801 prevented the EBS, in the absence of the antagonist
Carrara-Nascimento et al. (2011)	1.8 g/kg, 15 daily sessions	1.8 g/kg, for microdialysis procedures	Swiss mice, male, adolescent and adults	Adult, but not adolescent, mice developed EBS and exhibited reduced extracellular levels of Glu in the NAc after the challenge.
Kotlinska et al. (2006)	2.4 g/kg, 4 sessions, every 72-h	2.4 g/kg	Swiss mice, male	Acamprosate, the uncompetitive NMDA antagonists neramexane and MK-801, and the metabotropic mGluR5 antagonist MTEP inhibited the expression of EBS
Kruse et al. (2012)	2.5 g/kg, 11 daily sessions	2.0 g/kg	DBA/2J mice, male	GS39783 [GABA(B) positive allosteric modulator] did not significantly alter the expression, but potentiated the development, of EBS
Quadros et al. (2002a)	2.4 g/kg, 21 daily sessions	Expression was not evaluated	Swiss mice, male	Ethanol-treated mice that did not develop sensitization, but not those that sensitized, presented higher [^{3}H](+)MK-801 binding than controls in the nucleus accumbens core and the prefrontal cortex
Szumlinski et al. (2005)	3.0 g/kg, 8 sessions	Expression was not evaluated	Mice with null mutations of Homer2 and their wild-type (WT) mice	Wild type, but not Homer2 knock out mice, exhibited EBS after repeated administration of 3.0 g/kg

Table 2 (*Continued*)

Reference	Ethanol dose, length and schedule of development of EBS	Ethanol dose for the test day*	Rat/Mice/Other specie; and strain	Main Outcome and Additional Details
Endogenous Opioids				
Camarini et al. (2000b)	2.0 g/kg, 21 daily sessions	2.0 g/kg	Swiss mice, male	Naloxone (general opioid antagonist) blocked the development but not the expression of EBS
Pastor and Aragon (2006)	2.5 g/kg, 6 sessions, every 48-h	2.5 g/kg	Swiss mice, male	Naltrexone and CTOP (mu antagonist), but not naltrindole (delta antagonist), 3-methoxynaltrexone (mu3 antagonist) or naloxonazine (mu1 antagonist), blocked the development of EBS
Endocannabinoids				
Marinho et al. (2015)	1.8 g/kg, 1 session	1.8 g/kg	Swiss EPM-1 mice, Male	Rimonabant blocked the development of a single dose-induced EBS
Serotonin, Nitric Oxide and stress hormones				
Andrade et al. (2011)	2.2 g/kg, 21 daily sessions	2.2 g/kg	Swiss mice, male	SB-242084, a 5-HT2C antagonist, administered intra-accumbens but not systemically, blocked the expression of EBS in highly sensitized mice.
Bhutada et al. (2012)	2.0 g/kg, 4 sessions, every 72-h	2.0 g/kg	Swiss mice, male	Cilnidipine, a calcium channel blocker, intracerebroventricular, blocked the development and expression of EBS
Fee et al. (2007)	2.5 g/kg, 10 daily sessions	1.5 g/kg	DBA/2J mice, male	CP-154,526, a CRF_1 antagonist, blocked the expression but not the development of EBS
Ferraz and Boerngen-Lacerda (2008)	2.0 g/kg, 21 daily sessions	2.0 g/kg	Swiss mice, male	Mianserin, a 5-HT2 antagonist, blocked the development of EBS when co-administered with ethanol, but did not reverse EBS, once already established.
Itzhak and Martin (2000)	1.5 g/kg, 7 daily sessions	1.5 g/kg	DBA/2J mice, male	7-Nitroindazole, an inhibitor of the neuronal nitric oxide synthase, blocked both the development and expression of EBS.
Pastor et al. (2008)	2.5 g/kg, 10 daily sessions for studies using KO mice; 1.5 g/kg for studies using DBA/2J	1.5 g/kg	CRF_1, CRF_{1+2}, and CRF_2, Ucn_1 KO mice, male and female DBA/2J mice, female	EBS is absent in CRF_1 receptor KO, in CRF_{1+2} receptor double KO, but not in CRF_2 KO mice Ucn_1 (urocortin-1) KO mice developed EBS CP-154,526, a CRF1 antagonist, attenuated the development and prevented the expression of EBS in DBA mice (note that doses used in this study differ from those used in Fee et al., 2007) Mifepristone, a GR antagonist, did not block the expression of EBS in DBA mice.
Pastor et al. (2012)	1.5 g/kg, 10 daily sessions	1.5 g/kg	DBA/2J mice, female	Metyrapone, a corticosterone synthesis inhibitor, prevented the development but not the expression of EBS Spironolactone, a mineralocorticoid receptor antagonist, did not prevent the development or expression of EBS
Roberts et al. (1995)	1.5 g/kg, 10 daily sessions	1.5 g/kg	DBA/2J mice, female	Mifepristone (RU 38486), a glucocorticoid receptor antagonist, prevented the development of EBS and cross-sensitization between stress and ethanol
Umathe et al. (2009)	2.4 g/kg, 4 sessions, every 72-h	2.4 g/kg	Swiss mice, male	Ondansetron, a 5HT3 receptor antagonist, prevented the development and expression of EBS

* Challenge timeline is variable, according to each experiment. Some experiments analyze only the development but not the expression or vice-versa.

2.0–3.0 g/kg doses were effective. In this study, behavioral sensitization to lower doses (≤2.0 g/kg) was more evident during the first 10 min, which has been observed in several studies by our laboratory (Carrara-Nascimento et al., 2011; Faria et al., 2008; Rueda et al., 2012). For higher ethanol doses, the expression of sensitization was evident when locomotion was evaluated during the entire 30-min test session (Didone et al., 2008).

The number of and interval between injections that are necessary to induce sensitization can be highly variable. A single exposure to ethanol is insufficient for producing ethanol sensitization following a challenge dose of ethanol (Procopio-Souza et al., 2011), except under conditions of low levels of illumination (Fukushiro et al., 2010). For outbred Swiss mice, the protocol of 15 or 21 consecutive ethanol injections seems very reliable for the development of sensitization (Abrahao et al., 2009; Bellot et al., 1996; Camarini et al., 1995; Camarini et al., 2000a,b; Carrara-Nascimento et al., 2011; Faria et al., 2008; Ferraz and Boerngen-Lacerda, 2008; Masur and Boerngen, 1980; Quadros et al., 2002b; Rueda et al., 2012; Souza-Formigoni et al., 1999), although other protocols have also been effective (Kawakami et al., 2007; Kim and Souza-Formigoni, 2010; Pastor and Aragon, 2006).

Behavioral sensitization can be modulated by contextual cues. Studies that have focused on this topic usually employed separate chambers that featured distinctive textural and visual characteristics. The expression of behavioral sensitization is often significantly more robust and long-lasting in the context where animals were trained compared with an alternative context (Boehm et al., 2008; Cunningham and Noble, 1992). More persistent contextual alterations, namely the introduction of environmental enrichment (Rueda et al., 2012) or stressful stimuli (Roberts et al., 1995; Araujo et al., 2005; Rocha et al., 2010; Quadir et al., 2015), can block or facilitate, respectively, the development or expression of EBS.

Ethanol-induced behavioral sensitization has been reported in other species than rodents, including adolescent rhesus macaques (Schwandt et al., 2008) and zebrafish (Blaser et al., 2010; Tran and Gerlai, 2014). Congruent with mouse studies, zebrafish that were given continuous exposure to ethanol (*e.g.*, 1–2 weeks of constant immersion in 0.25% ethanol) became tolerant to ethanol-induced motor stimulation. In contrast, zebrafish given daily, 1-h immersion in 1% ethanol, exhibited behavioral sensitization (Tran and Gerlai, 2014).

The vast majority of EBS studies have measured motor activity. Sensitization, however, has been found when assessing drug-induced transmitter release (Ding et al., 2009) or sensitivity to the motivational effects of ethanol after chronic ethanol administration (Varlinskaya and Spear, 2010). Three very influential studies that were conducted in rats suggested the development of sensitization to the rewarding effect of ethanol after protracted exposure to ethanol (Bienkowski et al., 1995; Bozarth, 1990; Gauvin and Holloway, 1992). A similar increase in the rewarding effect of ethanol was recently reported in mice (Kurokawa et al., 2013).

Behavioral sensitization is a long-lasting phenomenon that can persist up to 1 year after amphetamine administration (Paulson et al., 1991). Ethanol-induced behavioral sensitization has been shown to last 23–29 days following the termination of ethanol treatment in outbred mice (Lessov and Phillips, 1998) and DBA/2J mice (Boehm et al., 2008), although contextual learning in the latter strain appears to be crucial for prolonging the persistence of sensitization. Animals that were given ethanol that was unpaired with the testing arena exhibited sensitization only through post-sensitization day 14. Notably, both studies (Boehm et al., 2008; Lessov and Phillips, 1998) were conducted in female mice. Some reports indicate that female mice develop more robust locomotor sensitization to psychostimulants than males (Becker and Hu, 2008), a finding that prompted researchers to use only females in several EBS studies (*e.g.*, Didone et al., 2008; Lessov and Phillips, 1998; Kawakami et al., 2007; Phillips et al., 1997; Quoilin et al., 2012).

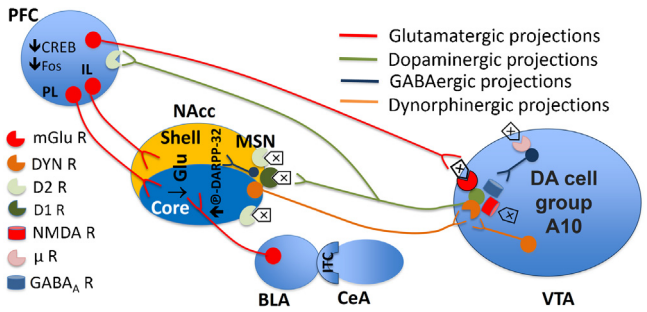

Fig. 2. The role of dopaminergic (DA) D1 receptors in ethanol-induced behavioral sensitization (EBS) is underscored by studies showing that D1 antagonists, administered systemically (Camarini et al., 2011) or intra-Nacc (Abrahao et al., 2011) block EBS. D2 antagonism intra-Nacc is also effective in blocking EBS. Sensitized mice show hyperlocomotion following D1 agonism, accompanied by increased phosphorylation of DARPP-32 (Abrahao et al., 2014). Attenuation of cAMP response element-binding (CREB) and Fos activity have been described in EBS in the PFC (Faria et al., 2008; Soares-Simi et al., 2013). Regarding glutamatergic system, an inverse relationship has been found between EBS and levels of glutamate in the Nacc (Kapasova and Szumlinski, 2008; Carrara-Nascimento et al., 2011). Moreover, non-selective NMDA and non-NMDA receptor antagonists, as metabotropic Glu R5 antagonists block EBS (Camarini et al., 2000a; Broadbent et al., 2003; Kotlinska et al., 2006). It is likely that the opioid system influences EBS via inhibition of dopamine release into the NAc, through opioid-GABA-dopamine interactions. Selective and non-selective mu opiod receptor antagonists prevent EBS (Camarini et al., 2000b; Pastor and Aragon, 2006). MSN—medium spine neurons; ⌧ indicates targets of receptor antagonists that block EBS. PFC—pré-frontal cortex; PL—prelimbic; IL—infralimbic; VTA—Ventral Tegmental Area; Nac—Nucleus accumbens; BLA—Basolateral Amygdala; CeA—Central Amygdala; ITC—Intercalated Amygdala neurons.

In summary, the most common protocol for EBS involves a lengthy induction or development phase, in which animals are given ≈2.0 g/kg ethanol, across daily trials (*e.g.*, Camarini et al., 1995). Important variations of the protocol are the use (or not, see Masur and Boerngen, 1980) of a challenge session, which can be applied immediately after the termination of the induction or after an incubation period. Moreover, certain inbred strains – notably, the DBA/2J – reliably exhibit EBS after only four trials (Broadbent and Harless, 1999). EBS can persist long after termination of the induction (Boehm et al., 2008) and is modulated by the contextual cues in which training took place (Faria et al., 2008). Long-term persistence and contextual modulation of EBS speak in favor of the notion that EBS is a valuable model for the study of addiction-related processes.

3. Neural basis of ethanol-induced behavioral sensitization

Current neuroscience research is guided, at least partially, by the notion that advancement in the understanding of drug addiction requires connecting the molecular and cellular correlates of chronic drug use with the behavioral manifestations of this exposure (Robbins et al., 2010). A wide array of neurochemical alterations accompanies behavioral sensitization. This section describes the roles of different transmitter systems in the induction and expression of EBS, based on the knowledge provided by studies that relied on the use of general and specific agonists and antagonists for a given system. Table 2 presents expanded information on a representative set of these studies. The role of mesocorticolimbic dopamine systems on EBS, and their interaction with glutamate, opioid and GABA systems has been depicted in Fig. 2.

Several studies have established that the main neural substrate of the expression of behavioral sensitization is the nucleus accumbens (NAc; Kalivas and Duffy, 1987; Vezina and Stewart, 1990). Rats that were given repeated microinfusions of amphetamine

in the VTA exhibited an increase in locomotor responses following systemic or intra-NAc challenge injections of amphetamine. Conversely, repeated amphetamine administration in the NAc increased locomotor activity but did not induce sensitization to systemic amphetamine injections (Carr and White, 1987; Dougherty and Ellinwood, 1981; Kalivas and Weber, 1988; Perugini and Vezina, 1994). A recent study found that high-frequency electrical stimulation (a technique that temporarily inactivates brain areas) in the NAc blocked the expression but not development of sensitization to ethanol (Nona et al., 2015).

Heightened dopamine release in the NAc (Kalivas and Duffy, 1987), the supersensitivity of D_1 receptors (Henry and White, 1991), the subsensitivity of D_2 autoreceptors (Henry et al., 1989; Pierce et al., 1995), and enhancement of the D_1 receptor-mediated activation of adenylyl cyclase (Cunningham et al., 1992; Miserendino and Nestler, 1995) have been shown to parallel behavioral sensitization to psychostimulants. It seems, therefore, that dopamine is a key, arguably the most important, transmitter system in the induction and expression of EBS. It is important to note, however, that transmitter systems other than the dopaminergic play a role in this phenomenon. Moreover, amphetamine sensitization has also been reported in the absence of a concomitant increase in dopamine release during its early stage (Wolf et al., 1993). The next section will focus on the role exerted by each transmitter system, beginning with dopamine and also including second-messenger cascades and hormones, in EBS.

3.1. Dopamine

Dopamine receptors are subdivided into two families: the D_1 family (D_1 and D_5, which are G protein-coupled receptors) and the D_2 family (D_2, D_3, and D_4, which are G_i protein-coupled receptors; Le Foll et al., 2009). Mice that were administered D_1 receptor antagonists systemically (Camarini et al., 2011) or intra-NAc (Abrahao et al., 2011) and mice that were genetically altered to not express D_1 receptors in the NAc (Bahi and Dreyer, 2012) exhibited the blunted acquisition and expression of behavioral sensitization. A recent study found that sensitized mice presented higher levels of locomotor activity following intra-NAc D_1 agonist administration compared with non-sensitized mice. This hyperresponsive locomotion in sensitized mice was accompanied by increased phosphorylation of dopamine and cyclic adenosine monophosphate (cAMP)-regulated phosphoprotein, relative molecular mass 32,000 (DARPP-32; Abrahao et al., 2014). Altogether, these data underscore the contribution of the D_1 receptor pathway in the NAc to EBS.

The literature is more conflicting regarding D_2 receptors. One experimental strategy has been to co-administer ethanol and D_1 or D_2 antagonists during chronic ethanol exposure when sensitization is gradually developing but not during the expression of sensitization. Another strategy is to administer the antagonist only during the challenge at the end of repeated drug exposure. Haloperidol, a mixed D_1/D_2 receptor antagonist with preference for D_2 receptors, did not block the development of sensitization (Broadbent et al., 1995). Systemic administration of the D_2 receptor antagonist sulpiride blocked the development but only attenuated the expression of sensitization (Camarini et al., 2011; but see Abrahao et al., 2012). Intra-NAc administration of a D_2 antagonist also attenuated EBS (Abrahao et al., 2012). The attenuation of dopamine transmission in the PFC is involved, in behavioral sensitization to cocaine, likely mediated by D_2 receptors (Beyer and Steketee, 2000; Sorg et al., 1997). Notably, however, sulpiride can also antagonize D_3 receptors, which may also play a role in this phenomenon.

D3 receptor exerts modulatory function upon behaviors by inhibiting the postsynaptic joint effect of D1 and other D2 receptors (Xu et al., 1997). They have received increasing attention because of their influence on motivation and motor function. An elegant study by Harrison and Nobrega (2009) found that repeated administration of the D_3 receptor antagonist U99194A prevented the development but not expression of EBS, and repeated administration of the D_3 receptor agonist PD128907 attenuated the expression of EBS. This study further underscored the role of D_3 receptors in EBS by showing that D_3 knockout mice were resistant to EBS. This phenomenon has been associated with intracellular postsynaptic events, in which both sensitized and control mice exhibited similar levels of D_3 receptor mRNA expression and D_3 receptor binding (Harrison and Nobrega, 2009).

The participation of dopaminergic systems in ethanol sensitization has also been evaluated by receptor binding studies. Sensitization did not induce alterations in D_1 receptor or dopamine transporter binding in several brain regions (Quadros et al., 2002b), whereas sensitized mice exhibited D_2 receptor upregulation in the anterior caudate putamen (Souza-Formigoni et al., 1999) and downregulation in the olfactory tubercle (Araujo et al., 2009) compared with non-sensitized mice. Repeated ethanol administration upregulated striatal D_4 receptors, regardless of whether the mice were sensitized (Quadros et al., 2005).

Repeated ethanol administration produced dopaminergic sensitization in the NAc in C57BL/6 but not DBA/2J mice (Kapasova and Szumlinski, 2008). Behavioral but not accumbal dopamine sensitization was reported in mice that were subjected to ethanol intoxication followed by withdrawal (Zapata et al., 2006). Repeated ethanol administration in rats did not induce dopamine tolerance or sensitization in the NAc (Rossetti et al., 1993). These findings raise the issue of whether behavioral sensitization to ethanol is necessarily correlated with dopamine sensitization.

The studies assessing a putative, causal link between changes in dopamine receptors and ethanol sensitization have provided conflicting evidence. Still, changes in dopamine receptors following this phenomenon may result in changes in downstream dopamine signaling (as discussed above). Mice that developed EBS exhibited lower expression of immediate early genes, cellular stimulation markers, and cAMP response element binding protein (CREB) activity in the PFC (Faria et al., 2008; Soares-Simi et al., 2013), which could be related to the attenuation of dopamine transmission. Briefly, the positive reports suggest that the dopaminergic system is – at least – an important neurobiological mediator of EBS. Data showing cross-sensitization between ethanol and the dopamine uptake inhibitor GBR 12909 suggest enhanced basal dopamine levels following EBS (Broadbent et al., 2005).

3.2. GABA and glutamate

Studies with psychostimulants support a modulatory role for the prefrontal cortex (PFC) in drug-induced behavioral sensitization. The PFC is a terminal region of the mesocorticolimbic dopamine system that features pyramidal glutamatergic neurons that are modulated by GABAergic interneurons. Ibotenic acid-induced lesions of glutamatergic neurons in the PFC prevented amphetamine-induced sensitization (Wolf et al., 1995), and 6-hydroxydopamine-induced lesions of the PFC led to greater behavioral sensitization to amphetamine (Banks and Gratton, 1995). The role of the PFC in EBS, however, is still unknown.

The PFC sends glutamatergic projections to the VTA and NAc. Because this region modulates dopamine cells and dopamine release in the NAc, alterations in glutamatergic transmission may contribute to the development and expression of behavioral sensitization. Kalivas (1995) proposed a model of interaction between dopamine and glutamate, in which the activation of NMDA receptors on VTA dopaminergic neurons modulates the initiation of sensitization, whereas an interaction between dopamine and non-NMDA glutamate receptors in the NAc is important for expression.

Although the more selective glutamatergic antagonist ifenprodil (selective for the NR2B subunit) did not alter the sensitized response to ethanol (Broadbent et al., 2003), this response was inhibited by systemic administration of the nonselective NMDA receptor antagonist MK-801 (Broadbent and Weitemier, 1999; Camarini et al., 2000a; Kotlinska et al., 2006), non-NMDA antagonists DNQX and GYKI 52466 (Broadbent et al., 2003), and metabotropic glutamate receptor 5 (mGluR5) antagonist MTEP (Kotlinska et al., 2006). mGluR5 is classified as a Group I mGluR (G_q protein-coupled receptor) that maintains cross-talk with NMDA receptors in several brain regions (Alagarsamy et al., 2001; Fitzjohn et al., 1996) through interactions with Homer protein and postsynaptic density (PSD) protein, termed Shank, which binds to the PSD-95-associated protein GKAP (Naisbitt et al., 1999). Such interactions might be important for the expression of ethanol sensitization, in which Homer2 deletion disrupted EBS (Szumlinski et al., 2005).

Ethanol inhibits NMDA-activated currents in central neurons (White et al., 1990), and more than one alcohol binding site may exist in the NMDA receptor complex. A recent study (Nona et al., 2014) suggested that sensitivity to the inhibitory effects of ethanol on glutamatergic function may differentiate subjects that are at risk for developing behavioral sensitization from those that are resistant to sensitization, despite similar drug exposure. Specifically, mice were categorized as low-sensitized based on locomotor activity scores and exhibited significantly more expression of the NR1 subunit in the NAc than high-sensitized animals and greater NR2A and NR2B levels than vehicle-treated controls. The upregulation of NR2A expression in the bed nucleus of the stria terminalis, an area associated with the regulation of fear and anxiety (Davis et al., 2010), was found in high-sensitized mice.

An inverse relationship has been demonstrated between the development of EBS and extracellular levels of glutamate in the NAc (Carrara-Nascimento et al., 2011; Kapasova and Szumlinski, 2008). C57BL/6J mice exhibited a sensitized glutamate response to ethanol following repeated treatment, whereas DBA2/J mice presented tolerance to this effect (Kapasova and Szumlinski, 2008). In another study, adult but not adolescent Swiss mice developed behavioral sensitization to repeated administration of 1.8 g/kg ethanol, and adolescents exhibited an increase in extracellular glutamate levels in the NAc following a subsequent ethanol challenge (Carrara-Nascimento et al., 2011).

Sensitization can be classified as conditioned and unconditioned, depending on whether the magnitude of the motor response is modulated by the environment where the drug is administered. The ability of MK-801 to block EBS may be attributable to disruption of the conditioned component of the phenomenon, although this is speculative and would explain only one part of this complex process. Glutamate-dependent cellular mechanisms are associated with drug-associated learning. NMDA antagonists prevent both behavioral sensitization to ethanol (Broadbent and Weitemier, 1999; Camarini et al., 2000a) and long-term potentiation, a form of neural plasticity that is involved in learning and memory and can be formed in dopamine neurons in the VTA or NAc (Jones and Bonci, 2005; Malenka, 2003).

Taking together, the available data indicates that individuals that show resistance to develop EBS exhibit higher extracellular levels of glutamate levels in the NAc (Kapasova and Szumlinski, 2008; Carrara-Nascimento et al., 2011) and higher expression of the NR1 subunit and [^{3}H](+)MK-801 binding levels in the NAc (Quadros et al., 2002a; Davis et al., 2010). Although speculative, these neurochemical markers could identify subjects that are resilient to the neuroplastic changes underlying addiction.

Notably, among the few pharmacological treatments for alcohol dependence that are approved by the United States Food and Drug Administration (FDA) is acamprosate, which has been shown to regulate the balance between GABA and glutamate neurotransmission (Pierrefiche et al., 2004). This agent has been shown to reduce alcohol drinking, reinstatement, and abstinence rates, and inhibit EBS (Kotlinska et al., 2006). For example, Kurokawa et al. (2013) found significantly greater ethanol-induced CPP in mice that were ethanol-dependent *via* continuous exposure to ethanol vapor compared with controls that were exposed only to air. This enhancement of CPP, which can be considered an index of sensitization to the rewarding effect of the drug, was associated with greater expression of protein kinase A (PKA) and phosphorylated CREB (p-CREB) in the limbic forebrain. Acamprosate (300 mg/kg) inhibited CPP in both nondependent and dependent mice and inhibited the overexpression of PKA and p-CREB (Kurokawa et al., 2013).

With regard to GABA, ethanol's actions on $GABA_A$ receptors depend on the subunit composition. Receptors that contain a combination of α4 (or α6), β3, and δ subunits are particularly sensitive to ethanol (Harris et al., 2008; Vengeliene et al., 2008). $GABA_A$ receptors are widely distributed in brain regions of the mesolimbic system that are involved in the expression and development of behavioral sensitization. Indeed, neuroadaptive changes in $GABA_A$ subunit composition may be responsible for behavioral sensitization to ethanol, in which repeated ethanol exposure increased $GABA_A$α1, −β2, and −γ2 subunit gene expression in the NAc in DBA/2J mice (Linsenbardt and Boehm, 2010).

The GABAergic system plays an important role in alcohol's reinforcing properties. For example, $GABA_A$ and $GABA_B$ receptor agonists blocked the stimulant effects of ethanol (Cott et al., 1976). The $GABA_B$ receptor agonist baclofen suppressed the acquisition of alcohol drinking, diminished the motivation to consume alcohol in rats, reduced the alcohol deprivation effect in alcohol-preferring sP rats (Colombo et al., 2003a,b), and reduced ethanol drinking in light-drinking but not uncontrolled-drinking mice (Villas Boas et al., 2012). Ethanol-induced behavioral sensitization is apparently also mediated by $GABA_B$ receptors. Coadministration of ethanol and the $GABA_B$ agonist baclofen prevented sensitization, whereas the $GABA_A$ agonist THIP failed to block the phenomenon (Broadbent and Harless, 1999). Moreover, Kruse et al. (2012) found that the positive allosteric modulation of $GABA_B$ receptors *via* GS39783 administration did not significantly alter the expression of EBS but potentiated its development. These effects may be explained by the location of $GABA_B$ receptors (pre- *vs.* postsynaptic). $GABA_B$ receptors are located at both pre- and postsynaptic sites. $GABA_B$ agonists exert bi-directional effects on VTA output, with low and high doses of GABA agonists leading to activation and inhibition, respectively, of dopamine neurons (Cruz et al., 2004). Thus, binding of GS39783 to $GABA_B$ receptors can stimulate or inhibit DA release from the VTA to NAc. In addition, $GABA_B$ receptors, that are G-protein coupled receptors, can phosphorylate a number of proteins, which favors long-lasting changes in the state of neuronal activity (Mott and Lewis, 1994). Overall, modulation of the stimulation of $GABA_B$ receptors can induce changes in neuronal activity that are not possible through activation of $GABA_A$ receptors.

So far, researches on the involvement of GABA systems on EBS have been limited to a handful of studies. These studies point to a role of $GABA_B$ receptors on mesolimbic dopaminergic pathway, controlling dopamine release in the NAc, and consequently the expression of EBS.

In addition to channel-coupled glutamate and GABA receptors, calcium channels are also primary targets of ethanol's actions. Cilnidipine, an L/N-type calcium channel blocker, attenuated both the acquisition and expression of ethanol sensitization (Bhutada et al., 2012). Interestingly, the deletion of N-type calcium channels reduced ethanol consumption in mice (Newton et al., 2004).

3.3. Endogenous opioids

Endogenous opioid systems play a key role in mediating alcohol's positive rewarding effects (Gianoulakis, 2004; Camarini et al., 2010). The activation of dopaminergic neurons by ethanol involves an interaction with endogenous opioids in the VTA (Gonzales and Weiss, 1998). This interaction between ethanol and opioids is illustrated by the observation that administration of opioid receptor antagonists suppresses ethanol-induced increases in dopamine in the NAc (Benjamin et al., 1993). A model has been proposed for the modulation of mesolimbic dopaminergic A10 neurons by the opioidergic system, in which A10 neurons in the VTA are stimulated by the μ opioid system *via* the inhibition of GABAergic interneurons, inducing the release of dopamine in the NAc (Di Chiara and North, 1992; Spanagel, 1995; Spanagel and Weiss, 1999). A long-loop GABAergic feedback projection also provides tonic inhibition of A10 neurons (Spanagel and Weiss, 1999). Dynorphin may regulate dopamine release in the NAc *via* negative feedback regulation by binding to κ opioid receptors (Hyman and Malenka, 2001; Shippenberg and Rea, 1997; Spanagel et al., 1992). This endogenous opioid peptide is expressed in a subset of medium spiny neurons and contributes to the dysphoric manifestations of drug withdrawal (Carlezon et al., 1998; Cole et al., 1995; Spanagel et al., 1992). Evidence suggests that the endogenous dynorphin/κ opioid system is upregulated by substantial ethanol exposure (Walker and Koob, 2008), and this neuroadaptation may be critical for the escalation to problematic drug use (Sirohi et al., 2012; Walker et al., 2012). Signaling by the dynorphin/κ opioid system has been traditionally associated with dysphoria. Studies suggest that this system may partially mediate the aversive post-ingestive effects of ethanol intoxication (Pautassi et al., 2012a).

Both nonspecific and selective opioid receptor antagonists have been shown to block EBS (Camarini et al., 2000b; Pastor and Aragon, 2006), confirming the hypothesis that manipulations of the opioid system might modify the behavioral sensitization to ethanol, likely through the inhibition of dopamine release in the NAc *via* opioid-GABA-dopamine interactions. Specifically, EBS requires the activation of μ but not δ opioid receptors (Pastor and Aragon, 2006). To our knowledge, the role of the nociceptin/orphanin FQ (N/OFQ) opioid receptor in sensitization to ethanol has been scarcely investigated, despite the possibility of its involvement in drug dependence (Sakoori and Murphy, 2008; Witkin et al., 2014). Heighted acute motor responses to ethanol (2.0 g/kg) have been observed in N/OFQ knockout mice (Sakoori and Murphy, 2008). The ethanol administration protocol that was employed in that study, however, did not yield greater motor responses across trials, either in wildtype or knockout mice, thus preventing discernment of the role of N/OFQ receptors in behavioral sensitization.

Ethanol promotes dopamine release in the NAc through the indirect modulation of GABAergic interneurons or direct actions on dopaminergic VTA neurons (Brodie et al., 1990). Either way, the main action of ethanol on dopamine release is mainly attributable to its modulation in the VTA rather than NAc. The inhibition of dopamine release by opioid antagonists is likely one of the mechanisms by which they block EBS. Remarkably, naltrexone is currently used for the treatment of alcohol addiction. However, remaining unclear is whether the blockade of sensitization processes may at least partially account for the beneficial effects of naltrexone on alcohol dependence.

3.4. Endocannabinoids

Up to date, two main endocannabinoids receptors have been described: CB1 and CB2. The former is mostly located in the central nervous system, whereas the latter can be primarily found in the immune system (Svízenská et al., 2008). The endocannabinoid system has been implicated in the regulation of motivational processes, including drug seeking behavior, by a dopamine-independent mechanism (Maldonado et al., 2006). Specifically, CB1 receptors seem to function as neuromodulators, mediating the inhibition of transmitter release (Pertwee, 2006) and preventing excitotoxicity (Blázquez et al., 2015). This system also participates in mechanisms underlying EBS. Studies on the role of endocannabinoid system on EBS demonstrate different patterns of cannabinoid receptor expression after chronic ethanol exposure, with increases on CB1 receptor expression in several encephalic nuclei (PFC, striatum, hippocampus, VTA and CeA) in sensitized mice on the 5th day of withdrawal. Ethanol challenge reversed the CB1 up-regulation (Coelhoso et al., 2013). The involvement of CB1 receptor on EBS has been confirmed by the use of rimonabant, a CB1 cannabinoid antagonist. Administration of rimonabant inhibited EBS (Marinho et al., 2015) and the cross-sensitization between nicotine and ethanol (Biała and Budzyńska, 2010). Ethanol-induced motor activity was also abolished after the administration of the highly selective CB1 antagonist PF-514273, although this drug did not alter the acquisition or expression of ethanol-induced CPP (Pina and Cunningham, 2014). Also important is a study conducted in CB1-/- mice. Unlike their wild-type counterparts, these animals did not exhibit changes in both NMDA and GABA receptors after chronic ethanol exposure (Warnault et al., 2007).

3.5. Serotonin, nitric oxide, estrogen and stress hormones

Ethanol affects a number of biochemical processes through its direct actions, comprising ligand-gate ion channel (*e.g.* NMDA, GABA, 5-hydroxytryptamine-3, nicotinic cholinergic, glycine), G-protein coupled receptors (*e.g.* CB1), enzymes (*e.g.* adenylyl cyclase, nitric oxide) and ion channels (*e.g.* L-type Ca^{2+} channels). Ethanol also exerts indirect actions which include effects on neurotransmitters (*e.g.* DA, serotonin), neuropeptides (*e.g.* NPY) and hormones (*e.g.* CRF system, estrogen) (Harris et al., 2008; Vengeliene et al., 2008). Some of these molecular targets are also involved in EBS, as discussed below.

The systemic administration of an antagonist of the serotoninergic system blocked the development of EBS, but did not reverse EBS once it was already established (Ferraz and Boerngen-Lacerda, 2008). The blockade of EBS expression was evident when a 5-HT_{2C} receptor antagonist was administered directly into the NAc but not systemically (Andrade et al., 2011). Similarly, fluoxetine inhibits the expression of sensitization to morphine-induced oral stereotypy in rats but does not affect its development (Wennemer and Kornetsky, 1999). The 5-HT_{2C} receptor exerts inhibitory control over the function of the mesoaccumbens dopamine pathway, and it has been considered a pharmacological target for the treatment of drug addiction (Berg et al., 2008). The serotonin receptor (5-HT_3) antagonist ondansetron significantly and dose-dependently blocked the development and expression of sensitization (Umathe et al., 2009). Conversely, selective serotonergic reuptake inhibitors facilitated EBS (Goeldner et al., 2005), confirming the important modulation of the phenomenon by this system.

Ethanol-induced behavioral sensitization is also influenced by nitric oxide (NO; Itzhak and Martin, 2000), corticotrophin-releasing factor (CRF; Fee et al., 2007), glucocorticoid receptors (Roberts et al., 1995), and calcium channel blockers (Bhutada et al., 2012). Cross-talk exists between NO and glutamate (D'Yakonova and D'Yakonova, 2008) and between NO and dopamine (Desvignes et al., 1999). Nitric oxide stimulates the release of both neurotransmitters. Thus, modulators of dopamine and glutamate systems, which are known to play a role in EBS, may represent important targets that can inhibit sensitization. In fact, 7-nitroindazole (7-NI), an inhibitor of neuronal nitric oxide synthase (nNOS), reduced EBS in DBA/2J mice (Itzhak and Martin, 2000) and Swiss mice (Rocha

et al., 2010). Behavioral sensitization may also be modulated by hormones that are associated with the hypothalamic-pituitary-adrenal (HPA) axis. Chronic stressors that alter activity of the HPA axis induced cross-sensitization with several drugs of abuse, including ethanol (Roberts et al., 1995; Rocha et al., 2010; Quadir et al., 2015), and pretreatment with a selective CRF receptor antagonist blocked the expression but not acquisition of locomotor sensitization to ethanol in DBA/2J mice (Fee et al., 2007), although Pastor et al. (2008) demonstrated that both development and expression of EBS can be attenuated by CP-154,526, using different doses. Moreover, CRF_1 receptor knockout mice but not CRF_2 knockout mice did not display sensitization to the stimulant effects of ethanol (Pastor et al., 2008).

Corticotropin-releasing factor activates the HPA axis and is a primary mediator of stress responses. Corticotropin-releasing factor releases adrenocorticotropic hormone (ACTH), which induces the secretion of glucocorticoids from the adrenal gland (Bale and Vale, 2004; Bilezikjian and Vale, 1987). Roberts et al. (1995) reported that the development of ethanol sensitization appears to be mediated by glucocorticoid receptors (GRs), in which both stress- and ethanol-induced sensitization was blunted by administration of the GR antagonist RU 38486. Mineralocorticoid antagonists do not prevent the acquisition or expression of ethanol sensitization (Pastor et al., 2012). These authors also found that CRF is particularly involved in neuroadaptive processes that are induced by repeated ethanol exposure, such as sensitization, but not its acute stimulant effect. Specifically, CRF_1 receptors, but not CRF_2 receptors or the urocortin peptide, are critically involved in ethanol sensitization (Pastor et al., 2008). Moreover, ethanol-induced increases in corticosterone levels are required for the acquisition but not expression of ethanol sensitization (Pastor et al., 2012).

As mentioned, expression of EBS is more reliable in females than in males (Becker and Hu, 2008). Estrogen is known to modulate striatal dopamine receptors and adenylyl cyclase activity (Ramos et al., 1987; Van Hartesveldt and Joyce, 1986; Becker and Cha, 1989) and plays a key role in mediating responses to cocaine in female rats (Segarra et al., 2010). For example, ovariectomy decreased striatal dopamine release (Becker, 1990) and attenuated cocaine-induced sensitization, which was restored after estrogen replacement (Becker and Hu, 2008; Sell et al., 2000). These effects demonstrate a role for estrogen in dopaminergic neurotransmission and EBS.

3.6. *Effects of ethanol-induced behavioral sensitization on downstream intracellular messenger cascades*

Behavioral sensitization to psychostimulants is accompanied by dopamine D_1 receptor supersensitivity in the NAc, which affects downstream G_s protein coupling and upregulates adenylyl cyclase and PKA. The downstream responses of PKA activation include the phosphorylation of CREB and consequent induction of CREB-regulated genes, such as immediate-early genes (Sheng and Greenberg, 1990), and the phosphorylation of DARPP-32 at the threonine (Thr^{34}) residue site. In fact, context-induced locomotor sensitization to cocaine is accompanied by an increase in cocaine-induced CREB and extracellular signal-regulated kinase (ERK) phosphorylation in a minority of NAc neurons (Marin et al., 2009), and ethanol-sensitized mice exhibit an increase in DARPP-32 phosphorylation after administration of a D_1 receptor agonist (Abrahao et al., 2014).

The long-term persistence of behavioral sensitization to psychostimulants has been related to the upregulation of transcription factors (Perrotti et al., 2005). One of the most studied of these has been ΔFosB, the truncated form of FosB. ΔFosB is expressed after chronic but not acute stimulation. Rats that were given access to intermittent ethanol self-administration (15 intake sessions, 3 days per week; Li et al., 2010) or repeated, experimenter-administered ethanol (Perrotti et al., 2005) exhibited significantly greater ΔFosB labeling in the striatum than control animals that drank only water or received vehicle injections. De Pauli et al. (2014) measured ΔFosB expression in several brain areas after termination of a 21-days EBS treatment. Significant differences were found in the motor cortex and VTA between sensitized and non-sensitized mice (upper and lower 30% of the distribution, respectively) 5 days after the last ethanol administration.

CREB is an important transcription factor that is implicated in learning, memory, and addiction processes. CREB binds to the cAMP response element (CRE) site that is localized in the promoter region of some genes (Impey et al., 2004; Zhang et al., 2005), and it is phosphorylated by PKA, calcium-calmodulin kinases (CaMKI and CaMKIV), and growth factor-associated kinases (Sun et al., 1996; Pende et al., 1997; Taylor et al., 2005), indicating that more than one signaling pathway converges to stimulate CREB.

Psychostimulants may activate CREB through the stimulation of dopamine receptors (Konradi et al., 1994; Cole et al., 1995). Studies have shown that repeated exposure to ethanol (either by experimenter-administered intraperitoneal injections or two-bottle choice self-administration) decreased PKA, G_s protein, and the levels of pCREB and increased G_i protein in the NAc shell (Pandey, 2004). These molecular changes have been associated with high ethanol consumption. Infusion of the PKA inhibitor Rp-cAMPS into the shell region of the NAc decreased CREB phosphorylation in rats, which switched their preference to consume more ethanol. Furthermore, basal CREB expression is lower in the NAc shell in C57BL/6 mice, which are an alcohol-preferring strain, compared with DBA/2 mice, which are an alcohol-non-preferring strain (Misra and Pandey, 2003). Ethanol-induced stimulation of VTA dopaminergic neurons is lower in C57BL/6 mice than in DBA mice (Brodie and Appel, 2000). Altogether, these findings suggest that C57BL/6 mice and animals that have lower CREB levels in the NAc shell have a propensity to drink high amounts of ethanol to achieve its rewarding effects.

We demonstrated that repeated ethanol administration to adolescent mice led to a significant decrease in c-Fos and Egr-1 levels (protein products of genes whose transcription is regulated by CREB) relative to acute ethanol administration in the PFC and NAc. In these mice, however, the repeated ethanol administration did not result in EBS. Instead, the adolescents showed context-dependent behavioral tolerance to ethanol (Faria et al., 2008; see Fig. 3). On the other hand, adult mice developed context-dependent sensitization to ethanol, which was associated with lower Fos protein levels in the hippocampus, as compared to adult mice that received an acute ethanol administration. In fact, ethanol blocks hippocampal Fos responses in conditioning paradigms (Melia et al., 1996).

Recently, the participation of CREB activity in behavioral sensitization to ethanol has been investigated. Regardless of the development of sensitization, repeated ethanol administration decreased CREB binding activity in the PFC and hippocampus in adolescent and adult mice (Soares-Simi et al., 2013). The suppression of CREB activity was more pronounced in the PFC in the adolescent group, and the most marked reduction in the hippocampus was detected in the adult group, which is consistent with the data from Faria et al. (2008).

Changes in CREB activity appear to be region-dependent. Sensitized mice displayed higher pCREB levels than non-sensitized or saline-control mice in the NAc and bed nucleus of the stria terminalis (Nona et al., 2013).

CREB is also a target of the Ca^{2+}/calmodulin-dependent protein kinaseII (CaMKII). CaMKII can activate CREB by phosphorylation at serine-133, indirectly, *via* activation of Ras/MAP kinase second messenger system, which occurs through ribosomal S6 kinase 2

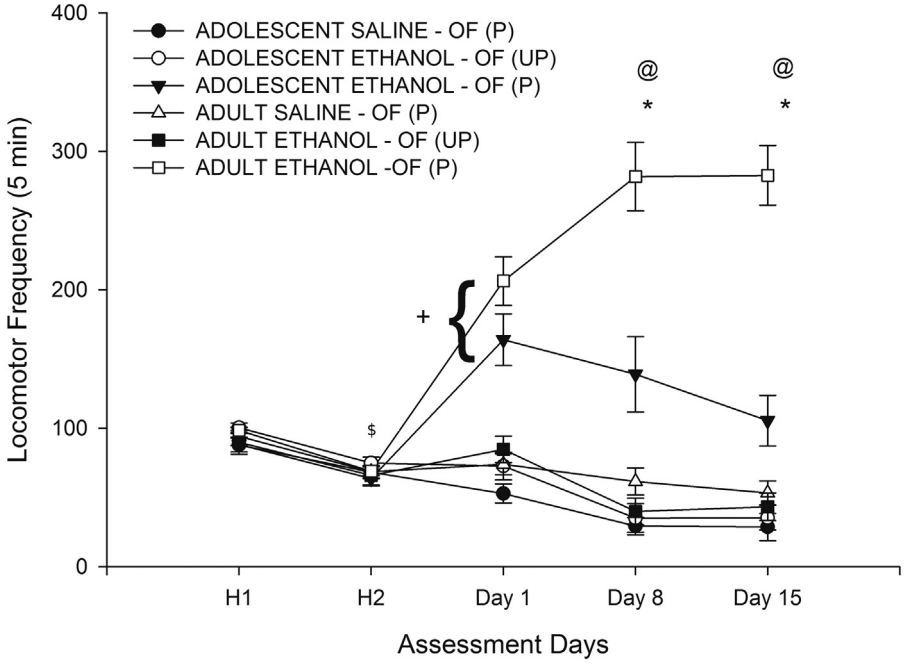

Fig. 3. Locomotor activity (*i.e.*, number of squares crossed with all four paws) in adolescent and adult Swiss mice given ethanol (2.0 g/kg) or saline (0.0 g/kg) injections for 15 consecutive days. The injections were performed in an open field or the home cage in paired (P) and unpaired (UP) groups, respectively. All of the mice received a saline injection on the first 2 days (H1 and H2). The $ sign indicates that locomotor activity on H2 was significantly lower compared with H1. The plus sign indicates that ethanol induced an acute stimulant locomotor response compared with mice that received saline in the homecage. The asterisk indicates a significantly greater locomotor response compared with day 1 following repeated ethanol injections. The @ sign indicates that locomotor activity in the adult group was greater than in the adolescent group. Vertical bars indicate the standard error of the mean. The figure and legend were adapted from Faria et al. (2008), with permission from Elsevier.

(Xing et al., 1996; Pende et al., 1997). It can also inhibit CREB activity by phosphorylation at serine-142 (Sun et al., 1996). CaMKII plays an important role in the expression and development of behavioral sensitization to psychostimulants (Pierce et al., 1998; Licata and Pierce, 2003) and has been proposed to represent the biochemical link between the development and expression of behavioral sensitization to psychostimulants (Licata and Pierce, 2003). More recently, it was demonstrated that the α-isoform of CaMKII (αCaMKII), which is capable of autophosphorylation, plays a role in the establishment of alcohol drinking behavior and also in the development of EBS. It has been suggested that these effects are dependent on alcohol-induced DA increase in the nucleus accumbens, triggered by αCaMKII autophosphorylation (Easton et al., 2013). Mijakowska et al. (2015) confirmed the role of αCaMKII in mechanisms of ethanol-induced sensitization, but not in the voluntary alcohol consumption. Differences in housing conditions (isolation *vs.* grouped housing) might explain the discrepancies between the two studies. Moreover, they also showed that αCaMKII regulates PSD-95 expression in the hippocampus and amygdala following EBS, implicating these two brain regions in ethanol-induced locomotor sensitization.

An epigenetic study by Legastelois et al. (2013) demonstrated that the histone deacetylase inhibitor sodium butyrate prevented and reversed EBS. This effect was observed when EBS was induced by a low dose of ethanol (1.0 g/kg), but was absent when EBS was induced by a moderate ethanol dose (2.0 g/kg). Pretreatment with this inhibitor altered the expression of genes that are associated with growth factors and neuroendocrine-related transduction pathways in mice that were treated with the low dose of ethanol, especially brain-derived neurotrophic factor (BDNF), in the striatum and PFC (Legastelois et al., 2013). BDNF is a neurotrophin that belongs to the family of growth factors. This finding is particularly interesting when considering the possible involvement of BDNF in EBS (Nona et al., 2013; Rueda et al., 2012).

Botia et al. (2012) reported differential responses of some epigenetic-related genes (*i.e.*, genes involved in histone modification and DNA methylation) to ethanol-induced transcriptional changes after ethanol challenge between animals that were classified as "resistant to sensitization" and "sensitized mice," suggesting the involvement of epigenetic mechanisms in this phenomenon. Immediate early genes are also altered following EBS. Sensitized animals exhibit brain, region-dependent, changes in *homer1A* mRNA expression (dos Santos et al., 2009) and c-Fos expression (the *c-fos* protein product, Faria et al., 2008), demonstrating the regulation of genes during ethanol sensitization.

4. Relationship between ethanol-induced behavioral sensitization and motivational effects of ethanol and ethanol drinking

As already discussed, a paramount question in current addiction research is how to discriminate between subjects that will engage in problematic drug use from those that, despite similar level of drug exposure, will maintain a social, non-pathological drug use. Several rodent studies have suggested that certain behavioral traits (*e.g.*, impulsivity, novelty seeking and anxiety response) are significant predictors of subsequent engagement in problematic ethanol drinking. Using this approach, Radwanska and Kaczmarek (2012) found that high levels of anxiety and impulsivity are associated with high levels of alcohol drinking in mice. These studies underscore the relevance of analyzing individual differences at the behavioral level and the relationship between these phenotypes and ethanol drinking. Specifically, a proclivity for alcohol intake has been found in rodents that exhibit greater response to novelty (Nadal et al., 2002), greater ethanol-induced acute motor activation (Acevedo et al., 2010), and greater anxiety reactivity (Spanagel et al., 1995). The ingestion of mildly sweetened ethanol but not

sucrose alone or water was higher in adolescent rats that exhibited a higher frequency of rearing behavior and higher percentage of open arm entries in the elevated plus maze (Acevedo et al., 2014). However, several studies have failed to predict ethanol intake as a function of similar variables (Bienkowski et al., 2001). A study by Schramm-Sapyta et al. (2008) gave adolescent rats 3 days of overnight forced access to 10% ethanol. The animals were subsequently assessed for 10 days in a two-bottle choice test (8% ethanol *vs*. water). Ethanol consumption during the early forced-access phase but not open field activity, novel object exploration, anxiety patterns, or basal corticosterone levels was associated with alcohol consumption during the two-bottle choice test.

The present section of this article will critically analyze a tacit assumption of most EBS studies; namely, that increasing knowledge of EBS brings us closer to understanding the transition from controlled to problematic ethanol drinking and seeking. However, whether sensitization and ethanol drinking or ethanol's motivational effects are positively linked is a question that remains unanswered.

A positive relationship has been found between the rapid development of EBS and ethanol self-administration in female adolescent rhesus macaques, although only when tested in a social setting (Schwandt et al., 2008). C57BL/6J mice displayed higher ethanol consumption relative to drinking levels prior to sensitization and compared with non-sensitized controls (Lessov et al., 2001a,b). DBA/2J mice that were sensitized to ethanol consumed more ethanol than animals that were pretreated with saline or only acute ethanol (Camarini and Hodge, 2004).

A caveat of the studies so far described (*e.g.*, Camarini and Hodge, 2004) is that it is difficult to dissect if animals display greater ethanol consumption due to the development of EBS or as a function of having been pre-exposed to ethanol. Ethanol pre-exposure, regardless of its ability to induce EBS, can enhance subsequent ethanol consumption (see Fabio et al., 2014). This caveat is circumvented by studies that assess differences in ethanol-induced sensitization in lines of mice that are selectively bred for high or low alcohol preference. High alcohol-preferring (HAP) and low alcohol-preferring (LAP) mice exhibited similar acute activating effects of ethanol and developed fairly similar tolerance to the sedative effects of ethanol, but only HAP mice exhibited ethanol-induced sensitization (Grahame et al., 2000). These results are consistent with the hypothesis by Hunt and Lands (1992) that neuroadaptations that underlie sensitization may be important in alcohol drinking or that sensitization may be a marker of risk for alcohol-related problems. The results of the study by Abrahao et al. (2013) further supported this hypothesis. After 21 days of ethanol treatment, some sensitized mice exhibited a two-fold increase in ethanol-induced ambulatory activity relative to baseline (pretreatment scores), whereas non-sensitized mice exhibited as much activity as during the beginning of the treatment. These significant differences in EBS were associated with subsequent ethanol drinking in a restricted 2 h session during the dark phase. Sensitized mice drank approximately 2.0 g/kg ethanol, which was significantly more than the approximately 1.0 g/kg drunk in non-sensitized mice. Moreover, ethanol sensitization was associated with reduced NMDA expression in the NAc and functional impairment of NMDA receptors in the NAc *core*, demonstrated by lower surface expression of NMDA GluN1 subunit protein in the NAc and higher AMPA/NMDA ratio in the NAc *core* cells from ethanol sensitized mice, compared to controls or non-sensitized mice (Abrahao et al., 2013). In another study (Tarragon et al., 2012), ethanol drinking facilitated or potentiated the subsequent development of EBS, depending on the mouse strain. Conflicting results, however, have been reported. In one study, animals that were classified as high-sensitized or non-sensitized displayed similar ethanol consumption (Frozino-Ribeiro et al., 2008). Moreover, adult and adolescent mice that were pretreated with ethanol consumed more ethanol than mice that were pretreated with saline, regardless of differences in the magnitude of EBS in both groups (Carrara-Nascimento et al., 2014).

Another approach involves assessing changes in ethanol's sensitivity that occur when mice or rats that are selectively bred for high alcohol preference escalate alcohol intake across days. Using this approach in crossed HAP (cHAP) mice, Matson et al. (2014) found that the increase in ethanol self-administration was associated with progressively less ethanol-induced ataxia but not locomotor sensitization or pharmacokinetic tolerance. An important difference with previous studies was the obvious difference in the method of ethanol administration (forced *vs*. self-administered) and use of selectively bred mice. Nonetheless, divergence between EBS and ethanol intake has been reported in outbred mice. Legastelois et al. (2014) observed similar EBS in Swiss mice that had access to 10% ethanol or water. Moreover, in this study, ethanol self-administration after ethanol pre-exposure was greater in subjects that had exhibited lower levels of motor sensitization. These "resistant" mice, however, exhibited a significant reduction of ethanol intake when the drug was adulterated with quinine, which has a bitter, aversive taste that is usually rejected by rodents, whereas sensitized mice kept drinking despite quinine adulteration.

Altogether, it seems that most (*e.g.*, Lessov et al., 2001a,b; Camarini and Hodge, 2004) but not all (Frozino-Ribeiro et al., 2008; Carrara-Nascimento et al., 2014) of the studies reviewed support a link between the development of EBS and ethanol drinking scores. Differences between studies in terms of method of ethanol administration and strains under analysis, among others, may explain the inconsistencies found in their conclusions. An important study by Fidler et al. (2012) shed some light to this question. Using DBA/2J mice, (which are known for developing robust sensitization and for avoiding ethanol drinking), these researchers showed that pre-exposure to passive infusions of ethanol resulted in these mice changing their usually low level of ethanol intake for a pattern characterized by sustained ethanol self-administration (by intragastric route of administration). This study strengthen the hypothesis that a high susceptibility to develop EBS may influence the motivation to self-administer ethanol.

Another important question is whether sensitization and ethanol-induced appetitive learning are associated. According to Robinson and Berridge's theory (1993, 2001, 2003, 2004, 2008), repeated exposure to addictive drugs should make the brain more sensitive to drugs and increase drug-seeking behavior and responsivity to drug-related stimuli. In an intriguing experiment, Risinger et al. (1992) gave mice ethanol alone or combined with the dopamine receptor antagonist haloperidol. The antagonist blocked ethanol-induced motor activity during conditioning but had no effect on ethanol-induced CPP. This experiment illustrated an interesting feature of CPP, namely that ethanol-induced locomotor activity can be measured during conditioning trials. This allows assessment of the correlation between the development of sensitization and subsequent expression of ethanol-induced CPP. Cunningham et al. (2002b) took advantage of this feature and found that home cage pre-exposure to ethanol (0.0, 2.0, or 4.0 g/kg ethanol injections in the home cage every 48 h) induced EBS in DBA/2J mice but did not alter the rewarding effect of ethanol. More recently, no association was found between the magnitude of ethanol-induced CPP and EBS in Swiss mice, regardless of the behavioral sensitization protocol that preceded or followed CPP training (Pildervasser et al., 2014). Notably, another study (Bocklisch et al., 2013) upregulated dopamine transmission in the VTA using high-frequency stimulation. This manipulation heightened behavioral sensitization to cocaine but blunted the development of cocaine-induced CPP.

Altogether, these experiments suggest a dissociation between the acquisition of EBS and ability of ethanol to induce CPP. The

extent to which this refutes the possibility of an association between behavioral sensitization and increased perception of the rewarding effects of ethanol is still a matter of debate. Conditioned place preference is subjected to ceiling effects and may fail to detect subtle graded effects. The expression of ethanol-induced CPP has been suggested to be incompatible with the development of context-dependent sensitization (Pautassi et al., 2009). Conditioned increases in locomotion when animals are tested for CPP may interfere with the conditioned approach and contextual permanence in the ethanol-related chamber, and thus obscure the expression of CPP. Moreover, a recent study found that repeated administration of salsolinol, the condensation product of acetaldehyde and dopamine, led to behavioral sensitization and enhanced alcohol intake (Quintanilla et al., 2007), suggesting that behavioral sensitization, CPP, and ethanol intake may be associated, at least under certain conditions.

Ethanol pre-exposure may indirectly increase sensitivity to the appetitive effects of ethanol through a reduction of the aversive effects of ethanol. Place conditioning can also be used in mice to test sensitivity to the aversive effects of ethanol. For reasons that have yet to be fully understood, the same dose that normally produces CPP when administered before conditioned stimulus (CS) exposure yields aversive conditioning if administered after CS exposure (Cunningham et al., 2002a). Pre-exposure to ethanol in the study by Cunningham et al. (2002b) attenuated subsequent ethanol-induced conditioned place aversion. This result is consistent with a study in which repeated ethanol administration inhibited the acquisition of taste aversion by ethanol and the prototypical emetic agent lithium chloride (LiCl). At high doses, ethanol appears to share some of the aversive effects of LiCl (Arias et al., 2010c).

Age differences in the sensitivity to the rewarding effect of ethanol after ethanol pre-exposure using a CPP procedure have been reported. Tolerance to the rewarding effect of ethanol was detected in adult sensitized mice, whereas ethanol pre-exposure during adolescence induced a sensitized rewarding effect of ethanol (Carrara-Nascimento et al., 2014). Interestingly, adolescent mice are less sensitive to ethanol-induced CPP than adult mice, although young animals express CPP after stress exposure (Song et al., 2007). This age-related difference parallels the apparent reduction of the sensitivity to ethanol-induced sensitization that was found in adolescent Swiss and DBA/2J mice compared with their adult counterparts (Carrara-Nascimento et al., 2011, 2014; Song et al., 2007).

As we tacitly acknowledged in the aforementioned studies, not all mice that are given intermittent ethanol exhibit ethanol-induced motor stimulation or ethanol sensitization. The variability in the stimulant response was first reported by Masur and dos Santos (1988). Mice were given weekly injections of 0.0, 0.8, 1.2, 1.6, 2.0, 2.4, or 3.0 g/kg ethanol. One third of the ethanol-treated mice (*i.e.*, 13 of 41) did not exhibit drug-induced motor stimulation at any of the doses tested compared with vehicle-treated counterparts. This inherent variability in sensibility to EBS can be both an obstacle and an advantage. Traditional statistical analyses contrast mean treatment differences, between experimental and control groups, *vs.* "random error" or overall sample variability. If only a certain group of experimental animals exhibits EBS, as in the report by Masur and dos Santos (1988), it is likely that ethanol-induced differences become relatively small and overall variability relatively high, thus leading to a null result. Yet, if the experimental group is broken down into high- and low-responders or into a sensitized *vs.* a non-sensitized group, the possibility of finding treatment (*i.e.*, ethanol-induced) differences is maximized. Perhaps more important, this approach opens the door for analyzing other variables, neural or behavioral, that are associated with this differential expression of EBS. In this sense, the analyses of differences between "sensitized" and "non-sensitized" mice can shed some light on the neural underpinnings of ethanol-induced behavioral sensitization. For example and as mentioned above, mice that were "resistant" in terms of the development of EBS exhibited a significant decrease in ethanol intake after adding an aversive, bitter taste to the alcohol solution (Legastelois et al., 2014). However, this quinine adulteration did not alter ethanol drinking in sensitized counterparts. Similarly, De Pauli et al. (2014) reported the differential expression of ΔFosB, an index of neuronal plasticity, in sensitized *vs.* non-sensitized Swiss mice.

One important difference and potential source of the lack of replication across laboratories is the way in which "sensitized" and "non-sensitized" animals are classified across studies. A very stringent criterion was employed by Legastelois et al. (2014). Animals were classified as sensitized if their increase in locomotor activity between the first and last days of treatment exceeded a coefficient of variance that was measured in the basal control condition. Most studies, however, typically employ median-split, quartile-split, or similar triage procedures. A widely used procedure (Souza-Formigoni et al., 1999; Abrahao et al., 2009; De Pauli et al., 2014) is to take high- and low-sensitized mice from the upper and lower 30% of the distribution of locomotor scores, as observed during the 21st day of ethanol treatment. These ethanol-sensitized animals exhibited a significant increase and decrease in D_2 and NMDA receptor binding, respectively, in specific limbic areas compared with non-sensitized mice (Quadros et al., 2002a; Souza-Formigoni et al., 1999). Similar "sensitized" mice exhibited a greater response to intra-NAc administration of dopamine D_1 or D_2 receptor agonists compared with non-sensitized counterparts (Abrahao et al., 2012; Abrahao et al., 2011). These results, obtained from subpopulations of "high" and "low" responders in the 21-day sensitization protocol, are interesting because they support the notion that sensitization to ethanol is associated with specific neuroadaptive changes. A study that used heterogeneous mice (*i.e.*, without "high" and "low" divisions), however, reported that EBS was not associated with alterations in NMDA receptor-mediated processes (Meyer and Phillips, 2007).

5. Acute motor effects of ethanol, insensitivity of rats to EBS, and parallels between the pharmacological basis of EBS and acute motor effects of ethanol

In EBS studies, emphasis is placed on the gradual increase in motor activity as a function of trials or how a history of ethanol exposure affects responsiveness during a challenge test. Numerous studies, however, have focused on the initial motor effect of ethanol. The rationale is that the nature of the first experience with the drug may be associated with vulnerability to alcohol-related problems later in life, in which such subjects are more sensitive to ethanol's appetitive effects and may be more prone to develop alcohol use disorders (Schramm-Sapyta et al., 2006, 2009). In this section we will briefly review how ethanol-induced acute motor activation came to be considered a proxy for ethanol-induced reinforcement. We will then describe the acute motor activating of ethanol in rodents, with a focus on species- (*i.e.*, rat *vs.* mice) and age-related differences in these effects. The notion that rats are mostly insensitive to EBS will be analyzed and alternatives to circumvent this issue will be proposed. Age-related differences in the acute motor effects of ethanol will be discussed in the broader context of developmental differences that may put younger, particularly adolescent, subjects at risk for the development of exacerbated alcohol consumption.

The rationale for considering the acute motor effects of ethanol a measure of motivational reactivity can be traced to the seminal work of Wise and Bozarth (Wise and Bozarth, 1987). The implications of dopamine in reward, and more particularly in the mescorticolimbic pathway, had been already suggested (Wise,

1978) and Wise and Bozarth (1987) expanded on the importance of this system proposing that drugs can be addictive because they stimulate dopaminergic fibers of the midbrain that project from limbic to cortical regions. Wise and Bozarth's psychomotor stimulant theory asserted that all drugs of abuse have motor stimulant properties and these stimulant actions have a shared biological mechanism, which would be homologous with that underlying the processing of the reinforcing effects of natural and pharmacological reinforcers. It is important to remark, however, that a low-level of response to ethanol has been also associated with increased probability of alcohol-related problems (Schuckit et al., 2004).

In mice, ethanol exerts biphasic effects on acute motor activity, enhancing locomotion at doses ≤2.0 g/kg (Faria et al., 2008) and inducing motor depression at higher doses (Quoilin et al., 2012). An ontogenetic analysis (Quoilin et al., 2010) indicated that younger mice were significantly more sensitive to the stimulant effects of ethanol, an effect that can likely be explained by a decrease in sensitivity to the sedative and hypnotic effects of ethanol. In other words, it is possible that the sedative and hypnotic effects of ethanol serve as a figurative break on the acute, motor activating effects of the drug. In the study by Quoilin et al. (2010), greater ethanol-induced locomotor activity in younger mice was accompanied by a shorter latency to regain the righting reflex compared with more mature counterparts. These results resemble previous work with rats (Silveri and Spear, 1998), which found diminished ethanol-induced sleep time in younger subjects than in older subjects. This reduction of sensitivity to ethanol-induced sedation in preweanling and adolescent animals was accompanied by higher blood alcohol levels at awakening and the greater expression of acute tolerance. Altogether, these studies suggest that the activating effects of ethanol in adult animals may be masked by greater sensitivity to ethanol-induced sedation.

Differential sensitivity to ethanol's effects during development is a broader phenomenon. Adolescent rats have been shown to be less sensitive to the ethanol-induced suppression of social activity and dysphoric effects of ethanol that result in CTA. However, they are more sensitive to ethanol-induced social facilitation (Varlinskaya and Spear, 2004), ethanol-induced autonomic activation (Ristuccia and Spear, 2008), and ethanol-induced appetitive reinforcement (Pautassi et al., 2008a). These sentences state the contradictory findings about the role of ethanol in the regulation of social interactions. It needs to be remarked that ethanol exerts biphasic effects during the course of the toxic process. During the onset of the intoxication, when blood ethanol levels are rising, the drug induces psychomotor activation and social facilitation (Risinger and Cunningham, 1992); whereas in later stages of the intoxication, characterized by peak blood ethanol levels, the drug suppress motor activity and social interaction, and induces sedation (Crabbe et al., 1982; Pautassi et al., 2009).

There is a dearth of research addressing age as a moderator of alcohol-related effects in humans (see Squeglia et al., 2014), yet some findings are congruent with those observed in animals. Human adolescents who abuse alcohol are not likely to report hangover effects when they discontinue the consumption of the drug (Martin and Winters, 1998), a finding reminiscent of differences in sensibility to alcohol withdrawal between adolescent and adult Sprague-Dawley rats (Varlinskaya and Spear, 2004). The differential pattern of reactivity to ethanol in adults *vs*. adolescents has been suggested to put adolescents at risk for the initiation and escalation of ethanol consumption (Spear and Varlinskaya, 2010). For example, lower sensitivity to ethanol-induced ataxia and dysphoria may allow adolescents to drink higher amounts of ethanol and for more extended periods of time. One postulation is that high sensitivity to the stimulant effects of ethanol is an indicator of a "sensitized brain" in adolescents. This could explain the lower susceptibility to ethanol-induced behavioral sensitization in adolescent mice. In other words, the already high sensitivity to the stimulant effects of ethanol would prevent further escalation in the psychomotor response to the drug (*i.e*. a so-called "ceiling effect").

The finding of differential effects of ethanol across development prompted researchers to analyze the stimulating effects of ethanol in immature (*i.e*., preweanling and adolescent) rats. Arias et al. (2008) analyzed locomotion in preweanling rats after 2.5 or 0.5 g/kg ethanol administration. The high but not low ethanol dose induced biphasic effects. Specifically, animals that were given 2.5 g/kg ethanol exhibited significant motor activation during the ascending limb of the blood ethanol curve and motor depression at later stages of intoxication that are characterized by peak blood ethanol levels. Ethanol-induced motor activation in preweanling animals was later replicated and found to vary during the course of infancy, with younger rats (*i.e*., 8- or 12-day-old) exhibiting higher drug-induced stimulation than older (15-day-old) rats (Arias et al., 2009c). Since then, several studies have used preweanling rats to assess the effects of dopamine (Arias et al., 2010a) and opioid (Arias et al., 2010b) receptor antagonists (and other pharmacological treatments, such as baclofen; Arias et al., 2009d) on ethanol-induced acute locomotor activation. Importantly, these stimulatory effects appear to be more pronounced in a novel testing environment (Arias et al., 2009a). This indicates that ethanol-induced locomotor activity in preweanling animals does not solely depend on the pharmacological consequences of ethanol but instead on interactions between these effects and the stress of novelty. Subsequent experiments revealed that social stress (*i.e*., 4 h of social isolation before testing) and exogenous administration of corticosterone (but not antagonism of the κ opioid system; see Pautassi et al., 2012b) exacerbated the expression of ethanol-induced locomotor activity in infant rats (Arias et al., 2010d). Similarly, work by Fernandez et al. (2013) indicated that a lack of maternal care during postnatal day (PND) 1–13 heightened reactivity to the motor stimulating effects of 1.25 g/kg ethanol.

Rats readily display ethanol-induced CTA (Acevedo et al., 2010; Arias et al., 2009b; Davis and Riley, 2010) and can learn to actively work to self-administer the drug (Ponce et al., 2008). Rats, however, have been deemed a species that is mostly insensitive to EBS (Chuck et al., 2006; Masur and Boerngen, 1980; Masur et al., 1986) and to a certain extent ethanol-induced CPP (Cunningham et al., 1993). This has led researchers to analyze only the acute, activating motor effects of ethanol in rats, and sometimes even these effects have been difficult to observe. The insensitivity of rats to EBS was illustrated by recent study by our group (Fabio and Pautassi, unpublished data). Adolescent male and female rats were given 2.5 g/kg ethanol (i.g.) or vehicle every other day from PND28 to PND36. Another group of adolescents was given ethanol on PND28 and PND32 and vehicle on the remaining days. Ethanol-induced locomotion was measured 5–20 min after ethanol administration in chambers (30 × 30 × 30 cm) that were equipped with photocell beams, which tracked the rat's movement in real-time and recorded the distance traveled (cm). As shown in Fig. 4 and confirmed by repeated-measures analysis of variance, significant ethanol-induced motor activation was found with the first administration on PND28, and this effect that was similar in males and females. Subsequent measurements on PND32 and PND36, however, indicated a reduction of the motor stimulating effects as a function of repeated ethanol exposure.

Differences in acute and chronic sensitivity to the locomotor effects of ethanol between mice and rats could reflect differences in the mode of ethanol administration, time of testing, or type of chamber that is used for motor assessment. In an important experiment, heterogeneous mice and rats were trained in the same CPP procedure and monitored for the development of motor sensitization (or lack thereof) across conditioning trials (Cunningham et al., 1993). Ethanol (1.5 g/kg) increased motor activation in mice,

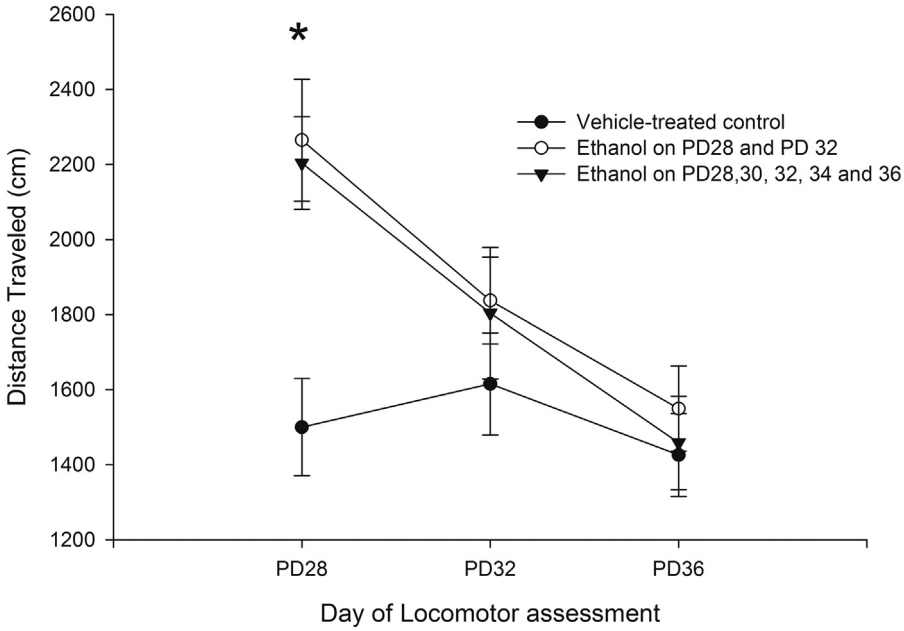

Fig. 4. Mean activity (distance traveled) in male and female adolescent rats during a 12-min test. The rats were given ethanol (2.5 g/kg, i.g.) every other day from postnatal day (PD) 28–36. Another group of adolescents was given ethanol on PD28 and PD32 and vehicle on the remaining days. Vehicle-treated controls were administered 0.0 g/kg ethanol across days. Analysis of variance and *post hoc* tests indicated significant ethanol-induced motor activation only during the first administration on PD28. The asterisk indicates a significant difference between a given group and the vehicle-treated control group. Vertical bars indicate the SEM.

which was associated with robust CPP. Neither of these phenomena was evident in rats (Cunningham et al., 1993). This elegant experiment, however, had some caveats, such as the lack of a dose-response curve (rats may be more sensitive to ethanol-induced CPP at lower doses; Philpot et al., 2003) and the lack of titration of blood ethanol concentrations in both species. The results, however, further supported the notion that rats are not as sensitive to EBS or to ethanol-induced CPP as mice are. It should be noted, however, that acute sensitivity to ethanol-induced motor activation can be observed in infant and adolescent rats (Arias et al., 2008; Pautassi et al., 2011a), and this coincides with an increase in the sensitivity to ethanol-induced conditioned tactile preference (Molina et al., 2007) and high ethanol intake (Sanders and Spear, 2007). Moreover, a recent study specified some conditions that may favor the expression of EBS in infant rats. Castello et al. (2015) gave rats ethanol (2.5 g/kg) in a distinctive activity chamber or their home cage every day during PND8-12 (induction phase), and the animals were tested for EBS on PD15. Sensitization was observed in the challenge test, although only in males that had been given ethanol in the homecage during induction.

As mentioned above, mouse studies suggest that the modulation of dopamine and opioid receptors and acetaldehyde levels can block ethanol-induced sensitization (Abrahao et al., 2011; Camarini et al., 2000b, 2011; Kim and Souza-Formigoni, 2010; Pastor and Aragon, 2006). Similarly, preweanling rats that were given systemic administration of SCH23390 or sulpiride (D_1 and D_2 receptor antagonists, respectively) at doses that failed to alter baseline locomotion suppressed the stimulatory effects of 2.5 g/kg ethanol (Arias et al., 2010a). In another set of studies, μ and δ opioid receptor antagonists blocked ethanol-mediated locomotor stimulation in 14-day-old rats, but only μ receptor antagonism blocked ethanol intake in rats and EBS in mice (Arias et al., 2010b; Pastor and Aragon, 2006). κ Opioid receptor antagonism did not affect ethanol-induced locomotor activity or intake, whereas κ receptor agonism increased the motor-depressing effects of ethanol (Pautassi et al., 2012a). At least some of the rewarding effects of ethanol have been suggested to be mediated by acetaldehyde, the primary metabolite of ethanol (Correa et al., 2009). Consistent with this postulation, central administration of an acetaldehyde-chelating agent (D-penicillamine [DP]) blocked ethanol-induced locomotor activity in preweanling rats (Pautassi et al., 2011b). Systemic administration of DP also inhibited ethanol-induced conditioned tactile preference. Altogether, these results suggest substantial overlap between the mechanisms that regulate the motor-stimulating effects of ethanol in preweanling rats and predisposition for ethanol-induced reinforcement and intake.

The studies summarized above indicate other important differences in the sensitivity to ethanol between preweanling and adult rats. Infant rats exhibit ethanol-induced conditioned tactile preference that is comparable to ethanol-induced CPP in mice (Nizhnikov et al., 2009). When assessed using the consumption-off-the-floor procedure, these animals also drink high amounts of ethanol (*e.g.*, 2.0–3.0 g/kg in 20 min) without the need for lengthy initiation procedures (Sanders and Spear, 2007). These intake levels were similar to those found in adult rats that were derived from lines that were selected for their propensity to drink ethanol, such as alcohol-preferring (P) rats that were originally bred at the University of Indiana (Pautassi et al., 2011c; Truxell and Spear, 2004; Truxell et al., 2007). P rats and other lines that are bred for high alcohol intake, such the University of Chile B (UChB) line, also exhibit ethanol-induced locomotor activation, particularly at low doses. For example, Quintanilla (1999) observed greater ethanol-induced locomotor enhancement in UChB rats than in low-preferring UChA (University of Chile abstemious) rats at 0.25 and 0.5 g/kg ethanol, an effect that is likely explained by the fact that the latter line is more sensitive to ethanol-induced sedation. These results are consistent with those found in P rats and Maudsley Reactive (MR) rats, which exhibited increases in locomotor activity after 0.12 and 0.25 g/kg ethanol administration (Waller et al., 1986). Some of these preferring strains also exhibited ethanol-induced CPP, although in some cases extensive pre-exposure to the drug likely induces tolerance to the aversive effects or sensitiza-

tion to the appetitive effects of the drug (Quintanilla and Tampier, 2011).

Less is known about the stimulatory effects of ethanol in adolescent rats. Recent work revealed that intragastric ethanol administration (2.0–2.5 g/kg) significantly increased locomotor activity during the rising phase of blood ethanol concentrations, an effect that was reduced by naloxone administration (Acevedo et al., 2010; Pautassi et al., 2011a). One of these studies screened adolescents for sensitivity to ethanol-induced motor stimulation. Using a median-split procedure, these researchers divided the animals into high and low responders (HR and LR, respectively). Subsequent assessments indicated greater ethanol intake in HR adolescents than in LR adolescents, an effect that was more pronounced in females (Acevedo et al., 2010).

As discussed above, a strategy for analyzing the functional relevance of ethanol-induced sensitization has been to correlate its expression with other indices of ethanol-induced motivational learning, such as CPP, or assess whether interventions that block ethanol-induced CPP also inhibit behavioral stimulation that is induced by the drug. A similar approach has been employed in immature rats but using acute psychomotor stimulation as the predictor variable. Ethanol-induced first- or second-order CPP has been observed in infant and adolescent rats, and these conditioned preferences can be blocked by general opioid antagonism (Nizhnikov et al., 2009; Pautassi et al., 2011a), drugs that block μ or δ opioid receptors (Nizhnikov et al., 2009), and inactivation of acetaldehyde, the primary metabolite of ethanol (Pautassi et al., 2011b). Intriguingly, most of these pharmacological interventions also disrupted the acute psychomotor effects of ethanol (Pautassi et al., 2011a,b). A recent study by Acevedo et al. (2013) reported ethanol-induced CPP and CTA in adolescents. This sensitivity to ethanol-induced appetitive and aversive reinforcement, however, was not associated with sensitivity to ethanol-induced locomotor stimulation.

Altogether, the studies described in this section suggest that the dearth of EBS studies on rats may obey to their relatively insensitivity to this phenomenon. At the same time, however, the studies reviewed also suggest that the assessment of ethanol's acute motor effects is an interesting, valuable alternative to the lengthier protocol of EBS, particularly if the researcher is working with an immature (*i.e.*, infant or adolescent) animal rat model. For the most part, studies conducted in infant (*e.g.*, Pautassi et al., 2011b) and adolescent (*e.g.*, Pautassi et al., 2011b) rats have indicated a relationship between the acute stimulatory effects of ethanol with motivational reactivity to ethanol or with ethanol intake (Acevedo et al., 2010). Moreover, manipulations know to alter EBS (*e.g.*, blockade of dopaminergic or opioidergic transmission) also affect the acute motor stimulating effects of ethanol in young rats (Arias et al., 2010a, 2009d).

6. Summary, conclusions, and closing comments

Ethanol-induced behavioral sensitization was first described more than 30 years ago (Masur and Boerngen, 1980). Since then, it has attracted enormous interest as a potential behavioral marker of the transition from a "normal" to an addicted state. There are still many discrepancies with regard to the neurochemical mechanisms that underlie its acquisition and expression. Most of the literature on behavioral sensitization has been conducted with the psychostimulants cocaine and amphetamine. These drugs usually yield more reliable and graded effects than ethanol, arguably because of their specific mechanisms of action, as opposed to the nonspecific effects of ethanol. Nevertheless, during the last decades, there has been substantial progress in understanding the neurochemical and environmental factors that influence ethanol-induced behavioral sensitization.

Ethanol has direct and indirect facilitative effects on the dopamine reward system (Brodie et al., 1990; Di Chiara and Imperato, 1988; Gessa et al., 1985; Imperato and Di Chiara, 1988; Kohl et al., 1998; Mereu and Gessa, 1985; Yim et al., 1998). According to incentive sensitization theory (Robinson and Berridge, 2001), ethanol can induce behavioral sensitization. Both direct-acting dopamine receptor agonists (*e.g.*, apomorphine and bromocriptine) and indirect dopamine receptor agonists (*e.g.*, amphetamine and cocaine) induce sensitization. The time course of sensitization that is induced by these categories of agonism is different. Direct and indirect dopamine agonists induce short- and long-lasting sensitization, respectively (Bedingfield et al., 1996). The key role of mesoaccumbens dopamine transmission in behavioral sensitization to psychostimulants led to the general hypothesis of a common circuitry model of the neural mechanisms of behavioral sensitization to all drugs of abuse. Under this framework, the VTA plays a prominent role in the development of sensitization, whereas the medial PFC and NAc mediate its expression (Pierce and Kalivas, 1997). The participation of D_1/D_2 receptors in these processes, however, appears to vary according to the psychostimulant. For example, D_1 receptors appear to be important for the development and expression of amphetamine-induced sensitization (Kuribara and Uchihashi, 1993a,b; Vezina and Stewart, 1989; Kuribara, 1995). The D_1 receptor antagonist SCH23390 blocks the expression but not development of cocaine-induced sensitization (Kuribara and Uchihashi, 1993a; Mattingly et al., 1994). Moreover, neuroadaptations in the glutamatergic system appear to be critical for cocaine-induced sensitization, but less clear is whether similar processes occur during sensitization to amphetamine (Vanderschuren and Kalivas, 2000). AMPA receptors appear to be involved in sensitization to indirect but not direct dopamine agonists (Bedingfield et al., 1996).

Although the discrepancies in the literature should not be dismissed and warrant further studies and consideration, overall, the blockade of dopamine (Abrahao et al., 2011; Bahi and Dreyer, 2012; Camarini et al., 2011) or glutamate (Broadbent et al., 2003; Broadbent and Weitemier, 1999; Camarini et al., 2000a; Kotlinska et al., 2006) receptor signaling is related to the inhibition of EBS. The role of dopamine receptors in EBS is clear, but the participation of D_1 receptors seems to be more convincing than the role of D_2 receptors. Several studies reported enhanced dopamine release in the NAc that accompanies behavioral sensitization to several drugs of abuse, including cocaine, amphetamine, nicotine, and morphine (Camarini et al., 2008; Kalivas and Duffy, 1990; Parsons and Justice, 1993; Robinson et al., 1998; Shoaib et al., 1994). The data on ethanol-induced dopaminergic sensitization, however, is contradictory, and sometimes tolerance has been found (Zapata et al., 2006; Spanagel and Weiss, 1999). Functional dissociations may exist between the development of behavioral and neural sensitization to the repeated administration of ethanol. Behaviors other than locomotion might be linked to dopaminergic sensitization, such as ethanol intake. For example, repeated ethanol administration in sensitization-resistant C57BL/6J mice resulted in mesoaccumbal dopaminergic sensitization (Kapasova and Szumlinski, 2008) and an increase in ethanol consumption (Camarini and Hodge, 2004; Lessov et al., 2001a,b). Furthermore, an inverse relationship was found between accumbal glutamate and EBS (Carrara-Nascimento et al., 2011; Kapasova and Szumlinski, 2008). This contrasts with the literature on psychostimulant-stimulated glutamate release in the NAc, which suggests a direct relationship between behavioral sensitization and glutamatergic sensitization (Baker et al., 2002; Pierce et al., 1996; Reid and Berger, 1996). The antagonistic action of ethanol on NMDA receptors should be considered to evaluate neuroadaptations to chronic ethanol exposure.

The blockade of D_1 and D_2 receptors also suppresses ethanol-induced acute motor stimulation in infant rats (Arias et al., 2010a). Immature infant and adolescent rats do not appear to exhibit behavioral sensitization (but see Castello et al., 2015). Unlike their adult counterparts, however, they are highly sensitive to the acute motor-activating effects of ethanol (Arias et al., 2008; Acevedo et al., 2010; Acevedo et al., 2013), particularly during the ascending limb of the blood ethanol curve. The time course of acute ethanol-induced motor activation in infant and adolescent rats coincides with the time course of its appetitive hedonic effects, as measured by CPP (Molina et al., 2007; Pautassi et al., 2008b).

The release of dopamine in the NAc can be mediated by opioid-GABA-dopamine interactions in the VTA (Gonzales and Weiss, 1998; Spanagel et al., 1992). Another important target for alcohol action is the opioid system. Ethanol increases the release of opioids, which inhibit GABAergic neurons, leading to the disinhibition of dopaminergic neurons and consequently increasing dopamine release in the NAc (Xiao et al., 2007). Ethanol also increases opioid peptide release in the NAc (Olive et al., 2001). The studies discussed in this review indicate that nonspecific and selective opioid antagonists inhibit ethanol-induced behavioral sensitization (Camarini et al., 2000b; Pastor and Aragon, 2006). Ethanol-induced acute motor activation and ethanol-induced CPP in infant and adolescent rats similarly depend on the integrity of the endogenous opioid system (Arias et al., 2009d; Nizhnikov et al., 2009; Pautassi et al., 2011a). Opioid antagonists also reversed ethanol-induced dopamine release in the NAc (Gonzales and Weiss, 1998) and prevented ethanol-induced CPP in adolescent and infant rats (Nizhnikov et al., 2009; Pautassi et al., 2011a).

Other neurotransmitter systems are likely involved in EBS. For example, a recent study suggested the involvement of nicotinic acetylcholine receptors in this phenomenon (Bhutada et al., 2010). More studies are needed, however, to provide conclusive evidence of the involvement of this and other transmitter systems.

Whether sensitization to ethanol is positively linked to ethanol drinking, similar to the way in which sensitization to amphetamine is correlated with self-administration, is still debatable. However, given the possibility of confirming such links, studies of molecular targets that are involved in ethanol sensitization could identify new approaches for the treatment of alcoholism. Dopaminergic dysfunction may play a role in ethanol preference (McBride and Li, 1998). Alcohol-preferring (P) rats, high-alcohol-drinking (HAD) rats, and Fawn-Hooded rats (which are considered "alcohol-preferring") exhibit lower dopamine levels compared with their non-alcohol-preferring counterparts. Opioid receptor antagonists suppressed ethanol seeking behavior in rats (Gonzales and Weiss, 1998) and attenuated alcohol self-administration in a two-bottle choice paradigm (Tomie et al., 2013). Ionotropic or metabotropic glutamate receptor antagonists reduced ethanol consumption and relapse behavior (McMillen et al., 2005; Sanchis-Segura et al., 2006; Vengeliene et al., 2005). Although no consensus has been reached regarding a causal relationship between ethanol sensitization and subsequently greater alcohol intake, several studies have found an overlap in the neurocircuitry of both behaviors (Steketee and Kalivas, 2011).

Alterations in dopaminergic and NMDA systems as a function of repeated ethanol exposure were also observed in rats. The Guerri group exposed adolescent animals to a 3.0 g/kg ethanol dose on two consecutive days, with a 2-day interval between exposures, over a period of 2 weeks (Pascual et al., 2007). This protocol activated glial innate immune receptors, which in turn stimulated a signaling cascade (*e.g.*, the transcription factor nuclear factor κB) that is responsible for inducing inflammation and neuronal damage (Pla et al., 2014). When tested in adulthood, these animals exhibited cognitive dysfunction, alterations in glutamatergic and dopaminergic systems, and increased ethanol intake (Pascual et al., 2009). Future studies should analyze the potential contributions of ethanol-induced activation of microglia and inflammation signaling cascades to the development and expression of behavioral sensitization.

In the present review, we indicated that dopamine D_1 and D_2, opioid, and glutamate receptor antagonists effectively block EBS. This review also underscored that the outcome of experimental designs that attempt to assess EBS may vary as a function of several parameters, including dose, post-administration time of testing, species, genetic strain, sex, and age. Adolescent mice appear to be less sensitive than adult mice to ethanol-induced sensitization (Carrara-Nascimento et al., 2011; Faria et al., 2008; Stevenson et al., 2008) but more sensitive to the acute motor-stimulating effects of ethanol (Quoilin et al., 2012). This high sensitivity to the stimulant effects of ethanol may be a consequence of a blunted response to the sedative effects of ethanol. This combination of age-specific sensitivity raises the hypothesis that adolescents may have a "sensitized brain" with regard to the stimulant effects of ethanol. We hypothesize that lower sensitivity to neuroadaptive processes during the induction of sensitization may contribute to higher vulnerability to dependence in adult individuals that are exposed to the drug during adolescence (Grant and Dawson, 1997). Dopaminergic system-related dysfunction has also been correlated with higher ethanol preference (Brodie and Appel, 2000; Misra and Pandey, 2003; Pandey, 2004; Rassnick et al., 1992; McBride and Li, 1998). Although speculative, lower neuronal activity in brain regions that are involved in motivation in adolescent *vs.* adult mice, as assessed by immediate early gene expression (Faria et al., 2008) and CREB expression (Soares-Simi et al., 2013), may provide additional information that can explain the higher risk of substance abuse in adolescents. These findings are in agreement with those reported with cocaine, in which drug-pretreated adolescent mice displayed lower dopamine peaks than adults in response to a cocaine challenge injection, although adolescents exhibited a more rapid onset of peak dopamine than adults (Camarini et al., 2008).

Environmental factors are also critical in the regulation of behavioral sensitization. Repeated stress induces cross-sensitization to ethanol (Roberts et al., 1995; Rocha et al., 2010; Quadir et al., 2015), and CRF_1 receptors appear to play a crucial role in ethanol-induced sensitization (Pastor et al., 2008). Environmental enrichment can block and reverse ethanol sensitization (Rueda et al., 2012), suggesting that a positive environment can be important as a non-pharmacological approach for the treatment of ethanol abuse and dependence.

In summary, this review was guided by the hypothesis that neuroadaptations occur during the transition from a non-addicted/nondependent state to an addicted state. These neuroadaptations may ultimately be responsible for drug craving and relapse (Pierce and Kalivas, 1997; Robinson and Berridge, 2008; Spanagel and Weiss, 1999; Vanderschuren and Kalivas, 2000). Although EBS is a model that does not reflect addictive-like behavior from the perspective that its expression eventually results in the loss of control over drug intake, experimental evidence indicates that behavioral sensitization is a valuable benchmark for assessing neuroplastic changes that occur in the brain after repeated exposure to ethanol that may underlie the escalation of ethanol intake. Overall, this phenomenon is proposed to contribute to drug relapse. Thus, elucidation of the mechanisms that are involved in behavioral sensitization may be useful for identifying potential pharmacological and non-pharmacological approaches for alcoholism treatment and relapse prevention.

Conflict of interest

The authors declare that there are no conflicts of interest.

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