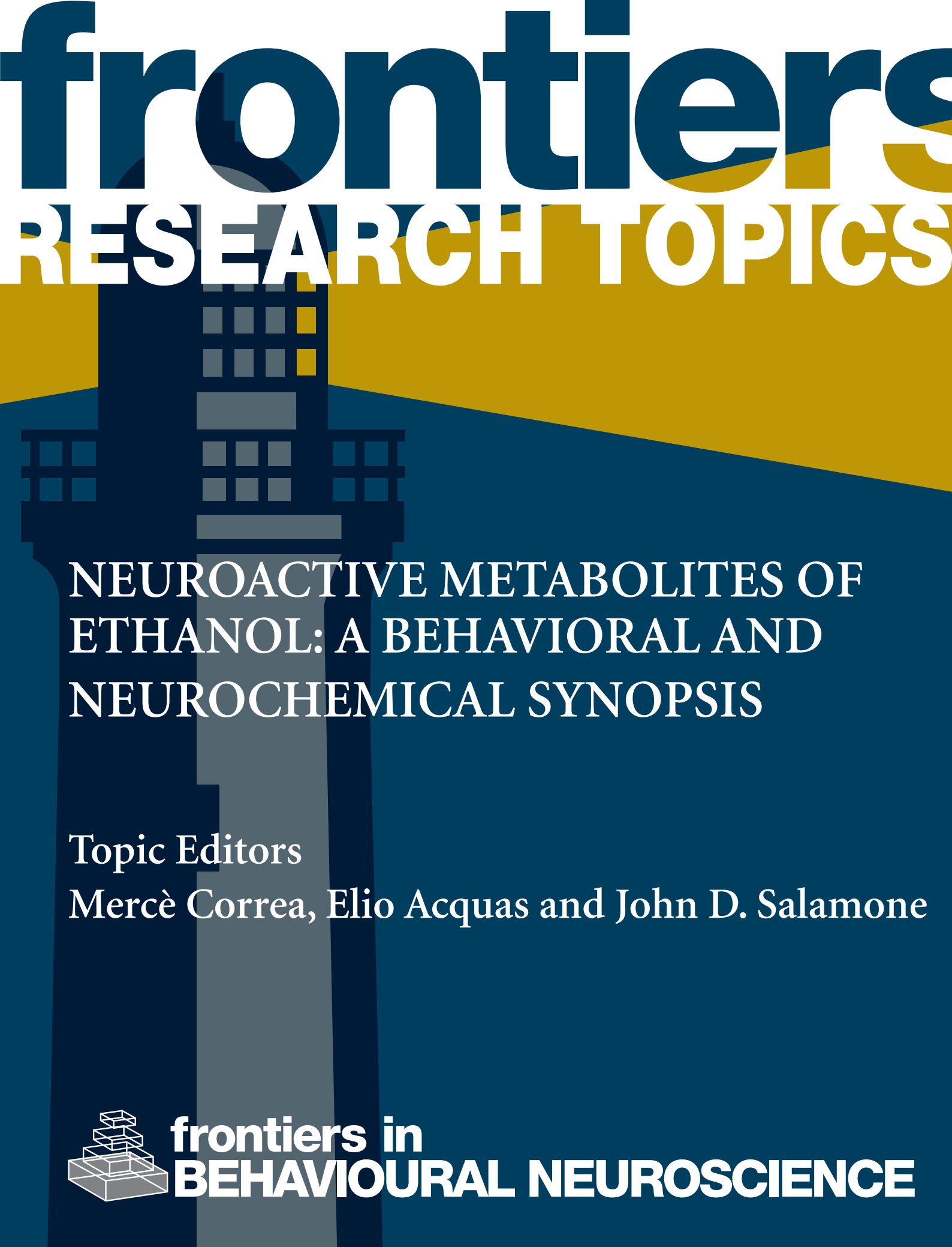
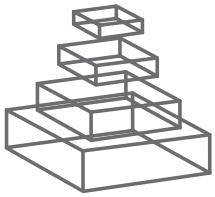
frontiers
RESEARCH TOPICS



NEUROACTIVE METABOLITES OF ETHANOL: A BEHAVIORAL AND NEUROCHEMICAL SYNOPSIS

Topic Editors
Mercè Correa, Elio Acquas and John D. Salamone

frontiers in
BEHAVIOURAL NEUROSCIENCE



frontiers

Cover image provided by Ibbl sarl, Lausanne CH

ISSN 1664-8714
ISBN 978-2-88919-375-2
DOI 10.3389/978-2-88919-375-2

ABOUT FRONTIERS

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

FRONTIERS JOURNAL SERIES

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing.
All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

DEDICATION TO QUALITY

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view.
By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

WHAT ARE FRONTIERS RESEARCH TOPICS?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area!
Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: researchtopics@frontiersin.org

NEUROACTIVE METABOLITES OF ETHANOL: A BEHAVIORAL AND NEUROCHEMICAL SYNOPSIS

Topic Editors:
Mercè Correa, University Jaume I, Spain
Elio Acquas, University of Cagliari, Italy
John D. Salamone, University of Connecticut, Storrs, USA

Ethanol is a very elusive drug, which has mechanisms of action that are diverse and relatively non-selective. Moreover, ethanol has been demonstrated to be a biologically active substance by itself, but also a pro-drug of the neuroactive metabolites, acetaldehyde and acetate. Acetaldehyde has traditionally been known as a toxic substance with several effects on multiple systems. However, in the last few decades evidence has accumulated to reveal the specific and, in some instances, distinct neural actions of acetaldehyde and acetate that are in part responsible for some of the observed psychoactive effects of ethanol.

The present issue will address these challenges to provide an up-to-date synopsis of the behavioral and neurophysiological impact of the two direct metabolites of ethanol, acetaldehyde and acetate. In doing so, this issue will present human and rodent evidence on their behavioral and neurophysiological impact, either when administered alone as drugs, or when metabolically-derived from their parent compound.

Emphasis will be placed to stress the importance of the different enzymatic systems that intervene to produce these metabolites, either peripherally and/or directly in the brain. Similarly, this Research Topic will be aimed at addressing some of the possible mechanisms of action of acetaldehyde and acetate in different brain areas and in different intracellular systems. Furthermore, the issue will lay out some of the suggested mechanisms of action of ethanol and of its metabolites by which they form adducts with other molecules and neurotransmitters such as dopamine and opioids (which lead to salsolinol and tetrahydropapaveroline, respectively), and their impact on the synthesis and actions of neuromodulators such as adenosine and the cannabinoid system.

Table of Contents

frontiers in
BEHAVIORAL NEUROSCIENCE

EDITORIAL
published: 22 July 2014
doi: 10.3389/fnbeh.2014.00249

The renaissance of acetaldehyde as a psychoactive compound: decades in the making

Mercè Correa[1]*, Elio Acquas[2] and John D. Salamone[3]

[1] *Department of Psicobiology, University Jaume I, Castelló, Spain*
[2] *Department of Life and Environmental Sciences, University of Cagliari, Cagliari, Italy*
[3] *Department of Psychology, University of Connecticut, Storrs, CT, USA*
**Correspondence: correa@psb.uji.es*

Edited and reviewed by:
Nuno Sousa, University of Minho, Portugal

Keywords: ethanol, catalase, acetate, dopamine, salsolinol, opioids, addiction, drug abuse

As with many events in the history of science, the development of the hypothesis that acetaldehyde is a plausible psychoactive substance with specific central effects (not related to its toxicity) has not been either incremental or progressive. Rather, it has evolved through a process of fits and starts. Initial clinical observations suggesting that accumulation of acetaldehyde could be used as a therapy for alcoholism did not lead to a highly effective treatment, and in fact, it was noted early on that small amounts of ethanol consumed under these conditions (i.e., blockade of aldehyde dehydrogenase) could be perceived as being even more pleasurable (Chevens, 1953). Although some laboratory data in animals appeared at that time (Carpenter and Macleod, 1952), it took a decade for the pre-clinical studies to focus on the potential importance of acetaldehyde. Since Myers proposed in the late 60's that acetaldehyde could be a mediator of some of the effects of ethanol (Myers and Veale, 1969), advances in this field have gone through a push-pull process. His seminal discoveries about acetaldehyde self-administration and neural effects were met with skepticism because of the lack of replicability of results at that time, and the many unsolved questions related to measurements of brain acetaldehyde. In the late 70's, work conducted mainly by Amit's group (e.g., Amit et al., 1977), put acetaldehyde back in the picture. To replicate some of the early discoveries and expand the range of behaviors for which acetaldehyde could be shown to be a mediator of ethanol's effects, this laboratory started to assess the impact of interfering with acetaldehyde metabolism. Again, this work met with considerable skepticism, in part because of issues such as the lack of penetrability of acetaldehyde into the brain due to the high content of aldehyde dehydrogenase (ALDH) in the blood brain barrier, and also the absence of the alcohol dehydrogenase (ADH) isoform that metabolizes ethanol in the brain (Lindros and Hillbom, 1979). More or less at that time, a newly characterized enzyme for the metabolism of ethanol was introduced to the cerebral enzymatic landscape; catalase (Cohen et al., 1980). This enzyme became the focus of the next wave of work by Aragon and Amit related to the acetaldehyde hypothesis (Aragon et al., 1985). *In vitro* and *in vivo* studies, mainly with one pharmacological tool and one genetic mutation, established the first plausible support for the idea that brain ethanol metabolism was possible, and had a clear impact on alcohol consumption (Aragon and Amit, 1993). In addition, late in the 90's the localization of high levels of catalase in catecholamine rich areas in the brain (Zimatkin and Lindros, 1996), identified possible neuroanatomical loci for acetaldehyde formation. Across the first few years of the new century, Aragon and his colleagues developed a series of pharmacological tools that more firmly established the idea that brain catalase activity was directly correlated with ethanol's stimulant effects (Correa et al., 2001). Since then, data have been accumulating at an expanding pace. Additional groups of researchers, most of which are represented in this issue, started to apply different approaches to the "acetaldehyde hypothesis," which led to a new wave of papers and substantial scientific advances in this field. Genetic, neuropharmacological, electrophysiological and cellular mechanisms for acetaldehyde actions have been established. Potential therapeutic tools have been used. Neural circuits and complex behaviors have been studied, and other mediators of ethanol's actions that depend upon acetaldehyde formation, metabolism, or conjugation have been analyzed.

The present group of articles provides a comprehensive and up-to-date overview of research related to the actions of acetaldehyde, with recent results from the many groups that have decisively contributed to the field. The first review by Peana and Acquas gives a clear perspective on the behavioral evidence showing how acetaldehyde is a neuroactive molecule able to exert key motivational effects that can lead to the development of addiction, and also explains the neurobiochemical correlates of those actions in different intracellular signaling systems and diverse brain areas. A special accent on acetaldehyde formation, metabolism and blockade in relation to its reinforcing characteristics is presented by Diana and colleagues. Emphasis is put on the use of thiol compounds, which sequester acetaldehyde, as therapeutic agents preventing those effects. A very interesting methodological approach using gene-specific manipulations to change enzymatic activity, both in the liver and in the brain, has led Israel and colleagues to explore the behavioral consequences of central versus peripheral acetaldehyde metabolism. Direct administration of ethanol or acetaldehyde either in the periphery or in the brain, as employed in the studies from Correa and Salamone's lab, describe c-Fos expression (a marker of neural activity) across a broad range of brain areas involved in the motivational actions of both compounds. At the molecular level, Weiger and colleagues present data indicating that acetaldehyde acts on maxi calcium-activated potassium channels, a key factor in electrical stimulation of neural cells.

The role of acetaldehyde/monoamine conjugates that form tetrahydroisoquinoline alkaloids is presented in the next group of articles. Special attention is paid to the conjugate of acetaldehyde with dopamine, salsolinol. This molecule has demonstrated to be central for the actions of ethanol in the mesolimbic system (Melis et al., 2013). Electrophysiological data presented by Xie, Ye and colleagues show how salsolinol modulates firing of dopamine neurons in brain slices from posterior ventral tegmental area via activation of opioid receptors located in neighboring GABAergic neurons. These data are discussed by Polache and Granero in a commentary, written from the perspective of their own extensive research on salsonilol-induced dopamine release in the accumbens in behaving animals. Deehan and Rodd, report on the role of salsonilol and other conjugates in acetaldehyde self-administration, in an integrative review of human findings and their own key animal data involving the mesolimbic dopaminergic system.

Although dopamine has been a major focus for studies of acetaldehyde's central actions, other neural systems are also involved. For instance, the role of the opioid system in the neurochemical and behavioral effects of acetaldehyde is reviewed by Font and Pastor, who not only discuss the effects of salsonilol on *mu* opiod receptors, but also summarize what is known about acetaldehyde formation in brain nuclei rich in catalase and beta-endorphine neurons. Elegant studies conducted by Cannizzaro's laboratory demonstrate that the endocannabinoid system modulates not only operant acetaldehyde self-administration, extinction, and relapse, but also its effects on the emission of punished responses during conflict experiments, all of which are key features of addiction. A relevant role for acetate, a metabolite of acetaldehyde, as a mediator of the sedative and ataxic effects of alcohol is presented by Correa and colleagues. These results are hypothesized to be mediated by the neuromodulator adenosine.

The work by March, Molina and colleagues is focused on a particulary important neurodevelopemental period; early ontogeny. At this stage, drugs have a substantial impact on the nervous system, and in the case of ethanol, its actions can be affected by the fact that rats show a high rate of brain catalase activity specifically in this period. Thus, these authors provide extensive results about vulnerability to abuse and addiction in newborn and infant rats, which is induced by early ethanol and acetaldehyde exposure. These data are discussed in a commentary by Zimatkin, an expert on brain enzymatic localization, in terms of the ideal scenario in brain pups for acetaldehyde accumulation (high catalase-low ALDH). Finally, on the other extreme of the neurodevelopmental process, Vaglini, Corsini and colleagues review data on the impact of acetaldehyde on Parkinson's desease. In this case the authors focus on the role of the other important brain enzymatic system for the central formation and degradation of acetaldehyde; cytochrome P450-2E1, which is highly concentrated in substantia nigra pars compacta, an area rich in dopamine cell bodies. Acetaldehyde acting as a substrate/inhibitor of this enzyme, may facilitate the susceptibility of these dopaminergic neurons to toxin-induced parkinsonism.

Many more exciting discoveries are yet to come, and new methodologies still need to be developed in order to overcome the obstacles that continue to obscure our understanding of the role of acetaldehyde. Especially important would be the need for reliable measurements of central acetaldehyde production under physiological conditions. However, we can certainly say that acetaldehyde, its metabolites and its conjugates are critical molecules for the complex set of effects observed after ethanol consumption, especially those related to the development of its pathological consumption.

ACKNOWLEDGMENTS

Mercè Correa received support from Plan Nacional de Drogas (2010I024), Spain. John D. Salamone was supported by NIH/NIMH (MH078023-01A1), USA. Elio Acquas received grants from the Italian Ministry of University and Research (MIUR, PRIN 2006), and the Regione Autonoma della Sardegna (RAS), Italy, Regional Law n°7/2007, n°F71J090006200002 and B71J11001480002_1.

REFERENCES

Amit, Z., Brown, Z. W., and Rockman, G. E. (1977). Possible involvement of acetaldehyde, norepinephrine and their tetrahydroisoquinoline derivatives in the regulation of ethanol seld-administration. *Drug Alcohol Depend.* 2, 495–500.

Aragon, C. M., and Amit, Z. (1993). Differences in ethanol-induced behaviors in normal and acatalasemic mice: systematic examination using a biobehavioral approach. *Pharmacol. Biochem. Behav.* 44, 547–554. doi: 10.1016/0091-3057(93)90165-P

Aragon, C. M., Sternklar, G., and Amit, Z. (1985). A correlation between voluntary ethanol consumption and brain catalase activity in the rat. *Alcohol.* 2, 353–356. doi: 10.1016/0741-8329(85)90074-6

Carpenter, R. K., and Macleod, L. D. (1952). The effects of ethyl alcohol and acetaldehyde on maze behaviour and motor co-ordination in rats. *J. Ment. Sci.* 98, 167–173.

Chevens, L.C. F. (1953). Antabuse addiction. *Br. J. Med.* 1, 1450–1451. doi: 10.1136/bmj.1.4825.1450-c

Cohen, G., Sinet, P. M., and Heikkila, R. (1980). Ethanol oxidation by rat brain *in vivo*. *Alcohol Clin. Exp. Res.* 4, 366–370.

Correa, M., Sanchis-Segura, C., and Aragon, C. M. (2001). Brain catalase activity is highly correlated with ethanol-induced locomotor activity in mice. *Physiol. Behav.* 73, 641–647. doi: 10.1016/S0031-9384(01)00511-X

Lindros, K. O., and Hillbom, M. E. (1979). Acetaldehyde in cerebrospinal fluid: its near-absence in ethanol-intoxicated alcoholics. *Med. Biol.* 57, 246–247.

Melis, M., Carboni, E., Caboni, P., and Acquas, E. (2013). Key role of salsolinol in ethanol actions on dopamine neuronal activity of the posterior ventral tegmental area. *Addict. Biol.* doi: 10.1111/adb.12097. [Epub ahead of print].

Myers, R. D., and Veale, W. L. (1969). Alterations in volitional alcohol intake produced in rats by chronic intraventricular infusions of acetaldehyde, paraldehyde or methanol. *Arch. Int. Pharmacodyn. Ther.* 180, 100–113.

Zimatkin, S. M., and Lindros, K. O. (1996). Distribution of catalase in rat brain: aminergic neurons as possible targets for ethanol effects. *Alcohol Alcohol.* 31, 167–174.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 05 June 2014; accepted: 01 July 2014; published online: 22 July 2014.
*Citation: Correa M, Acquas E and Salamone JD (2014) The renaissance of acetaldehyde as a psychoactive compound: decades in the making. Front. Behav. Neurosci. **8**:249. doi: 10.3389/fnbeh.2014.00249*
This article was submitted to the journal Frontiers in Behavioral Neuroscience.

frontiers in
BEHAVIORAL NEUROSCIENCE

REVIEW ARTICLE
published: 15 July 2013
doi: 10.3389/fnbeh.2013.00086

Behavioral and biochemical evidence of the role of acetaldehyde in the motivational effects of ethanol

Alessandra T. Peana[1]* and Elio Acquas[2]*

[1] *Department of Chemistry and Pharmacy, University of Sassari, Sassari, Italy*
[2] *Department of Life and Environmental Sciences - Pharmaceutical, Pharmacological and Nutraceutical Sciences Section, University of Cagliari, Cagliari, Italy*

Edited by:
John D. Salamone, University of Connecticut, USA

Reviewed by:
Merce Correa, Universitat Jaume I, Spain
Laura F. Hurtado, Universitat Jaume I, Spain

****Correspondence:***
Elio Acquas, Department of Life and Environmental Sciences - Pharmaceutical, Pharmacological and Nutraceutical Sciences Section, University of Cagliari, via Ospedale, 72 – I-09124 Cagliari, Italy
e-mail: acquas@unica.it;
Alessandra T. Peana, Department of Chemistry and Pharmacy, University of Sassari, Sassari, Italy
e-mail: apeana@uniss.it

Since Chevens' report, in the early 50's that his patients under treatment with the aldehyde dehydrogenase inhibitor, antabuse, could experience beneficial effects when drinking small volumes of alcoholic beverages, the role of acetaldehyde (ACD) in the effects of ethanol has been thoroughly investigated on pre-clinical grounds. Thus, after more than 25 years of intense research, a large number of studies have been published on the motivational properties of ACD itself as well as on the role that ethanol-derived ACD plays in the effects of ethanol. Accordingly, in particular with respect to the motivational properties of ethanol, these studies were developed following two main strategies: on one hand, were aimed to challenge the suggestion that also ACD may exert motivational properties on its own, while, on the other, with the aid of enzymatic manipulations or ACD inactivation, were aimed to test the hypothesis that ethanol-derived ACD might have a role in ethanol motivational effects. Furthermore, recent evidence significantly contributed to highlight, as possible mechanisms of action of ACD, its ability to commit either dopaminergic and opioidergic transmission as well as to activate the Extracellular signal Regulated Kinase cascade transduction pathway in reward-related brain structures. In conclusion, and despite the observation that ACD seems also to have inherited the elusive nature of its parent compound, the behavioral and biochemical evidence reviewed points to ACD as a neuroactive molecule able, on its own and as ethanol metabolite, to exert motivational effects.

Keywords: acetaldehyde, behavior, ethanol, dopaminergic transmission, extracellular signal regulated kinase, opioidergic transmission, salsolinol

INTRODUCTION

Acetaldehyde (ACD) is well-known as a toxic compound and on this property was grounded the rationale for the use of disulfiram, an aldehyde dehydrogenase inhibitor, to treat alcoholism. However, toxicity does not necessarily involve perceived aversive effects and at the basis of this treatment are its aversive effects (nausea, headache, hot flushes, etc.) that discourage consumption. In spite of this, however, in the early 50's it was reported that some patients under treatment with the aldehyde dehydrogenase inhibitor (ALDH), antabuse, could experience pleasurable effects while ingesting small amounts of ethanol and it was postulated that ACD might have positive emotional as well as motivational effects (Chevens, 1953). After a long period of obsolescence from this observation, in the last decades the research on the role of ACD in the effects of ethanol, both as ethanol's metabolite and as chemical with its own motivational properties, has seen a renewed interest. To address this issue, the first approach has been to consider ethanol as a pro-drug of ACD. Indeed, the most radical view suggested that ACD could be responsible for all of the effects of ethanol and that *alcoholism* might, instead, be termed *acetaldehydism* (Raskin, 1975). Notably, a consistent body of evidence, suggesting that to exert its motivational properties ethanol must be metabolized into ACD, has been collected by different approaches including catalase manipulations (Aragon et al., 1985, 1991; Aragon and Amit, 1992), the use of alcohol dehydrogenase (ADH) (Amit, 1977; Brown et al., 1979; Smith et al., 1984; Quertemont and De Witte, 2001; Peana et al., 2008a) or ALDH inhibitors (Amit, 1977; Spivak et al., 1987a,b; Suh et al., 2006), the use of knock-out mice for the CYP2E1 isoform (Suh et al., 2006; Correa et al., 2009a) and the use of lentiviral vectors to silence the cell genome encoding for catalase or ADH synthesis (Karahanian et al., 2011). These approaches generated a large number of studies, summarized in comprehensive reviews (Quertemont et al., 2005; Correa et al., 2012), showing that locomotor (Escarabajal and Aragon, 2002; Martí-Prats et al., 2010; Ledesma and Aragon, 2012), anxiolytic (Correa et al., 2008; Escrig et al., 2012) and, in particular, motivational (Peana et al., 2008a,b, 2009, 2010a) properties of ethanol could be prevented by inhibiting either its peripheral and central metabolism or by ACD inactivation. Notably, two further issues, one related to the questioned ability of ACD to cross the blood brain barrier [see Correa et al. (2012) for an extensive discussion on this issue] and another related to the role of enhanced ethanol plasma concentrations that may in turn reach the brain, require to be dealt with while taking into consideration the consequences of blockade of ethanol peripheral metabolism.

Another approach to address the role of ACD in the motivational properties of ethanol has been to consider it as a chemical

with neurobiological properties on its own. Indeed, also this line of investigation has generated a significant body of data that also converged toward the characterization of ACD as a neurochemical agent able to elicit locomotor activity (Correa et al., 2009b) and anxiolytic effects (Correa et al., 2008), to sustain drug discrimination (York, 1981; Redila et al., 2000, 2002; Quertemont and Grant, 2002), to affect cognition (Sershen et al., 2009), and to elicit motivational effects (York, 1981; Peana et al., 2008a, 2009, 2010b; Spina et al., 2010). Interestingly, the behavioral evidence for the characterization of ACD as a drug with motivational properties was gathered, from conditioned place preference (CPP) and self-administration studies, in parallel with electrophysiological, biochemical and immunohistochemical studies pointing also to the critical role of dopamine (DA) (Foddai et al., 2004; Melis et al., 2007; Enrico et al., 2009; Spina et al., 2010; Vinci et al., 2010; Sirca et al., 2011) and opioid (Pastor et al., 2004; Sánchez-Catalán et al., 2009; Peana et al., 2011) transmission as well as to the involvement of Extracellular signal Regulated Kinase (ERK) (Spina et al., 2010; Vinci et al., 2010) at the basis of ACD's motivational properties. The present review aims to recapitulate this evidence in support of the tenet of ACD as a molecule able to exert motivational effects in rodents (for a recent comprehensive review see Correa et al., 2012).

CONDITIONED PLACE PREFERENCE AND SELF-ADMINISTRATION STUDIES

The role of ACD in the positive motivational properties of ethanol has become an increasingly attractive matter of debate and many studies have attempted to establish whether ACD is necessary for the manifestation of the neurobiological and behavioral effects of ethanol. Such studies have been developed by the aid of compounds that increase as well as inhibit, both centrally and peripherally, the formation of ACD and by the aid of compounds able to sequester ACD into stable non-reactive adducts. ACD has been shown to elicit CPP after intracerebroventricular infusion (Smith et al., 1984) and after intragastric (Peana et al., 2008a) and intraperitoneal (Quertemont and De Witte, 2001) administration. Notably, under these conditions, ACD-elicited CPP shows a bell shaped dose-response curve similar to that of ethanol (Quertemont and De Witte, 2001; Peana et al., 2008a).

The isoforms of ADH, normally found in gastric and hepatic tissue, represent the main metabolic pathway by which ethanol is converted into ACD upon ingestion (Baraona et al., 1991). Hence, provided that ethanol is not a substrate of brain ADH isoforms, the effect of the ADH competitive inhibitor, 4-methylpyrazole (4-MP), mostly used in behavioral studies is restricted to the peripheral metabolism of ethanol (Escarabajal and Aragon, 2002). In this paragraph we will focus our attention mostly on studies in which, to mimic the route commonly used by humans, ethanol and ACD were administered orally. In a CPP study, we showed that pretreatment with 4-MP reduces intragastric ethanol- (1 g/kg) but not ACD- (20 mg/kg) induced CPP, suggesting that ACD, metabolically derived from ethanol, could be responsible for this effect (Peana et al., 2008a). As previously mentioned, however, the impact of these results is partly reduced by the observation that administration of 4-MP may also affect, indirectly, brain ACD (Spivak et al., 1987a,b). In fact, when ethanol's metabolic conversion is prevented in the periphery it can reach the brain in greater amounts and can be metabolized therein by alternative pathways (catalase) (Aragon et al., 1985, 1991). This observation is also in agreement with the study by Tambour et al. (2007) reporting that the administration of cyanamide, a catalase and ALDH inhibitor, could prevent the locomotor stimulant effects of ethanol in mice and that this effect could be prevented by previous administration of 4-MP (Sanchis-Segura et al., 1999; Tambour et al., 2007).

Evidence of the role of ACD in the motivational properties of ethanol has also been provided by CPP experiments with D-penicillamine, a compound, which acts by sequestering ACD into a non-reactive and stable adduct without altering ethanol metabolism (Nagasawa et al., 1978). Thus, peripheral administration of D-penicillamine, at doses devoid of motivational properties *per se*, prevents the acquisition of either ethanol- (Font et al., 2006a; Peana et al., 2008a) or ACD-induced CPP (Peana et al., 2008a). In addition, as in the case of the experiments with 4-MP, the specificity of pretreatment with D-penicillamine on ethanol-derived ACD was confirmed by its failure to affect morphine-elicited CPP. Further, indirect, evidence of the role of ACD in the motivational effects of ethanol comes from studies with L-cysteine, a thiol amino acid, also known for its ability to protect against ACD toxicity (Salaspuro, 2007). L-cysteine is a precursor of the antioxidant glutathione (Soghier and Brion, 2006) and binds ACD by way of cysteinylglycine, the first metabolite in glutathione breakdown (Kera et al., 1985). Notably, L-cysteine formulations have been developed to bind in the oral cavity ACD originating after heavy smoking and alcohol drinking (Salaspuro et al., 2002; Salaspuro, 2007). Such formulations offer a novel method for intervention studies aimed to fight the role of ACD in the pathogenesis of upper digestive tract cancer (Salaspuro, 2007), alcoholic cardiomyopathy, as well as against the chronic toxicity of ACD (Sprince et al., 1974). Accordingly, we found that L-cysteine reduces either ethanol- and ACD-elicited CPP (Peana et al., 2009) supporting the notion that the generation and accumulation of ACD actively contributes to ethanol-induced CPP.

Several studies have found that ACD supports its self-administration. This has been demonstrated in unselected rats self-administering ACD intravenously (Myers et al., 1982; Takayama and Uyeno, 1985), intracerebroventricularly (Brown et al., 1979) and into the VTA (McBride et al., 2002), the latter also in alcohol-preferring rats (Rodd-Henricks et al., 2002). Further evidence that ACD exerts positive motivational properties arises from the observation that rats also acquire and maintain oral ACD self-administration (Peana et al., 2010b; Cacace et al., 2012). Our work, in this regard, has been aimed to characterize the role that ethanol-derived ACD plays in the pharmacological properties of orally ingested ethanol. Thus, as in the CPP studies, we have found that inhibition of the metabolism of ethanol, by 4-MP, reduces oral ethanol self-administration behavior (Peana et al., 2008b) an effect that might also be attributed to an increased concentration of ethanol which falls on the right-hand side of its bell shaped dose response curve. On the other hand, we have recently demonstrated, for the first time that ACD shares with ethanol the ability to induce oral

self-administration behavior further supporting the hypothesis that ACD itself exerts motivational effects (Peana et al., 2010b). Notably, rats self-administering ACD show extinction behavior when ACD is discontinued while gradually reinstate operant responses when ACD is reintroduced (Peana et al., 2010b). In addition, studies directed toward the characterization of the role of ACD, provided evidence that ethanol-derived ACD plays an important role in ethanol's motivational effects. Accordingly, previous findings have reported that intracerebroventricular the structural analogue of L-cysteine, D-penicillamine, reduces voluntary ethanol consumption in rats indicating that the central inactivation of ACD also blocks ethanol intake (Font et al., 2006b). On the other hand, L-cysteine reduces the acquisition and maintenance of oral ethanol self-administration as well as the reinstatement of ethanol-drinking and ethanol-seeking behaviors (Peana et al., 2010a, 2013a). Others studies examined the motivational effects of ACD under the break point that serves as an index of animals' motivation to work for the reinforcer. In these experiments 0.2% v/v ACD's break point was not statistically different from the break point of 10% v/v ethanol in spite of a 50 times lower concentration (Peana et al., 2012). On the other hand, ACD consumatory responses were paralleled by a relevant increase in ACD blood but not brain concentrations (Peana et al., 2010b). Furthermore, as in CPP and ethanol self-administration studies, during oral ACD self-administration L-cysteine was shown to decrease acquisition, maintenance, deprivation effect as well as ACD break point without interfering with saccharin reinforcement (Peana et al., 2012).

The ability of L-cysteine to affect ethanol-induced motivation could reside in different mechanisms. The first is consistent with the conjugation/inactivation mechanism that would take place between ACD and the first metabolite of glutathione (Kera et al., 1985). Furthermore, being L-cysteine an analogue of L-glutamate (Thompson and Kilpatrick, 1996) it is posited to interact at presynaptic group I metabotropic glutamate receptors (mGluR) of the mGluR5 subtype to exert a positive modulatory control on synaptic glutamate release (Harman et al., 1984; Croucher et al., 2001). In support of these results is the finding that L-cysteine reduces ethanol-induced stimulation of DA transmission in the nucleus accumbens shell (Sirca et al., 2011). Finally, it is noteworthy in this regard that L-cysteine may cross the blood brain barrier through excitatory amino acid transporters (Chen and Swanson, 2003) thus leaving open the possibility that this compound might also act centrally.

The main system of central ethanol oxidation is mediated by the enzyme catalase (Aragon et al., 1991; Aragon and Amit, 1992; Zimatkin et al., 1998). By reacting with H_2O_2, brain catalase forms compound I (the catalase-H_2O_2 system), which is able to oxidize ethanol into ACD (Pastor et al., 2002; Ledesma and Aragon, 2012). Recently we showed that the H_2O_2 scavenging agent, alpha lipoic acid, dose-dependently reduces the maintenance and break point of oral ethanol self-administration under a progressive ratio schedule as well as the reinstatement of ethanol seeking behavior without suppressing saccharin self-administration (Peana et al., 2013b). On a similar vein, a recent study by Ledesma and Aragon (2013) demonstrated that alpha lipoic acid reduces the acquisition and reconditioning of ethanol-induced CPP in mice. Overall, these data support the suggestion that a decrease in cerebral H_2O_2 availability, i.e., a reduced metabolic activity of brain catalase, by alpha lipoic acid administration may inhibit oral ethanol self-administration further suggesting that the brain catalase-H_2O_2 system, and therefore centrally formed ACD, plays a key role in the motivational effects of ethanol.

ACETALDEHYDE, DOPAMINE, AND INTRACELLULAR SIGNALING

The acute administration of ethanol elicits DA transmission in the rat nucleus accumbens (Imperato and Di Chiara, 1986; Howard et al., 2008) and this is also true in men whereby these increases in the ventral striatum positively correlate with the psychostimulant effect of ethanol (Boileau et al., 2003). Thus, a physiologically active DA transmission may represent the prerequisite for ethanol and, in accordance with Chevens (1953), also for ACD being able to elicit motivational effects. In this regard, in light of the hypothesized role of DA in the motivational effects of ACD, an indirect support comes from the clinical observation that subjects administered the DA biosynthesis inhibitor, alpha-methyltyrosine, do not experience ethanol-induced stimulation and euphoria (Ahlenius et al., 1973). With these premises in mind this paragraph will present and discuss the preclinical studies that support the involvement of DA in the motivational effects of ACD.

The main line of experimental evidence of the involvement of DA in the effects of ACD was inspired by the known ability of ethanol to stimulate the firing rate of midbrain DA cells either *in vivo* (Gessa et al., 1985; Foddai et al., 2004) and *in vitro* (Brodie et al., 1990; Melis et al., 2007) as well as the release of DA from mesoaccumbens terminals (Imperato and Di Chiara, 1986; Howard et al., 2008). Indeed these data also inspired the experiments aimed to test whether ethanol-derived ACD, as well as ACD on its own, could stimulate the firing rate of DA neurons in the VTA (Foddai et al., 2004) and DA transmission in the nucleus accumbens (Ward et al., 1997; Melis et al., 2007; Enrico et al., 2009). Thus, in keeping with the well-established paradigm, successfully applied in behavioral (Peana et al., 2008a) and histochemical (Vinci et al., 2010) studies that envisioned the use of 4-MP to prevent ethanol peripheral metabolism, Foddai et al. (2004) showed that this ADH inhibitor prevents ethanol-elicited stimulation of firing rate of DA neurons in the VTA, demonstrating for the first time that metabolic conversion of ethanol into ACD plays a critical role in the ability of ethanol to activate mesencephalic DA cells (Foddai et al., 2004). This finding has been further extended by the study of Melis et al. (2007) in which, by recording VTA DA cells *ex vivo*, it was shown that ethanol and ACD similarly stimulate DA cells firing rate but also that the stimulatory effect of ethanol is prevented by inhibition of its metabolic conversion into ACD by the catalase inhibitor 3-aminotriazole (Melis et al., 2007).

Notably, all the above refers to the ability of ACD to affect DA cells function and transmission at the pre-synaptic level. Immunohistochemical data, however, have shown that the expression of phosphorylated Extracellular signal regulated

kinase (pERK), taken as an index of post-synaptic DA-dependent neuronal activation (Acquas et al., 2007; Ibba et al., 2009), is similarly increased in the nucleus accumbens after the acute oral administration of either ethanol (Ibba et al., 2009; Vinci et al., 2010) and ACD (Vinci et al., 2010). In addition, ethanol-elicited increase of pERK expression in the nucleus accumbens could be prevented either by 4-MP and D-penicillamine (Vinci et al., 2010). ERK phosphorylation may take place by a number of factors ranging from extracellular signals to increased intracellular Ca^{++} concentrations via the sequential activation of a kinase cascade (Sweatt, 2004). This activated kinase has been related to neuronal plasticity (Fasano and Brambilla, 2011) and to long-term behavioral events that may be triggered by addictive drugs (Valjent et al., 2004; Girault et al., 2007) such as acquisition of conditioned responses (Beninger and Gerdjikov, 2004) as well as reinstatement of ethanol seeking (Radwanska et al., 2008; Schroeder et al., 2008; Peana et al., 2013a) in self-administration experiments. Accordingly, we found that while acute ACD administration elicits pERK in the nucleus accumbens (and other nuclei of the extended amygdala) (Vinci et al., 2010), the intracerebroventricular administration of PD98059, an inhibitor of the mitogen-activating extracellular kinase (MEK), the kinase responsible of ERK phosphorylation, prevents the acquisition of ACD-elicited CPP (Spina et al., 2010). In addition, the involvement of DA in these studies is supported by the experiments with the DA D_1 antagonist SCH 39166 (Ibba et al., 2009; Spina et al., 2010; Vinci et al., 2010). In fact, we demonstrated that either oral ethanol- (Ibba et al., 2009) and ACD- (Vinci et al., 2010) elicited nucleus accumbens increases of pERK expression and ACD-elicited CPP (Spina et al., 2010) could be prevented by blockade of D_1 receptors by SCH 39166.

A further line of experimental evidence, though yet speculative, of the involvement of DA in the effects of ACD originates from the studies on the condensation product(s) of ACD which, in particular when condensates with DA, can either spontaneously and enzymatically (Chen et al., 2011) generate 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (salsolinol) (Yamanaka et al., 1970; Jamal et al., 2003). Interestingly, since its discovery, this molecule has been related to ethanol (Davis and Walsh, 1970; Davis et al., 1970) and it was hypothesized that at least the DA-mediated effects of ethanol could be attributed to salsolinol (Davis and Walsh, 1970; Davis et al., 1970). This compound, indeed, has recently been reported to stimulate *in vitro* DA cells firing rate in the posterior VTA (Xie and Ye, 2012; Xie et al., 2012) and also, when applied into the VTA *in vivo*, to elicit DA transmission in the rat nucleus accumbens (Rodd et al., 2003; Deehan et al., 2012) and sustain its self-administration (Rodd et al., 2008). Thus, these data seem to collectively point to salsolinol as a substance that may exert motivational effects by virtue of its ability to involve mesolimbic DA. However, the relationship between ACD, DA and salsolinol still awaits to be fully disclosed.

ACETALDEHYDE AND OPIOIDS

Acute administration of ethanol increases endogenous opioid (endorphin and enkephalin) release from brain slices, pituitary gland and increases blood levels of opioids in humans *in vivo* (see Herz, 1997 for a comprehensive review). Acute alcohol administration also enhances gene expression of both endorphin and enkephalin in selected brain areas of rats, whereas chronic ethanol administration reduces gene expression, making less of opioid peptides available for release (Herz, 1997). Since opioid transmission, in both the VTA and nucleus accumbens, regulates the release of DA from mesolimbic neurons, ethanol-induced opioid release may produce reinforcement by modulating DA transmission. Accordingly, opioid antagonists decrease the motivational properties of ethanol in rats self-administering ethanol by interfering with ethanol-dependent dopaminergic activation (Acquas et al., 1993; Benjamin et al., 1993; Di Chiara et al., 1996; Gonzales and Weiss, 1998) as the motivational effects of ethanol by opioid antagonists may involve an opioid-DA link (Di Chiara et al., 1996). However, another line of evidence for the involvement of the endogenous opioid system in the motivational effects of ethanol appears to be related to its first metabolite, ACD. In fact, there are several proofs to support that opioids are implicated in the motivational effects of ACD as well. The first arises from the early observation by Myers et al. (1984) demonstrating that naloxone decreases intravenous ACD self-administration. The second arises from the observation that naltrexone reduces oral ACD self-administration (Peana et al., 2011). In particular, we observed that this effect could be mediated by an involvement of μ_1 opioid receptors since naloxonazine, a μ_1 selective opioid receptor antagonist, reduced the maintenance phase of ACD self-administration (Peana et al., 2011). The mechanism by which opioid antagonists affect the motivational properties of ACD is unclear. In this regard one might consider that ACD on its own, and as a consequence of the metabolism of ethanol, activates neuronal firing of DA cells in the VTA (Foddai et al., 2004), stimulates DA transmission (Melis et al., 2007; Enrico et al., 2009; Sirca et al., 2011) and ERK phosphorylation in the nucleus accumbens (Vinci et al., 2010). This possibility appears in agreement with the observation that blockade of μ opioid receptors also prevents ERK phosphorylation in the nucleus accumbens (Peana et al., 2011). An alternative possibility, yet to be fully demonstrated, is offered by the suggestion that the condensation product between ACD and DA, salsolinol (see above), acts *via* stimulation of μ opioid receptors (Hipolito et al., 2009, 2010, 2012; Xie et al., 2012).

DISCUSSION AND CONCLUSIONS

The reviewed literature indicates that ACD has its own motivational properties as assessed by CPP and self-administration studies (Peana et al., 2008a, 2010b; Spina et al., 2010) and also that this property is grounded on its ability to involve mesolimbic DA (Foddai et al., 2004; Melis et al., 2007; Spina et al., 2010; Vinci et al., 2010), μ opioid receptors -mediated transmission (Peana et al., 2011) as well as phosphorylated ERK in the nucleus accumbens (Spina et al., 2010; Vinci et al., 2010; Peana et al., 2011). In addition, a wealth of experimental evidence supports that the motivational effects of ethanol are mediated by its metabolism into ACD either in the periphery or in the brain. Accordingly, this has been demonstrated by inhibiting the production of ACD in the periphery (inhibition of ADH), by inhibiting the generation of brain ACD (inhibition of brain catalase) or by reducing

ACD bioavailability (Font et al., 2006a,b; Peana et al., 2008a, 2009, 2010a, 2013a,b; Enrico et al., 2009; Sirca et al., 2011; Correa et al., 2012). All these observations support the tenet that the generation of central and peripheral, but not peripherally accumulated (Escrig et al., 2012), ACD actively participates in the positive motivational properties of ethanol and raise the possibility that its role can be exploited to devise novel pharmacological approaches that target alcohol abuse related problems.

REFERENCES

Acquas, E., Meloni, M., and Di Chiara, G. (1993). Blockade of delta-opioid receptors in the nucleus accumbens prevents ethanol-induced stimulation of dopamine release. *Eur. J. Pharmacol.* 230, 239–241. doi: 10.1016/0014-2999(93)90809-V

Acquas, E., Pisanu, A., Spiga, S., Plumitallo, A., Zernig, G., and Di Chiara, G. (2007). Differential effects of intravenous R,S-(±)-3,4-methylenedioxymethamphetamine (MDMA, ecstasy) and its S(+)- and R(-)-enantiomers on dopamine transmission and extracellular signal regulated kinase phosphorylation (pERK) in the rat nucleus accumbens shell and core. *J. Neurochem.* 102, 121–132. doi: 10.1111/j.1471-4159.2007.04451.x

Ahlenius, S., Carlsson, A., Engel, J., Svensson, T., and Södersten, P. (1973). Antagonism by alpha methyltyrosine of the ethanol-induced stimulation and euphoria in man. *Clin. Pharmacol. Ther.* 14, 586–591.

Amit, Z. (1977). Brain and liver aldehyde dehydrogenase: relations to ethanol consumption in wistar rats. *Neuropharmacology* 16, 781–784. doi: 10.1016/0028-3908(77)90137-X

Aragon, C. M., and Amit, Z. (1992). The effect of 3-amino-1,2,4-triazole on voluntary ethanol consumption: evidence for brain catalase involvement in the mechanism of action. *Neuropharmacology* 31, 709–712. doi: 10.1016/0028-3908(92)90150-N

Aragon, C. M., Sternklar, G., and Amit, Z. (1985). A correlation between voluntary ethanol consumption and brain catalase activity in the rat. *Alcohol* 2, 353–356. doi: 10.1016/0741-8329(85)90074-6

Aragon, C. M., Stotland, L. M., and Amit, Z. (1991). Studies on ethanol-brain catalase interaction: evidence for central ethanol oxidation. *Alcohol. Clin. Exp. Res.* 15, 165–169. doi: 10.1111/j.1530-0277.1991.tb01848.x

Baraona, E., Yokoyama, A., Ishii, H., Hernandez-Munoz, R., Takagi, T., Tsuchiya, M., et al. (1991). Lack of alcohol dehydrogenase isoenzyme activities in the stomach of Japanese subjects. *Life Sci.* 49, 1929–1934. doi: 10.1016/0024-3205(91)90295-M

Beninger, R. J., and Gerdjikov, T. (2004). The role of signaling molecules in reward-related incentive learning. *Neurotox. Res.* 61, 91–104. doi: 10.1007/BF03033301

Benjamin, D., Grant, E. R., and Pohorecky, L. A. (1993). Naltrexone reverses ethanol-induced dopamine release in the nucleus accumbens in awake, freely moving rats. *Brain Res.* 621,137–140. doi: 10.1016/0006-8993(93)90309-B

Boileau, I., Assaad, J. M., Pihl, R. O., Benkelfat, C., Leyton, M., Diksic, M., et al. (2003). Alcohol promotes dopamine release in the human nucleus accumbens. *Synapse* 49, 226–231. doi: 10.1002/syn.10226

Brodie, M. S., Shefner, S. A., and Dunwiddie, T. V. (1990). Ethanol increases the firing rate of dopamine neurons of the rat ventral tegmental area *in vitro*. *Brain Res.* 508, 65–69. doi: 10.1016/0006-8993(90)91118-Z

Brown, Z. W., Amit, Z., and Rockman, G. E. (1979). Intraventricular self-administration of acetaldehyde, but not ethanol, in naïve laboratory rats. *Psychopharmacology (Berl.)* 64, 271–276. doi: 10.1007/BF00427509

Cacace, S., Plescia, F., Barberi, I., and Cannizzaro, C. (2012). Acetaldehyde oral self-administration: evidence from the operant-conflict paradigm. *Alcohol. Clin. Exp. Res.* 36, 1278–1287. doi: 10.1111/j.1530-0277.2011.01725.x

Chen, A., Arshad, A., Qing, H., Wang, R., Lu, J., and Deng, Y. (2011). Enzymatic condensation of dopamine and acetaldehyde: a salsolinol synthase from rat brain. *Biologia* 66, 1183–1188. doi: 10.2478/s11756-011-0134-y

Chen, Y., and Swanson, R. A. (2003). The glutamate transporters EAAT2 and EAAT3 mediate cysteine uptake in cortical neuron cultures. *J. Neurochem.* 84, 1332–1339. doi: 10.1046/j.1471-4159.2003.01630.x

Chevens, L. C. F. (1953). Antabuse addiction. *Br. J. Med.* 1, 1450–1451. doi: 10.1136/bmj.1.4825.1450-c

Correa, M., Manrique, H. M., Font, L., Escrig, M. A., and Aragon, C. M. (2008). Reduction in the anxiolytic effects of ethanol by centrally formed acetaldehyde: the role of catalase inhibitors and acetaldehyde-sequestering agents. *Psychopharmacology* 200, 455–464. doi: 10.1007/s00213-008-1219-3

Correa, M., Salamone, J. D., Segovia, K. N., Pardo, M., Longoni, R., Spina, L., et al. (2012). Piecing together the puzzle of acetaldehyde as a neuroactive agent. *Neurosci. Biobehav. Rev.* 36, 404–430. doi: 10.1016/j.neubiorev.2011.07.009

Correa, M., Viaggi, C., Escrig, M. A., Pascual, M., Guerri, C., Vaglini, F., et al. (2009a). Ethanol intake and ethanol-induced locomotion and locomotor sensitization in Cyp2e1 knockout mice. *Pharmacogenet. Genomics* 19, 217–225. doi: 10.1097/FPC.0b013e328324e726

Correa, M., Arizzi-Lafrance, M. N., and Salamone, J. D. (2009b). Infusions of acetaldehyde into the arcuate nucleus of the hypothalamus induce motor activity in rats. *Life Sci.* 84, 321–327. doi: 10.1016/j.lfs.2008.12.013

Croucher, M. J., Thomas, L. S., Ahmadi, H., Lawrence, V., and Harris, J. R. (2001). Endogenous sulphur-containing amino acids: potent agonists at presynaptic metabotropic glutamate autoreceptors in the rat central nervous system. *Br. J. Pharmacol.* 133, 815–824. doi: 10.1038/sj.bjp.0704138

Davis, V. E., and Walsh, M. J. (1970). Alcohol, amines, and alkaloids: a possible biochemical basis for alcohol addiction. *Science* 167, 1005–1007. doi: 10.1126/science.167.3920.1005

Davis, V. E., Walsh, M. J., and Yamanaka, Y. (1970). Augmentation of alkaloid formation from dopamine by alcohol and acetaldehyde *in vitro*. *J. Pharmacol. Exp. Ther.* 174, 401–412.

Deehan, G. A. Jr., Engleman, E. A., Ding, Z. M., McBride, W. J., and Rodd, Z. A. (2012). Microinjections of acetaldehyde or salsolinol into the posterior ventral tegmental area increase dopamine release in the nucleus accumbens shell. *Alcohol. Clin. Exp. Res.* 37, 722–729. doi: 10.1111/acer.12034

Di Chiara, G., Acquas, E., and Tanda, G. (1996). Ethanol as a neurochemical surrogate of conventional reinforcers: the dopamine-opioid link. *Alcohol* 13, 13–17. doi: 10.1016/0741-8329(95)02034-9

Enrico, P., Sirca, D., Mereu, M., Peana, A. T., Lintas, A., Golosio, A., et al. (2009). Acetaldehyde sequestering prevents ethanol-induced stimulation of mesolimbic dopamine transmission. *Drug Alcohol Depend.* 100, 265–271. doi: 10.1016/j.drugalcdep.2008.10.010

Escarabajal, M. D., and Aragon, C. M. (2002). The effect of cyanamide and 4-methylpyrazole on the ethanol-induced locomotor activity in mice. *Pharmacol. Biochem. Behav.* 72, 389–395. doi: 10.1016/S0091-3057(01)00762-6

Escrig, M. A., Pardo, M., Aragon, C. M., and Correa, M. (2012). Anxiogenic and stress-inducing effects of peripherally administered acetaldehyde in mice: similarities with the disulfiram-ethanol reaction. *Pharmacol. Biochem. Behav.* 100, 404–412. doi: 10.1016/j.pbb.2011.10.002

Fasano, S., and Brambilla, R. (2011). Ras-ERK signaling in behavior: old questions and new perspectives. *Front. Behav. Neurosci.* 5:79. doi: 10.3389/fnbeh.2011.00079

Foddai, M., Dosia, G., Spiga, S., and Diana, M. (2004). Acetaldehyde increases dopaminergic neuronal activity in the VTA. *Neuropsychopharmacology* 29, 530–536. doi: 10.1038/sj.npp.1300326

Font, L., Aragon, C. M., and Miquel, M. (2006a). Ethanol-induced conditioned place preference, but not aversion, is blocked by treatment with D-penicillamine, an inactivation agent for acetaldehyde. *Psychopharmacology* 184, 56–64. doi: 10.1007/s00213-005-0224-z

Font, L., Aragon, C. M., and Miquel, M. (2006b). Voluntary ethanol consumption decreases after the inactivation of central acetaldehyde by d-penicillamine. *Behav. Brain Res.* 171, 78–86. doi: 10.1016/j.bbr.2006.03.020

Gessa, G. L., Muntoni, F., Vargiu, L., and Mereu, G. (1985). Low doses of ethanol activate dopaminergic neurons in the ventral tegmental area. *Brain Res.* 348, 201–203. doi: 10.1016/0006-8993(85)90381-6

Girault, J. A., Valjent, E., Caboche, J., and Herve, D. (2007). ERK2: a logical AND gate critical for drug-induced plasticity? *Curr.*

Opin. Pharmacol. 7, 77–85. doi: 10.1016/j.coph.2006.08.012

Gonzales, R. A., and Weiss, F. (1998). Suppression of ethanol-reinforced behavior by naltrexone is associated with attenuation of the ethanol-induced increase in dialysate dopamine levels in the nucleus accumbens. *J. Neurosci.* 18, 10663–10671.

Harman, L. S., Mottley, C., and Mason, R. P. (1984). Free radical metabolites of L-cysteine oxidation. *J. Biol. Chem.* 259, 5606–5611.

Herz, A. (1997). Endogenous opioid systems and alcohol addiction. *Psychopharmacology* 129, 99–111. doi: 10.1007/s002130050169

Hipolito, L., Sánchez-Catalán, M. J., Granero, L., and Polache, A. (2010). Locomotor stimulant effects of acute and repeated intrategmental injections of salsolinol in rats: role of mu-opioid receptors. *Psychopharmacology* 209, 1–11. doi: 10.1007/s00213-009-1751-9

Hipolito, L., Sánchez-Catalán, M. J., Martí-Prats, L., Granero, L., and Polache, A. (2012). Revisiting the controversial role of salsolinol in the neurobiological effects of ethanol: old and new vistas. *Neurosci. Biobehav. Rev.* 36, 362–378. doi: 10.1016/j.neubiorev.2011.07.007

Hipolito, L., Sánchez-Catalán, M. J., Zornoza, T., Polache, A., and Granero, L. (2009). Local salsolinol modulates dopamine extracellular levelsfrom rat nucleus accumbens: shell/core differences. *Neurochem. Int*. 55, 187–192. doi: 10.1016/j.neuint.2009.02.014

Howard, E. C., Schier, C. J., Wetzel, J. S., Duvauchelle, C. L., and Gonzales, R. A. (2008). The shell of the nucleus accumbens has a higher dopamine response compared with the core after non-contingent intravenous ethanol administration. *Neuroscience* 154, 1042–1053. doi: 10.1016/j.neuroscience.2008.04.014

Ibba, F., Vinci, S., Spiga, S., Peana, A. T., Assaretti, A. R., Spina, L., et al. (2009). Ethanol-induced extracellular signal regulated kinase: role of dopamine D1 receptors. *Alcohol. Clin. Exp. Res.* 33, 858–867. doi: 10.1111/j.1530-0277.2009.00907.x

Imperato, A., and Di Chiara, G. (1986). Preferential stimulation of dopamine release in the nucleus accumbens of freely moving rats by ethanol. *J. Pharmacol. Exp. Ther.* 239, 219–228.

Jamal, M., Ameno, K., Ameno, S., Okada, N., and Ijiri, I. (2003). *In vivo* study of salsolinol produced by a high concentration of acetaldehyde in the striatum and nucleus accumbens of free-moving rats. *Alcohol. Clin. Exp. Res.* 27(8 Suppl.), 79S–84S. doi: 10.1097/01.ALC.0000078617.33026.AD

Karahanian, E., Quintanilla, M. E., Tampier, L., Rivera-Meza, M., Bustamante, D., Gonzalez-Lira, V., et al. (2011). Ethanol as a prodrug: brain metabolism of ethanol mediates its motivationaleffects. *Alcohol. Clin. Exp. Res.* 35, 606–612. doi: 10.1111/j.1530-0277.2011.01439.x

Kera, Y., Kiriyama, T., and Komura, S. (1985). Conjugation of acetaldehyde with cysteiniylglycine, the first metabolite in glutathione breakdown by c-glutamyltranspeptidase. *Agents Actions* 17, 48–52. doi: 10.1007/BF01966681

Ledesma, J. C., and Aragon, C. M. (2013). Acquisition and reconditioning of ethanol-induced conditioned place preference in mice is blocked by the H_2O_2 scavenger alpha lipoic acid. *Psychopharmacology* 226, 673–685. doi: 10.1007/s00213-012-2831-9

Ledesma, J. C., and Aragon, C. M. G. (2012). Alpha lipoic acid, a scavenging agent for H_2O_2, reduces ethanol-stimulated locomotion in mice. *Psychopharmacology* 219, 171–180. doi: 10.1007/s00213-011-2407-0

Martí-Prats, L., Sánchez-Catalán, M. J., Hipólito, L., Orrico, A., Zornoza, T., Polache, A., et al. (2010). Systemic administration of D-penicillamine prevents the locomotor activation after intra-VTA ethanol administration in rats. *Neurosci. Lett.* 483, 143–147. doi: 10.1016/j.neulet.2010.07.081

McBride, W. J., Li, T. K., Deitrich, R. A., Zimatkin, S., Smith, B. R., and Rodd-Henricks, Z. A. (2002). Involvement of acetaldehyde in alcohol addiction. *Alcohol. Clin. Exp. Res.* 26, 114–119. doi: 10.1111/j.1530-0277.2002.tb02439.x

Melis, M., Enrico, P., Peana, A. T., and Diana, M. (2007). Acetaldehyde mediates alcohol activation of the mesolimbic dopamine system. *Eur. J. Neurosci.* 26, 2824–2833. doi: 10.1111/j.1460-9568.2007.05887.x

Myers, W. D., Ng, K. T., and Singer, G. (1982). Intravenous self-administration of acetaldehyde in the rat as a function of schedule, food deprivation and photoperiod. *Pharmacol. Biochem. Behav.* 17, 807–811. doi: 10.1016/0091-3057(82)90364-1

Myers, W. D., Ng, K. T., and Singer, G. (1984). Effects of naloxone and buprenorphine on intravenous acetaldehyde self-injection in rats. *Physiol. Behav*. 33, 449–455. doi: 10.1016/0031-9384(84)90168-9

Nagasawa, H. T., Goon, D. J., and DeMaster, E. G. (1978). 2,5,5-Trimethylthiazolidine-4-carboxylic acid, a D(-)-penicillamine-directed pseudometabolite of ethanol. Detoxication mechanism for acetaldehyde. *J. Med. Chem.* 21, 1274–1279. doi: 10.1021/jm00210a019

Pastor, R., Sanchis-Segura, C., and Aragon, C. M. (2002). Ethanol-stimulated behaviour in mice is modulated by brain catalase activity and H_2O_2 rate of production. *Psychopharmacology* 165, 51–59. doi: 10.1007/s00213-002-1241-9

Pastor, R., Sanchis-Segura, C., and Aragon, C. M. (2004). Brain catalase activity inhibition as well as opioid receptor antagonism increases ethanol-induced HPA axis activation. *Alcohol. Clin. Exp. Res.* 28, 1898–1906. doi: 10.1097/01.ALC.0000148107.64739.76

Peana, A. T., Assaretti, A. R., Muggironi, G., Enrico, P., and Diana, M. (2009). Reduction of ethanol-derived acetaldehyde induced motivational properties by L-cysteine. *Alcohol. Clin. Exp. Res.* 33, 43–48. doi: 10.1111/j.1530-0277.2008.00809.x

Peana, A. T., Enrico, P., Assaretti, A. R., Pulighe, E., Muggironi, G., Nieddu, M., et al. (2008a). Key role of ethanol-derived acetaldehyde in the motivational properties induced by intragastric ethanol: a conditioned place preference study in the rat. *Alcohol. Clin. Exp. Res.* 32, 249–258. doi: 10.1111/j.1530-0277.2007.00574.x

Peana, A. T., Assaretti, A. R., Muggironi, G., Enrico, P., and Diana, M. (2008b). "The peripheral competitive inhibitor of alcohol dehydrogenase, 4-methylpyrazole, reduces oral ethanol self-administration," in *ESBRA2008 Nordmann Award Meeting-Animal Models in Alcohol Research,* (Cagliari), 26–27.

Peana, A. T., Giugliano, V., Rosas, M., Sabariego, M., and Acquas, E. (2013a). Effects of L-cysteine on reinstatement of ethanol seeking behavior and on reinstatement-elicited ERK phosphorylation in the rat nucleus accumbens shell. *Alcohol. Clin. Exp. Res.* 37(Suppl. 1), E329–E337. doi: 10.1111/j.1530-0277.2012.01877.x

Peana, A. T., Muggironi, G., Fois, G., and Diana, M. (2013b). Alpha-lipoic acid reduces ethanol self-administration in rats. *Alcohol. Clin. Exp. Res.* doi: 10.1111/acer.12169. [Epub ahead of print].

Peana, A. T., Muggironi, G., Calvisi, G., Enrico, P., Mereu, M., Nieddu, M., et al. (2010a). L-cysteine reduces oral ethanol self-administration and reinstatement of ethanol-drinking behavior in rats. *Pharmacol. Biochem. Behav.* 94, 431–437. doi: 10.1016/j.pbb.2009.10.005

Peana, A. T., Muggironi, G., and Diana, M. (2010b). Acetaldehyde-motivationaleffects; a study on oral self-administration behavior. *Front. Psychiatry* 1:23. doi: 10.3389/fpsyt.2010.00023

Peana, A. T., Muggironi, G., Fois, G. R., Zinellu, M., Sirca, D., and Diana, M. (2012). Effect of (L)-cysteine on acetaldehyde self-administration. *Alcohol* 46, 489–497. doi: 10.1016/j.alcohol.2011.10.004

Peana, A. T., Muggironi, G., Fois, G. R., Zinellu, M., Vinci, S., and Acquas, E. (2011). Effect of opioid receptor blockade on acetaldehyde self-administration and ERK phosphorylation in the rat nucleus accumbens. *Alcohol* 45, 773–783. doi: 10.1016/j.alcohol.2011.06.003

Quertemont, E., and De Witte, P. (2001). Conditioned stimulus preference after acetaldehyde but not ethanol injections. *Pharmacol. Biochem. Behav.* 68, 449–454. doi: 10.1016/S0091-3057(00)00486-X

Quertemont, E., and Grant, K. A. (2002). Role of acetaldehyde in the discriminative stimulus effects of ethanol. *Alcohol. Clin. Exp. Res*. 26, 812–817. doi: 10.1111/j.1530-0277.2002.tb02609.x

Quertemont, E., Tambour, S., and Tirelli, E. (2005). The role of acetaldehyde in the neurobehavioral effects of ethanol: a comprensive review of animal studies. *Prog. Neurobiol.* 75, 247–274. doi: 10.1016/j.pneurobio.2005.03.003

Radwanska, K., Wrobel, E., Korkosz, A., Rogowski, A., Kostowski, W., Bienkowski, P., et al. (2008). Alcohol relapse induced by discrete cues activates components of AP-1 transcription factor and ERK pathway in the rat basolateral and central amygdala. *Neuropsychopharmacology* 33, 1835–1846. doi: 10.1038/sj.npp.1301567

Raskin, N. H., (1975). Editorial: alcoholism or acetaldehydism? *N. Engl. J. Med.* 292, 422–423. doi: 10.1056/NEJM197502202920811

Redila, V. A., Aliatas, E., Smith, B. R., and Amit, Z. (2002). Effects of ethanol on an acetaldehyde drug discrimination with a conditioned taste aversion procedure. *Alcohol*

28, 103–109. doi: 10.1016/S0741-8329(02)00270-7

Redila, V. A., Smith, B. R., and Amit, Z. (2000). The effects of aminotriazole and acetaldehyde on an ethanol drug discrimination with a conditioned taste aversion procedure. *Alcohol* 21, 279–285. doi: 10.1016/S0741-8329(00)00096-3

Rodd, Z. A., Bell, R. L., Zhang, Y., Goldstein, A., Zaffaroni, A., McBride, W. J., et al. (2003). Salsolinol produces motivational effects in the nucleus accumbens shell of alcohol-preferring (P) rats. *Alcohol. Clin. Exp. Res.* 27, 440–449. doi: 10.1097/01.ALC.0000056612.89957.B4

Rodd, Z. A., Oster, S. M., Ding, Z. M., Toalston, J. E., Deehan, G., Bell, R. L., et al. (2008). The motivational properties of salsolinol in the ventral tegmental area: evidence for regional heterogeneity and the involvement of serotonin and dopamine. *Alcohol. Clin. Exp. Res.* 32, 230–239. doi: 10.1111/j.1530-0277.2007.00572.x

Rodd-Henricks, Z. A., Melendez, R. I., Zaffaroni, A., Goldstein, A., McBride, W. J., and Lu, T. K. (2002). The motivationaleffects of acetaldehyde in the posterior ventral tegmental area of alcohol-preferring rats. *Pharmacol. Biochem. Behav.* 72, 55–64. doi: 10.1016/S0091-3057(01)00733-X

Salaspuro, V. (2007). Pharmacological treatments and stategies for reducing oral and intestinal acetaldehyde. *Novartis Found. Symp.* 285, 145–153. doi: 10.1002/9780470511848.ch11

Salaspuro, V., Hietala, J., Kaihovaara, P., Pihlajarinne, L., Marvola, M., and Salaspuro, M. (2002). Removal of acetaldehyde from saliva by a slow-release buccal tablet of L-cysteine. *Int. J. Cancer* 97, 361–364. doi: 10.1002/ijc.1620

Sánchez-Catalán, M. J., Hipólito, L., Zornoza, T., Polache, A., and Granero, L. (2009). Motor stimulant effects of ethanol and acetaldehyde injected into the posterior ventral tegmental area of rats: role of opioid receptors. *Psychopharmacology* 204, 641–653. doi: 10.1007/s00213-009-1495-6

Sanchis-Segura, C., Miquel, M., Correa, M., and Aragon, C. M. (1999). Daily injections of cyanamide enhance both ethanol-induced locomotion and brain catalase activity. *Behav. Pharmacol.* 10, 459–465. doi: 10.1097/00008877-199909000-00004

Schroeder, J. P., Spanos, M., Stevenson, J. R., Besheer, J., Salling, M., and Hodge, C. W. (2008). Cue-induced reinstatement of alcohol-seeking behavior is associated with increased ERK(1/2) phosphorylation in specific limbic brain regions: blockade by the mGluR5 antagonist MPEP. *Neuropharmacology* 55, 546–554. doi: 10.1016/j.neuropharm.2008.06.057

Sershen, H., Shearman, E., Fallon, S., Chakraborty, G., Smiley, J., and Lajtha, A. (2009). The effects of acetaldehyde on nicotine-induced transmitter levels in young and adult brain areas. *Brain Res. Bull.* 79, 458–462. doi: 10.1016/j.brainresbull.2009.04.005

Sirca, D., Enrico, P., Mereu, M., Peana, A. T., and Diana, M. (2011). L-cysteine prevents ethanol-induced stimulation of mesolimbic dopamine transmission. *Alcohol. Clin. Exp. Res.* 35, 862–869. doi: 10.1111/j.1530-0277.2010.01416.x

Smith, B. R., Amit, Z., and Splawinsky, J. (1984). Conditioned place preference induced by intraventricular infusions of acetaldehyde. *Alcohol* 1, 193–195. doi: 10.1016/0741-8329(84)90097-1

Soghier, L. M., and Brion, L. P. (2006). Cysteine, cystine or N-acetylcysteine supplementation in parenterally fed neonated. *Cochrane Database Syst. Rev.* 18:CD004869. doi: 10.1002/14651858.CD004869.pub2

Spina, L., Longoni, R., Vinci, S., Ibba, F., Peana, A. T., Muggironi, G., et al. (2010). Role of dopamine D1 receptors and extracellular signal regulated kinase in the motivational properties of acetaldehyde as assessed by place preference conditioning. *Alcohol. Clin. Exp. Res.* 34, 607–616. doi: 10.1111/j.1530-0277.2009.01129.x

Spivak, K., Aragon, C. M., and Amit, Z. (1987a). Alterations in brain aldehyde dehydrogenase activity modify ethanol-induced conditioned taste aversion. *Alcohol. Clin. Exp. Res.* 11, 513–517. doi: 10.1111/j.1530-0277.1987.tb00163.x

Spivak, K., Aragon, C. M., and Amit, Z. (1987b). Alterations in brain aldehyde dehydrogenase activity modify the locomotor effects produced by ethanol in rats. *Alcohol Drug Res.* 7, 481–491.

Sprince, H., Parker, C. M., Smith, G. G., and Gonzales, L. J. (1974). Protection against acetaldehyde toxicity in the rat by L-cysteine, thiamin and L-2-methylthiazolidine-4-carboxylic acid. *Agents Actions* 4, 125–130. doi: 10.1007/BF01966822

Suh, J. J., Pettinati, H. M., Kampman, K. M., and O'Brien, C. P. (2006). The status of disulfiram: a half of a century later. *J. Clin. Psychopharmacol.* 26, 290–302. doi: 10.1097/01.jcp.0000222512.25649.08

Sweatt, J. D. (2004). Mitogen-activated protein kinases in synaptic plasticity and memory. *Curr. Opin. Neurobiol.* 14, 311–317. doi: 10.1016/j.conb.2004.04.001

Takayama, S., and Uyeno, E. T. (1985). Intravenous self-administration of ethanol and acetaldehyde by rats. *Yakubutsu Seishin Kodo* 5, 329–334.

Tambour, S., Closon, C., Tirelli, E., and Quertemont, E. (2007). Effects of cyanamide and acetaldehyde accumulation on the locomotor stimulant and sedative effects of ethanol in mice. *Behav. Pharmacol.* 18, 777–784. doi: 10.1097/FBP.0b013e3282f158ca

Thompson, G. A., and Kilpatrick, I. C. (1996). The neurotransmitter candidature of sulphur-containing excitatory amino acids in the mammalian central nervous system. *Pharmacol. Ther.* 72, 25–36. doi: 10.1016/S0163-7258(96)00097-6

Valjent, E., Pages, C., Herve, D., Girault, J. A., and Caboche, J. (2004). Addictive and non-addictive drugs induce distinct and specific patterns of ERK activation in mouse brain. *Eur. J. Neurosci.* 19, 1826–1836. doi: 10.1111/j.1460-9568.2004.03278.x

Vinci, S., Ibba, F., Longoni, R., Spina, L., Spiga, S., and Acquas, E. (2010). Acetaldehyde elicits ERK phosphorylation in the rat nucleus accumbens and extended amygdala. *Synapse* 64, 916–927. doi: 10.1002/syn.20811

Ward, R. J., Colantuoni, C., Dahchour, A., Quertemont, E., and De Witte, P. (1997). Acetaldehyde-induced changes in monoamine and amino acid extracellular microdialysate content of the nucleus accumbens. *Neuropharmacology* 36, 225–232. doi: 10.1016/S0028-3908(97)00007-5

Xie, G., Hipólito, L., Zuo, W., Polache, A., Granero, A., Krnjevic, K., et al. (2012). Salsolinol stimulates dopamine neurons in slices of posterior ventral tegmental area indirectly by activating μ-opioid receptors. *J. Pharmacol. Exp. Ther.* 341, 43–50. doi: 10.1124/jpet.111.186833

Xie, G., and Ye, J. H. (2012). Salsolinol facilitates glutamatergic transmission to dopamine neurons in the posterior ventral tegmental area of rats. *PLoS ONE* 7:e36716. doi: 10.1371/journal.pone.0036716

Yamanaka, Y., Walsh, M. J., and Davis, V. E. (1970). Salsolinol, an alkaloid derivative of dopamine formed *in vitro* during alcohol metabolism. *Nature* 227, 1143–1144. doi: 10.1038/2271143a0

York, J. L. (1981). The ethanol stimulus in rats with differing ethanol preferences. *Psychopharmacology* 74, 339–343. doi: 10.1007/BF00432743

Zimatkin, S. M., Liopo, A. V., and Deitrich, R. A. (1998). Distribution and kinetics of ethanol metabolism in rat brain. *Alcohol. Clin. Exp. Res.* 22, 1623–1627. doi: 10.1111/j.1530-0277.1998.tb03958.x

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 30 April 2013; paper pending published: 01 June 2013; accepted: 27 June 2013; published online: 15 July 2013.

Citation: Peana AT and Acquas E (2013) Behavioral and biochemical evidence of the role of acetaldehyde in the motivational effects of ethanol. Front. Behav. Neurosci. 7:86. doi: 10.3389/fnbeh.2013.00086

frontiers in
BEHAVIORAL NEUROSCIENCE

MINI REVIEW ARTICLE
published: 17 July 2013
doi: 10.3389/fnbeh.2013.00087

Ethanol-derived acetaldehyde: pleasure and pain of alcohol mechanism of action

Giulia Muggironi, Giulia R. Fois and Marco Diana*

Department of Chemistry and Pharmacy, 'G. Minardi' Laboratory of Cognitive Neuroscience, University of Sassari, Sassari, Italy

Edited by:
Merce Correa, Universitat Jaume I, Spain

Reviewed by:
John D. Salamone, University of Connecticut, USA
Laura F. Hurtado, Universitat Jaume I, Spain

****Correspondence:***
Marco Diana, Department of Chemistry and Pharmacy, 'G. Minardi' Laboratory of Cognitive Neuroscience, University of Sassary, Via Muroni, 23, 07100 Sassari, Italy
e-mail: dsfdiana@uniss.it

Acetaldehyde (ACD), the first metabolite of ethanol (EtOH), has been implicated in several actions of alcohol, including its reinforcing effects. Previously considered an aversive compound, ACD was useful in alcoholic's pharmacological treatment aimed at discouraging alcohol drinking. However, it has recently been shown that EtOH-derived ACD is necessary for EtOH-induced place preference and self-administration, thereby suggesting a possible involvement of ACD in EtOH motivational properties. In addition, EtOH-stimulating properties on DA neurons are prevented by pharmacological blockade of local catalase H_2O_2 system, the main metabolic step for biotransformation of EtOH into ACD within the central nervous system. It was further shown that pretreatment with thiol compounds, like L-Cysteine or D-Penicillamine, reduced EtOH and ACD-induced motivational effects, in fact preventing self-administration of both EtOH and ACD, thus suggesting a possible role for ACD as a biomarker useful in evaluating potential innovative treatments of alcohol abuse. These findings suggest a key role of ACD in the EtOH reinforcing effects. In the present paper we review the role of EtOH-derived ACD in the reinforcing effects of EtOH and the possibility that ACD may serve as a therapeutically targetable biomarker in the search for novel treatments in alcohol abuse and alcoholism.

Keywords: ethanol, acetaldehyde, ethanol metabolism, catalase, biomarkers, pharmacological

INTRODUCTION

A recurring emergent theory in the alcohol field is that the reinforcing properties of alcohol are not produced by the ethanol (EtOH) molecule itself, but may depend upon the action of EtOH metabolites/products within the central nervous system (CNS) (Deitrich, 2004; Quertemont et al., 2005c; Correa et al., 2012).

This stance proposes EtOH as a pro-drug, and metabolism of EtOH to acetaldehyde (ACD) within the CNS could mediate most, if not all, of the CNS effects of EtOH (Quertemont et al., 2005a). The reinforcing properties of alcohol are most likely generated through a complex series of peripheral and central effects of both alcohol and its metabolites. Therefore a better understanding for how the metabolites/products of alcohol contribute to the reinforcing properties of alcohol is important for the development of efficacious pharmacotherapies for alcohol abuse and alcoholism.

BRIEF HISTORY OF ACD AND ALCOHOLISM

ACD, in EtOH addiction, has been classically considered as an aversive EtOH by-product useful in the pharmacological therapy of alcoholics (Diana et al., 2003; Suh et al., 2006).

Elevation of ACD peripheral blood levels, after disulfiram administration causes a number of typical effects, known as flushing syndrome (Suh et al., 2006) including anxiogenic effects and endocrine stress responses (Escrig et al., 2012). Over the last decades, several lines of research have described the aversive effects of alcohol following the ingestion of compounds which significantly affected alcohol-related behaviors in both preclinical as well as clinical populations. Hald and Jacobsen (1948), reported similar aversive symptoms (redness of the face, increased heart rate, sleepiness, etc.) following treatment with tetraethylthiuramdisulfide and alcohol consumption, thereby suggesting that blocking Aldehyde Dehydrogenase (ALDH) resulted in a sharp increase in blood levels of ACD, which in turn, produced an increase in the aversive side effects of drinking. Tetraethylthiuramdisulfide has since been given the name disulfiram (marketed as antabuse) and was the first compound approved for the treatment of alcoholism by the US FDA.

The primary pharmacological action of disulfiram involves the enzyme ALDH that is responsible for converting ACD to acetate in metabolizing EtOH. Disulfiram inhibits ALDH and thus increases the concentration of ACD. Most of the adverse effects that characterize alcohol sensitivity were attenuated efficiently by 4-methylpyrazole (4-MP), ADH inhibitor, following ALDH inhibition by both disulfiram and cyanamide (Stowell et al., 1980; Kupari et al., 1983).

In sharp contrast with this notion, Chevens (1953) observed that patients don't have aversive effects by taking low doses of EtOH when under disulfiram treatment, and it has also been reported that ALDH inhibitors may potentiate the euphoric and pleasurable effects of low doses of EtOH (Brown et al., 1983).

Given the clinical implications of the early antabuse studies, several theories emerged associating alcoholism and ACD (Carpenter and MacLeod, 1952; Myers and Veale, 1969;

Davis et al., 1970; Walsh et al., 1970; Griffiths et al., 1974). The most strident theories suggested that ACD was responsible for all the effects associated with alcohol and that alcoholism would be more appropriately termed acetaldehydism (Walsh et al., 1970; Raskin, 1975).

ETOH METABOLISM

EtOH is first metabolized into ACD through several enzymatic and nonenzymatic mechanisms, the main enzymatic pathways being alcohol dehydrogenase (ADH), cytochrome *P*4502E1 (CYP2E1) and catalase H_2O_2 system. In the periphery, ACD is formed from EtOH through the action of ADH primarily in the liver. In the brain, ADH is inactive (Zimatkin et al., 1998), and formation of ACD from EtOH is achieved primarily through the action of another enzyme, catalase H_2O_2 system (Sippel, 1974; Zimatkin, 1991).

A prerequisite for the involvement of ACD in EtOH behavioral effects is the occurrence of pharmacologically significant levels of ACD in the brain after alcohol consumption.

The levels of ACD in the CNS have profound effects in mediating the reinforcing actions of EtOH. ACD derived from the peripheral metabolism of EtOH penetrates from blood to brain with difficulty because of the metabolic barrier presented by ALDH across the Blood-Brain Barrier (BBB) (Eriksson and Sippel, 1977; Deitrich, 1987; Zimatkin, 1991; Hunt, 1996; Quertemont and Tambour, 2004). In addition, in the liver ALDH rapidly converts ACD into acetate and very low levels of ACD are detected in blood after the administration of moderate doses of EtOH (Quertemont and Tambour, 2004). Further research indicated that high levels of peripherally administered ACD results in detection of ACD in the brain within minutes (Ward et al., 1997). Therefore, peripheral ACD may over saturate the peripheral ALDH, allowing some percentage of ACD to enter the brain (Quertemont et al., 2005b). However, this mechanism does not provide an absolute protection of the brain because high blood concentrations allow ACD to cross the BBB. Additional local metabolic pathways (e.g., CYP2E1) can also result in the formation of ACD from EtOH within the brain (Zakhari, 2006) and pharmacologically significant amounts of ACD can be generated *in situ* thereby producing effects that are difficult to ascribe to peripheral mechanisms.

A plausible source of ACD in the brain is the *in situ* synthesis from some of the EtOH that escapes peripheral metabolism. ACD can be formed in the brain through the peroxidatic activity of catalase H_2O_2 system and by oxidation via other oxidizing enzymes such as CYP2E1.

Indeed, production of ACD during EtOH oxidation *in situ* was found and confirmed in several laboratories (Aragon et al., 1992; Gill et al., 1992; Hamby-Mason et al., 1997; Zimatkin et al., 1998; Person et al., 2000). Although ADH is not expressed in the brain (Zimatkin and Buben, 2006; Deitrich, 2011), ACD can nevertheless be generated by the action of catalase H_2O_2 system and to a minor extent by CYP2E1, both enzymes present in the brain (Aragon and Amit, 1992; Zimatkin et al., 2006; Deitrich, 2011). In vitro studies indicate that catalase H_2O_2 system generates 60 to 70% of brain ACD while CYP2E1 some 15 to 20% (Zimatkin et al., 2006).

In a study in mice, Correa et al. found that when catalase H_2O_2 system-mediated metabolism of EtOH into ACD is blocked (Correa et al., 2008) there is a suppressive effect of the anxiolytic actions of EtOH (Correa et al., 2008), suggesting that centrally formed ACD contributes to the anxiolytic effects of EtOH. Additionally, it has been reported that when catalase H_2O_2 system activity is pharmacologically reduced, via 3-aminotriazole (3-AT), rats reduce their intake and preference for EtOH (Koechling and Amit, 1994), a decreased voluntary EtOH intake in UChB rats is observed (Tampier et al., 1995) and EtOH-induced conditioned place preference (CPP) in mice (Font et al., 2008) is blocked. Furthermore the presence of 3-AT induced a concentration-dependent reduction of the amount of ACD generated after incubation. Homogenates of perfused brains of rats treated with AT or cyanamide (another H_2O_2-dependent catalase blocker) also showed a dose-dependent reduction of ACD (Aragon and Amit, 1992).

Recently, Karahanian et al. (2011) developed lentiviral vectors that coded for an shRNA designed to inhibit the synthesis of catalase H_2O_2 system. The single stereotaxic administration of an anticatalase-lentiviral vector into the ventral tegmental area (VTA), which reduced catalase H_2O_2 system levels by 70 to 80% (Quintanilla et al., 2012; Tampier et al., 2013), virtually abolished the voluntary EtOH consumption (up to 95%) by UChB rats. The lentiviral anticatalase shRNA administration also abolished the increases in dopamine release in nucleus accumbens (Acb) induced by the acute administration of EtOH. These effects strongly support a role of catalase H_2O_2 system and thus ACD in the central metabolism and in the motivational properties of EtOH.

REINFORCING PROPERTIES OF ACD

ACD itself possesses reinforcing properties, which suggests that some of the behavioral pharmacological effects attributed to EtOH may be a result of the formation of ACD, supporting the involvement of ACD in EtOH addiction (Brown et al., 1979). On this account, the positive reinforcing properties generally attributed to EtOH may in fact be mediated centrally by its metabolite. ACD, *per se*, would then be responsible for many biological effects which are not clearly distinguishable from those of EtOH (Quertemont et al., 2005c; Font et al., 2006a,b; Peana et al., 2008, 2009, 2010b; Correa et al., 2012).

ACD induces CPP in rats after intracerebroventricular administration (Smith et al., 1984), is self-administered directly into the cerebral ventricles (Brown et al., 1979) and into the (VTA) (McBride et al., 2002) whereas Rodd-Henricks et al., (2002) reported ACD self-administration into VTA in alcohol-preferring rats.

Further ACD induces positive motivational effects not only by central administration but also when administered peripherally. In fact, studies have shown that ACD induces CPP in rats after intraperitoneal administration (Quertemont and De Witte, 2001) and rats self-administer ACD intravenously (Myers et al., 1984; Takayama and Uyeno, 1985). Importantly, ACD induces CPP after intragastric administration (Peana et al., 2008), and is orally self-administered (Peana et al., 2010b) thereby mimicking the commonly employed route of administration of alcoholic

beverages in humans. Further, ACD induces conditioned stimulus preference (Quertemont and De Witte, 2001), and directly enhances the activity of putative dopamine (DA) neurons in the rat VTA in vivo (Foddai et al., 2004). In addition, blockade of alcohol dehydrogenase with 4-MP prevents EtOH-induced CPP, oral EtOH self-administration and stimulation of the mesolimbic DA system (Foddai et al., 2004; Melis et al., 2007; Peana et al., 2008). As 4-MP administration mainly prevents peripheral ACD formation, thereby reducing ACD available to penetrate the brain (Isse et al., 2005), and provokes a consequent increase in blood EtOH levels (Waller et al., 1982), it is possible that the lack of EtOH-induced CPP could be ascribed to high blood EtOH concentrations (Melis et al., 2007). However, reduction of pharmacologically active ACD, by administration of the ACD-sequestering agent D-penicillamine (DP), which does not increase blood EtOH concentrations, also prevents spontaneous EtOH drinking and strongly sustain the hypothesis that some of the behavioral (Font et al., 2006b) and rewarding (Font et al., 2006a) effects of EtOH are mediated by ACD.

ACD ACTIONS IN THE VTA

Most abused drugs, including EtOH, stimulate the release of DA in several limbic regions (Di Chiara, 2002). Therefore, the reinforcing properties of ACD may be mediated by increasing the release of DA in terminal areas.

Through utilization of the intracranial self-administration (ICSA) paradigm, Rodd-Henricks et al. (2002) established that rats will readily self-administer ACD directly into the posterior ventral tegmental area (pVTA) at concentrations that were 1000-fold lower than that for EtOH (Rodd-Henricks et al., 2002; Rodd et al., 2005, 2008). It appears that the pVTA is significantly more sensitive to the reinforcing properties of ACD compared to EtOH. Alcohol preferring rats display the highest levels of ICSA for ACD doses that are approximately 2,000-fold lower than the optimal dose of EtOH (Rodd-Henricks et al., 2002; Rodd et al., 2005, 2008). Responding/infusion data from the ICSA experiments exhibit an inverted "U-shaped" dose–response curve for ACD, in which lower and higher doses do not produce reliable responding (Rodd-Henricks et al., 2002; Rodd et al., 2005, 2008), suggesting that the reinforcing effects of ACD within the pVTA appears to involve activation of DA neurons (Rodd et al., 2005, 2008). In line with this, Melis et al. (2007) found that ACD is essential for EtOH-increased microdialysate DA levels in the Nucleus Accumben shell (AcbSh) and that this effect is mimicked by intra-VTA ACD administration that produced an increase in DA release in the AcbSh to 150% that of baseline.

ACD has excitatory actions on neurons of the VTA as clearly demonstrated by the effects on DA release and on the firing frequency of individual VTA neurons. In experiments using in vivo recording methods, ACD was injected intravenously, and a dose-dependent increase in firing of dopaminergic VTA neurons was reported (Foddai et al., 2004). Thus, ACD parallels the effects observed with EtOH, but at 50 times lower concentrations. The effects of EtOH on VTA neuronal activity were blocked by systemic pretreatment with the ADH inhibitor 4-MP, but this drug had no effect on ACD induced excitation (Foddai et al., 2004), suggesting that the excitatory effects of EtOH on the VTA are mediated by ACD. Sequestration of ACD in vivo by administration of DP is sufficient to block the effects of intragastrically administered EtOH or ACD (Enrico et al., 2009). These key results indicate that ACD-induced activation of dopaminergic VTA neurons mimics EtOH-induced excitation (Diana et al., 2008), and is produced at much lower concentrations compared to EtOH (Brodie et al., 1990; Brodie and Appel, 1998). Furthermore, EtOH applied in the presence of a catalase H_2O_2 system inhibitor, 3-AT, failed to produce its characteristic excitation of the VTA. Further, in exploring the mechanism of ACD excitation of VTA neurons, Melis et al. (2007) examined the effect of ACD on two ion currents, A-current and h-current. An A-current represents a rapidly-inactivating potassium current that contributes to spike after hyperpolarization and is involved in the regulation of firing frequency of dopaminergic VTA neurons (Koyama and Appel, 2006). The authors noted a rightward voltage shift produced by ACD on I_A (Melis et al., 2007). Also noted was a significant increase in h-current produced by acutely applied ACD; this is reminiscent of the effect of EtOH, which has been shown to acutely increase I_h of VTA neurons (Brodie and Appel, 1998; Okamoto et al., 2006). The most parsimonious explanation suggests that EtOH is metabolized to ACD by local catalase H_2O_2 system in the VTA, and the authors of these studies suggest that, in general, EtOH actions on the VTA are mediated by ACD (Deehan et al., 2013a,b).

Overall, it seems most likely that ACD is a crucial component of the overall effects of EtOH on dopaminergic neurons of the VTA; the essential action of ACD could be parallel to EtOH, or it could enhance EtOH-induced changes. Blockade of the formation of ACD can reduce the response of dopaminergic VTA neurons to EtOH, and could serve as a platform for the development of agents that reduce the rewarding and reinforcing actions of EtOH.

ACD AS A BIOMARKER

The results reviewed above suggest that enzymatic manipulations of EtOH metabolism would diminish its rewarding properties, possibly discouraging drinking (**Figure 1**). There could be several mechanisms by which reduction of ACD levels could reduce alcohol intake. For example, advantage can be obtained by exploiting the ACD-chelating properties of thiol compounds (Nagasawa et al., 1980). Indeed, administration of the ACD-sequestering agent DP, reduces voluntary EtOH consumption, ACD motivational properties (Font et al., 2005, 2006b) and free-choice EtOH drinking behavior in mice (Font et al., 2006a), acting centrally to reduce EtOH-derived acetaldehyde (Font et al., 2005; Serrano et al., 2007). Further, L-cysteine, prevented EtOH and ACD-induced conditioned place preference (Peana et al., 2009), reduced oral EtOH and ACD self-administration (Peana et al., 2010a, 2012), and blunted both EtOH and ACD-induced stimulation of DA release in the AcbSh (Sirca et al., 2011).

In addition, modulation of catalase H_2O_2 system by enzymatic inhibition (Melis et al., 2007), or H_2O_2 scavenging may reduce ACD formation in the CNS and the motivational properties of EtOH (Ledesma et al., 2012; Ledesma and Aragon, 2012, 2013). Since the enzyme catalase takes H_2O_2, as a co-substrate

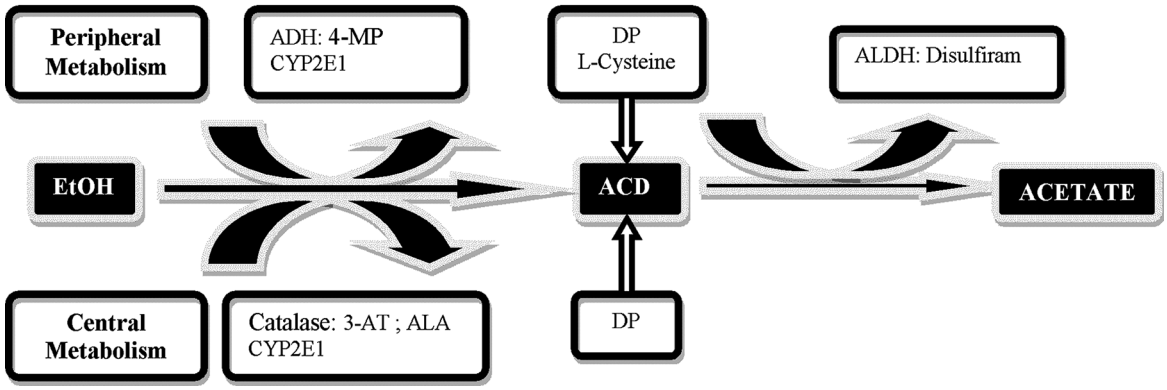


FIGURE 1 | Schematic representation of central and peripheral ethanol (EtOH) metabolic pathways and possible pharmacological targets. EtOH is metabolized into acetaldehyde (ACD) through several pathways, the main enzyme being alcohol dehydrogenase (ADH), inhibited by 4-methylpyrazole (4-MP); CYP2E1 and catalase H_2O_2 system inactivated by 3-aminotriazole (3-AT) an enzymatic inhibitor and by alpha lipoic acid (ALA) a radical scavenger for H_2O_2. ACD is subsequently oxidized into acetate by aldehyde dehydrogenase (ALDH) inhibited by disulfiram. An additional strategy is represented by sequestration agents of ACD, D-Penicillamine (DP) and L-Cysteine.

to form compound I, the central production of ACD derived from the metabolism of EtOH in the brain (Cohen et al., 1980; Sinet et al., 1980), may be affected by pharmacological manipulation of this system. Accordingly, pretreatment with alpha lipoic acid, scavenger of H_2O_2, reduces the acquisition and reconditioning of ethanol-induced CPP in mice (Ledesma and Aragon, 2013) and reduces EtOH self administration in rats (Peana et al., 2013).

These considerations suggest further experiments to probe the use of these molecules as potential experimental therapies and could help in devising new effective pharmacologic treatments in alcoholism.

CONCLUSIONS

It is hypothesized that many neuropharmacological, neurochemical, neurotoxic, and behavioral effects of EtOH are mediated by the first metabolite of EtOH, ACD (Hunt, 1996; Deitrich, 2004; Quertemont and Tambour, 2004; Quertemont et al., 2005b,c; Zimatkin et al., 2006). In addition, the present observations suggest that the positive motivational properties following EtOH administration, and the EtOH-induced enhancement of DAergic transmission, require EtOH's first metabolite, ACD.

The important distinction between the central and the peripheral effects of ACD lays the groundwork for considering that many of the central effects of EtOH could in fact be dependent on the actions of its first metabolite, ACD. Although peripherally accumulated ACD is a potential toxic and deterrent substance, high levels of this substance can reach the brain and generate positive effects that can promote later consumption.

At last, targeting ACD, instead of EtOH, may offer new potential biomarkers in the search for novel compounds to reduce excessive alcohol intake, abuse and ultimately alcoholism. In general, targeting drug metabolism may reveal new ways to treat addictive disorders not limited to alcohol abuse but possibly useful in other addictions such as tobacco (Pianezza et al., 1998), heroin and cocaine dependence (Kreek et al., 2005) and other chemical dependencies (reviewed in Bough et al. (2013) and references therein).

REFERENCES

Aragon, C. M., and Amit, Z. (1992). The effect of 3-amino-1,2,4-triazole on voluntary ethanol consumption: evidence for brain catalase involvement in the mechanism of action. *Neuropharmacology* 31, 709–712. doi: 10.1016/0028-3908(92)90150-N

Aragon, C. M., Rogan, F., and Amit, Z. (1992). Ethanol metabolism in rat brain homogenates by a catalase-H_2O_2 system. *Biochem. Pharmacol.* 44, 93–98. doi: 10.1016/0006-2952(92)90042-H

Bough, K. J., Lerman, C., Rose, J. E., McClernon, F. J., Kenny, P. J., Tyndale, R. F., et al. (2013). Biomarkers for smoking cessation. *Clin. Pharmacol. Ther.* 93, 526–538. doi: 10.1038/clpt.2013.57

Brodie, M. S., and Appel, S. B. (1998). The effects of ethanol on dopaminergic neurons of the ventral tegmental area studied with intracellular recording in brain slices. *Alcohol. Clin. Exp. Res.* 22, 236–244. doi: 10.1111/j.1530-0277.1998.tb03644.x

Brodie, M. S., Shefner, S. A., and Dunwiddie, T. V. (1990). Ethanol increases the firing rate of dopamine neurons of the rat ventral tegmental area in vitro. *Brain Res.* 508, 65–69. doi: 10.1016/0006-8993(90)91118-Z

Brown, Z. W., Amit, Z., and Rockman, G. E. (1979). Intraventricular self-administration of acetaldehyde, but not ethanol, in naive laboratory rats. *Psychopharmacology (Berl)* 64, 271–276. doi: 10.1007/BF00427509

Brown, Z. W., Amit, Z., Smith, B. R., Sutherland, E. A., and Selvaggi, N. (1983). Alcohol-induced euphoria enhanced by disulfiram and calcium carbimide. *Alcohol. Clin. Exp. Res.* 7, 276–278. doi: 10.1111/j.1530-0277.1983.tb05459.x

Carpenter, R. K., and MacLeod, L. D. (1952). The effects of ethyl alcohol and acetaldehyde on maze behaviour and motor co-ordination in rats. *J. Ment. Sci.* 98, 167–173. doi: 10.1192/bjp.98.410.167

Chevens, L. C. (1953). Antabuse addiction. *Br. Med. J.* 1, 1450–1451. doi: 10.1136/bmj.1.4825.1450-c

Cohen, G., Sinet, P. M., and Heikkila, R. (1980). Ethanol oxidation by rat brain in vivo. *Alcohol. Clin. Exp. Res.* 4, 366–370. doi: 10.1111/j.1530-0277.1980.tb04833.x

Correa, M., Manrique, H. M., Font, L., Escrig, M. A., and Aragon, C. M. (2008). Reduction in the anxiolytic effects of ethanol by centrally formed acetaldehyde: the role of catalase inhibitors and acetaldehyde-sequestering agents. *Psychopharmacology (Berl)* 200, 455–464. doi: 10.1007/s00213-008-1219-3

Correa, M., Salamone, J. D., Segovia, K. N., Pardo, M., Longoni, R., Spina, L., et al. (2012). Piecing together the puzzle of acetaldehyde as a neuroactive agent. *Neurosci. Biobehav. Rev.* 36, 404–430. doi: 10.1016/j.neubiorev.2011.07.009

Davis, V. E., Walsh, M. J., and Yamanaka, Y. (1970). Augmentation of alkaloid formation from dopamine by alcohol and acetaldehyde in vitro. *J. Pharmacol. Exp. Ther.* 174, 401–412.

Deehan, G. A., Brodie, M. S., and Rodd, Z. A. (2013a). What is in that drink: the biological actions of ethanol, acetaldehyde, and salsolinol. *Curr. Top. Behav. Neurosci.* 13, 163–184. doi: 10.1007/7854_2011_198

Deehan, G. A., Engleman, E. A., Ding, Z. M., McBride, W. J., and Rodd, Z. A. (2013b). Microinjections of acetaldehyde or salsolinol into the posterior ventral tegmental area increase dopamine release in the nucleus accumbens shell. *Alcohol. Clin. Exp. Res.* 37, 722–729. doi: 10.1111/acer.12034

Deitrich, R. (2011). Ethanol as a prodrug: brain metabolism of ethanol mediates its reinforcing effects–a commentary. *Alcohol. Clin. Exp. Res.* 35, 581–583. doi: 10.1111/j.1530-0277.2011.01454.x

Deitrich, R. A. (1987). Specificity of the action of ethanol in the central nervous system: behavioral effects. *Alcohol Alcohol. Suppl.* 1, 133–138.

Deitrich, R. A. (2004). Acetaldehyde: déjà vu du jour. *J. Stud. Alcohol.* 65, 557–572.

Di Chiara, G. (2002). Nucleus accumbens shell and core dopamine: differential role in behavior and addiction. *Behav. Brain Res.* 137, 75–114. doi: 10.1016/S0166-4328(02)00286-3

Diana, M., Brodie, M., Muntoni, A., Puddu, M. C., Pillolla, G., Steffensen, S., et al. (2003). Enduring effects of chronic ethanol in the CNS: basis for alcoholism. *Alcohol. Clin. Exp. Res.* 27, 354–361. doi: 10.1097/01.ALC.0000057121.36127.19

Diana, M., Peana, A. T., Sirca, D., Lintas, A., Melis, M., and Enrico, P. (2008). Crucial role of acetaldehyde in alcohol activation of the mesolimbic dopamine system. *Ann. NY Acad. Sci.* 1139, 307–317. doi: 10.1196/annals.1432.009

Enrico, P., Sirca, D., Mereu, M., Peana, A. T., Lintas, A., Golosio, A., et al. (2009). Acetaldehyde sequestering prevents ethanol-induced stimulation of mesolimbic dopamine transmission. *Drug Alcohol Depend.* 100, 265–271. doi: 10.1016/j.drugalcdep.2008.10.010

Eriksson, C. J., and Sippel, H. W. (1977). The distribution and metabolism of acetaldehyde in rats during ethanol oxidation-I. The distribution of acetaldehyde in liver, brain, blood and breath. *Biochem. Pharmacol.* 26, 241–247. doi: 10.1016/0006-2952(77)90310-0

Escrig, M. A., Pardo, M., Aragon, C. M., and Correa, M. (2012). Anxiogenic and stress-inducing effects of peripherally administered acetaldehyde in mice: similarities with the disulfiram-ethanol reaction. *Pharmacol. Biochem. Behav.* 100, 404–412. doi: 10.1016/j.pbb.2011.10.002

Foddai, M., Dosia, G., Spiga, S., and Diana, M. (2004). Acetaldehyde increases dopaminergic neuronal activity in the VTA. *Neuropsychopharmacology* 29, 530–536. doi: 10.1038/sj.npp.1300326

Font, L., Aragon, C. M., and Miquel, M. (2006a). Ethanol-induced conditioned place preference, but not aversion, is blocked by treatment with D-penicillamine, an inactivation agent for acetaldehyde. *Psychopharmacology (Berl)* 184, 56–64. doi: 10.1007/s00213-005-0224-z

Font, L., Aragon, C. M., and Miquel, M. (2006b). Voluntary ethanol consumption decreases after the inactivation of central acetaldehyde by d-penicillamine. *Behav. Brain Res.* 171, 78–86. doi: 10.1016/j.bbr.2006.03.020

Font, L., Miquel, M., and Aragon, C. M. (2005). Prevention of ethanol-induced behavioral stimulation by D-penicillamine: a sequestration agent for acetaldehyde. *Alcohol. Clin. Exp. Res.* 29, 1156–1164. doi: 10.1097/01.ALC.0000171945.30494.AF

Font, L., Miquel, M., and Aragon, C. M. (2008). Involvement of brain catalase activity in the acquisition of ethanol-induced conditioned place preference. *Physiol. Behav.* 93, 733–741. doi: 10.1016/j.physbeh.2007.11.026

Gill, K., Menez, J. F., Lucas, D., and Deitrich, R. A. (1992). Enzymatic production of acetaldehyde from ethanol in rat brain tissue. *Alcohol. Clin. Exp. Res.* 16, 910–915. doi: 10.1111/j.1530-0277.1992.tb01892.x

Griffiths, R., Bigelow, G., and Liebson, I. (1974). Suppression of ethanol self-administration in alcoholics by contingent time-out from social interactions. *Behav. Res. Ther.* 12, 327–334. doi: 10.1016/0005-7967(74)90007-2

Hald, J., and Jacobsen, E. (1948). A drug sensitizing the organism to ethyl alcohol. *Lancet* 2, 1001–1004. doi: 10.1016/s0140-6736(48)91514-1

Hamby-Mason, R., Chen, J. J., Schenker, S., Perez, A., and Henderson, G. I. (1997). Catalase mediates acetaldehyde formation from ethanol in fetal and neonatal rat brain. *Alcohol. Clin. Exp. Res.* 21, 1063–1072. doi: 10.1111/j.1530-0277.1997.tb04255.x

Hunt, W. A. (1996). Role of acetaldehyde in the actions of ethanol on the brain–a review. *Alcohol* 13, 147–151. doi: 10.1016/0741-8329(95)02026-8

Isse, T., Matsuno, K., Oyama, T., Kitagawa, K., and Kawamoto, T. (2005). Aldehyde dehydrogenase 2 gene targeting mouse lacking enzyme activity shows high acetaldehyde level in blood, brain, and liver after ethanol gavages. *Alcohol. Clin. Exp. Res.* 29, 1959–1964. doi: 10.1097/01.alc.0000187161.07820.21

Karahanian, E., Quintanilla, M. E., Tampier, L., Rivera-Meza, M., Bustamante, D., Gonzalez-Lira, V., et al. (2011). Ethanol as a prodrug: brain metabolism of ethanol mediates its reinforcing effects. *Alcohol. Clin. Exp. Res.* 35, 606–612. doi: 10.1111/j.1530-0277.2011.01439.x

Koechling, U. M., and Amit, Z. (1994). Effects of 3-amino-1,2,4-triazole on brain catalase in the mediation of ethanol consumption in mice. *Alcohol* 11, 235–239. doi: 10.1016/0741-8329(94)90036-1

Koyama, S., and Appel, S. B. (2006). A-type K+ current of dopamine and GABA neurons in the ventral tegmental area. *J. Neurophysiol.* 96, 544–554.

Kreek, M. J., Nielsen, D. A., Butelman, E. R., and LaForge, K. S. (2005). Genetic influences on impulsivity, risk taking, stress responsivity and vulnerability to drug abuse and addiction. *Nat. Neurosci.* 8, 1450–7. doi: 10.1038/nn1583

Kupari, M., Lindros, K., Hillbom, M., Heikkilä, J., and Ylikahri, R. (1983). Cardiovascular effects of acetaldehyde accumulation after ethanol ingestion: their modification by beta-adrenergic blockade and alcohol dehydrogenase inhibition. *Alcohol. Clin. Exp. Res.* 7, 283–288. doi: 10.1111/j.1530-0277.1983.tb05461.x

Ledesma, J. C., and Aragon, C. M. (2012). α-Lipoic acid, a scavenging agent for H_2O_2, reduces ethanol-stimulated locomotion in mice. *Psychopharmacology (Berl)* 219, 171–180. doi: 10.1007/s00213-011-2407-0

Ledesma, J. C., and Aragon, C. M. (2013). Acquisition and reconditioning of ethanol-induced conditioned place preference in mice is blocked by the H_2O_2 scavenger alpha lipoic acid. *Psychopharmacology (Berl)* 226, 673–685. doi: 10.1007/s00213-012-2831-9

Ledesma, J. C., Font, L., and Aragon, C. M. (2012). The H_2O_2 scavenger ebselen decreases ethanol-induced locomotor stimulation in mice. *Drug Alcohol Depend.* 124, 42–49. doi: 10.1016/j.drugalcdep.2011.12.003

McBride, W. J., Li, T. K., Deitrich, R. A., Zimatkin, S., Smith, B. R., and Rodd-Henricks, Z. A. (2002). Involvement of acetaldehyde in alcohol addiction. *Alcohol. Clin. Exp. Res.* 26, 114–119. doi: 10.1111/j.1530-0277.2002.tb02439.x

Melis, M., Enrico, P., Peana, A. T., and Diana, M. (2007). Acetaldehyde mediates alcohol activation of the mesolimbic dopamine system. *Eur. J. Neurosci.* 26, 2824–2833. doi: 10.1111/j.1460-9568.2007.05887.x

Myers, R. D., and Veale, W. L. (1969). Alterations in volitional alcohol intake produced in rats by chronic intraventricular infusions of acetaldehyde, paraldehyde or methanol. *Arch. Int. Pharmacodyn. Ther.* 180, 100–113.

Myers, W., Ng, K., and Singer, G. (1984). Ethanol preference in rats with a prior history of acetaldehyde self-administration. *Experientia* 40, 1008–1010. doi: 10.1007/BF01946483

Nagasawa, H. T., Elberling, J. A., and DeMaster, E. G. (1980). Structural requirements for the sequestration of metabolically generated acetaldehyde. *J. Med. Chem.* 23, 140–143. doi: 10.1021/jm00176a007

Okamoto, T., Harnett, M. T., and Morikawa, H. (2006). Hyperpolarization-activated cation current (Ih) is an ethanol target in midbrain dopamine neurons of mice. *J. Neurophysiol.* 95, 619–626. doi: 10.1152/jn.00682.2005

Peana, A. T., Assaretti, A. R., Muggironi, G., Enrico, P., and Diana, M. (2009). Reduction of ethanol-derived acetaldehyde induced motivational properties by L-cysteine. *Alcohol. Clin. Exp. Res.* 33, 43–48. doi: 10.1111/j.1530-0277.2008.00809.x

Peana, A. T., Enrico, P., Assaretti, A. R., Pulighe, E., Muggironi, G., Nieddu, M., et al. (2008). Key role of ethanol-derived acetaldehyde in the motivational properties induced by intragastric ethanol: a conditioned place preference study in the rat. *Alcohol. Clin. Exp. Res.* 32, 249–258. doi: 10.1111/j.1530-0277.2007.00574.x

Peana, A. T., Muggironi, G., Calvisi, G., Enrico, P., Mereu, M., Nieddu, M., et al. (2010a). l-Cysteine reduces oral ethanol self-administration and reinstatement of ethanol-drinking behavior in rats. *Pharmacol. Biochem. Behav.* 94, 431–437. doi: 10.1016/j.pbb.2009.10.005

Peana, A. T., Muggironi, G., and Diana, M. (2010b). Acetaldehyde-reinforcing effects: a study on oral self-administration behavior. *Front. Psychiatry* 1, 23. doi: 10.3389/fpsyt.2010.00023

Peana, A. T., Muggironi, G., Fois, G. R., and Diana, M. (2013). Alpha-Lipoic Acid Reduces Ethanol Self-Administration in Rats. *Alcohol. Clin. Exp. Res.* doi: 10.1111/acer.12169. [Epub ahead of print]

Peana, A. T., Muggironi, G., Fois, G. R., Zinellu, M., Sirca, D., and Diana, M. (2012). Effect of (L)-cysteine on acetaldehyde self-administration. *Alcohol* 46, 489–497. doi: 10.1016/j.alcohol.2011.10.004

Person, R. E., Chen, H., Fantel, A. G., and Juchau, M. R. (2000). Enzymic catalysis of the accumulation of acetaldehyde from ethanol in human prenatal cephalic tissues: evaluation of the relative contributions of CYP2E1, alcohol dehydrogenase, and catalase/peroxidases. *Alcohol. Clin. Exp. Res.* 24, 1433–1442. doi: 10.1111/j.1530-0277.2000.tb02114.x

Pianezza, M. L., Sellers, E. M., and Tyndale, R. F. (1998). Nicotine metabolism defect reduces smoking. *Nature* 393, 750.

Quertemont, E., and De Witte, P. (2001). Conditioned stimulus preference after acetaldehyde but not ethanol injections. *Pharmacol. Biochem. Behav.* 68, 449–454. doi: 10.1016/S0091-3057(00)00486-X

Quertemont, E., Eriksson, C. J., Zimatkin, S. M., Pronko, P. S., Diana, M., Pisano, M., et al. (2005a). Is ethanol a pro-drug? Acetaldehyde contribution to brain ethanol effects. *Alcohol. Clin. Exp. Res.* 29, 1514–1521. doi: 10.1097/01.alc.0000175015.51329.45

Quertemont, E., Grant, K. A., Correa, M., Arizzi, M. N., Salamone, J. D., Tambour, S., et al. (2005b). The role of acetaldehyde in the central effects of ethanol. *Alcohol. Clin. Exp. Res.* 29, 221–234. doi: 10.1097/01.ALC.0000156185.39073.D2

Quertemont, E., Tambour, S., and Tirelli, E. (2005c). The role of acetaldehyde in the neurobehavioral effects of ethanol: a comprehensive review of animal studies. *Prog. Neurobiol.* 75, 247–274. doi: 10.1016/j.pneurobio.2005.03.003

Quertemont, E., and Tambour, S. (2004). Is ethanol a pro-drug? The role of acetaldehyde in the central effects of ethanol. *Trends Pharmacol Sci.* 25, 130–134. doi: 10.1016/j.tips.2004.01.001

Quintanilla, M. E., Tampier, L., Karahanian, E., Rivera-Meza, M., Herrera-Marschitz, M., and Israel, Y. (2012). Reward and relapse: complete gene-induced dissociation in an animal model of alcohol dependence. *Alcohol. Clin. Exp. Res.* 36, 517–522. doi: 10.1111/j.1530-0277.2011.01606.x

Raskin, N. H. (1975). Editorial: Alcoholism or acetaldehydism? *N. Engl. J. Med.* 292, 422–423. doi: 10.1056/NEJM197502202920811

Rodd, Z. A., Bell, R. L., McQueen, V. K., Davids, M. R., Hsu, C. C., Murphy, J. M., et al. (2005). Prolonged increase in the sensitivity of the posterior ventral tegmental area to the reinforcing effects of ethanol following repeated exposure to cycles of ethanol access and deprivation. *J. Pharmacol. Exp. Ther.* 315, 648–657. doi: 10.1124/jpet.105.084350

Rodd, Z. A., Oster, S. M., Ding, Z. M., Toalston, J. E., Deehan, G., Bell, R. L., et al. (2008). The reinforcing properties of salsolinol in the ventral tegmental area: evidence for regional heterogeneity and the involvement of serotonin and dopamine. *Alcohol. Clin. Exp. Res.* 32, 230–239. doi: 10.1111/j.1530-0277.2007.00572.x

Rodd-Henricks, Z. A., Melendez, R. I., Zaffaroni, A., Goldstein, A., McBride, W. J., and Li, T. K. (2002). The reinforcing effects of acetaldehyde in the posterior ventral tegmental area of alcohol-preferring rats. *Pharmacol. Biochem. Behav.* 72, 55–64. doi: 10.1016/S0091-3057(01)00733-X

Serrano, E., Pozo, O. J., Beltrán, J., Hernández, F., Font, L., Miquel, M., et al. (2007). Liquid chromatography/tandem mass spectrometry determination of (4S,2RS)-2,5,5-trimethylthiazolidine-4-carboxylic acid, a stable adduct formed between D-(-)-penicillamine and acetaldehyde (main biological metabolite of ethanol), in plasma, liver and brain rat tissues. *Rapid Commun. Mass Spectrom.* 21, 1221–1229.

Sinet, P. M., Heikkila, R. E., and Cohen, G. (1980). Hydrogen peroxide production by rat brain in vivo. *J. Neurochem.* 34, 1421–1428. doi: 10.1111/j.1471-4159.1980.tb11222.x

Sippel, H. W. (1974). The acetaldehyde content in rat brain during ethanol metabolism. *J. Neurochem.* 23, 451–452. doi: 10.1111/j.1471-4159.1974.tb04380.x

Sirca, D., Enrico, P., Mereu, M., Peana, A. T., and Diana, M. (2011). L-cysteine prevents ethanol-induced stimulation of mesolimbic dopamine transmission. *Alcohol. Clin. Exp. Res.* 35, 862–869. doi: 10.1111/j.1530-0277.2010.01416.x

Smith, B. R., Amit, Z., and Splawinsky, J. (1984). Conditioned place preference induced by intraventricular infusions of acetaldehyde. *Alcohol* 1, 193–195. doi: 10.1016/0741-8329(84)90097-1

Stowell, A. R., Lindros, K. O., and Salaspuro, M. P. (1980). Breath and blood acetaldehyde concentrations and their correlation during normal and calcium carbimide-modified ethanol oxidation in man. *Biochem. Pharmacol.* 29, 783–787. doi: 10.1016/0006-2952(80)90557-2

Suh, J. J., Pettinati, H. M., Kampman, K. M., and O'Brien, C. P. (2006). The status of disulfiram: a half of a century later. *J. Clin. Psychopharmacol.* 26, 290–302. doi: 10.1097/01.jcp.0000222512.25649.08

Takayama, S., and Uyeno, E. T. (1985). Intravenous self-administration of ethanol and acetaldehyde by rats. *Yakubutsu Seishin Kodo* 5, 329–334.

Tampier, L., Quintanilla, M. E., Karahanian, E., Rivera-Meza, M., Herrera-Marschitz, M., and Israel, Y. (2013). The alcohol deprivation effect: marked inhibition by anticatalase gene administration into the ventral tegmental area in rats. *Alcohol. Clin. Exp. Res.* doi: 10.1111/acer.12101. [Epub ahead of print]

Tampier, L., Quintanilla, M. E., and Mardones, J. (1995). Effects of aminotriazole on ethanol, water, and food intake and on brain catalase in UChA and UChB rats. *Alcohol* 12, 341–344. doi: 10.1016/0741-8329(95)00014-I

Waller, M. B., McBride, W. J., Lumeng, L., and Li, T. K. (1982). Effects of intravenous ethanol and of 4-methylpyrazole on alcohol drinking in alcohol-preferring rats. *Pharmacol. Biochem. Behav.* 17, 763–768. doi: 10.1016/0091-3057(82)90359-8

Walsh, M. J., Truitt, E. B., and Davis, V. E. (1970). Acetaldehyde mediation in the mechanism of ethanol-induced changes in norepinephrine metabolism. *Mol. Pharmacol.* 6, 416–424.

Ward, R. J., Colantuoni, C., Dahchour, A., Quertemont, E., and De Witte, P. (1997). Acetaldehyde-induced changes in monoamine and amino acid extracellular microdialysate content of the nucleus accumbens. *Neuropharmacology* 36, 225–232. doi: 10.1016/S0028-3908(97)00007-5

Zakhari, S. (2006). Overview: how is alcohol metabolized by the body? *Alcohol Res. Health* 29, 245–254.

Zimatkin, S. M. (1991). Histochemical study of aldehyde dehydrogenase in the rat CNS. *J. Neurochem.* 56, 1–11. doi: 10.1111/j.1471-4159.1991.tb02555.x

Zimatkin, S. M., and Buben, A. L. (2006). A novel method for evaluation of ethanol oxidation in living brain. *Bull. Exp. Biol. Med.* 142, 382–385. doi: 10.1007/s10517-006-0371-1

Zimatkin, S. M., Liopo, A. V., and Deitrich, R. A. (1998). Distribution and kinetics of ethanol metabolism in rat brain. *Alcohol. Clin. Exp. Res.* 22, 1623–1627. doi: 10.1111/j.1530-0277.1998.tb03958.x

Zimatkin, S. M., Pronko, S. P., Vasiliou, V., Gonzalez, F. J., and Deitrich, R. A. (2006). Enzymatic mechanisms of ethanol oxidation in the brain. *Alcohol. Clin. Exp. Res.* 30, 1500–1505. doi: 10.1111/j.1530-0277.2006.00181.x

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 02 May 2013; accepted: 29 Jun 2013; published online: 17 July 2013.
Citation: Muggironi G, Fois GR and Diana M (2013) Ethanol-derived acetaldehyde: pleasure and pain of alcohol mechanism of action. Front. Behav. Neurosci. 7:87. doi: 10.3389/fnbeh.2013.00087

frontiers in
BEHAVIORAL NEUROSCIENCE

REVIEW ARTICLE
published: 08 July 2013
doi: 10.3389/fnbeh.2013.00080

Gene specific modifications unravel ethanol and acetaldehyde actions

Yedy Israel[1,2]*, Mario Rivera-Meza[1], Eduardo Karahanian[3], María E. Quintanilla[1], Lutske Tampier[1], Paola Morales[1] and Mario Herrera-Marschitz[1]

[1] Faculty of Medicine, Molecular and Clinical Pharmacology Program, University of Chile, Santiago, Chile
[2] Department of Pharmacological and Toxicological Chemistry, University of Chile, Santiago, Chile
[3] Faculty of Medicine, Center for Biomedical Research, Diego Portales University, Santiago, Chile

Edited by:
Merce Correa, University Jaume I, Spain

Reviewed by:
Merce Correa, University Jaume I, Spain
Etienne Quertemont, University of Liege, Belgium

***Correspondence:**
Yedy Israel, Molecular and Clinical Pharmacology Program, Laboratory of Genetics of Alcoholism, Institute of Biomedical Sciences, University of Chile, Faculty of Medicine, Independencia 1027, Santiago RM 8380453, Chile
e-mail: yisrael@uchile.cl

Ethanol is metabolized into acetaldehyde mainly by the action of alcohol dehydrogenase in the liver, while mainly by the action of catalase in the brain. Aldehyde dehydrogenase-2 metabolizes acetaldehyde into acetate in both organs. Gene specific modifications reviewed here show that an increased liver generation of acetaldehyde (by transduction of a gene coding for a high-activity liver alcohol dehydrogenase ADH1*B2) leads to increased blood acetaldehyde levels and aversion to ethanol in animals. Similarly aversive is an increased acetaldehyde level resulting from the inhibition of liver aldehyde dehydrogenase-2 (ALDH2) synthesis (by an antisense coding gene against *aldh2* mRNA). The situation is diametrically different when acetaldehyde is generated in the brain. When the brain ventral tegmental area (VTA) is endowed with an increased ability to generate acetaldehyde (by transfection of liver rADH) the reinforcing effects of ethanol are increased, while a highly specific inhibition of catalase synthesis (by transduction of a shRNA anti catalase mRNA) virtually abolishes the reinforcing effects of ethanol as seen by a complete abolition of ethanol intake in rats bred for generations as high ethanol drinkers. Data shows two divergent effects of increases in acetaldehyde generation: aversive in the periphery but reinforcing in the brain.

Keywords: alcohol dehydrogenase, catalase, aldehyde dehydrogenase, reinforcement, aversion

INTRODUCTION: THE ETHANOL MOLECULE

Ethanol became part of our ecology over 200 million years ago when yeast started fermenting carbohydrates in fruits and grains, generating ethanol (Ratcliff et al., 2012). Animals were subsequently exposed to ethanol in these naturally fermented products. It has been proposed (Duddley, 2000; Dominy et al., 2004) that animals that perceived ethanol as a pleasant substance had an evolutionary advantage since they also increased the intake of calories from these sources.

The pleasant effects of alcohol explain why humans engaged in manufacturing it. The book of Anni, in 1700 BC Egypt describes an overt intoxication and the rules of proper behavior to be followed in a beer shop. Subsequently, its distillation is described in 900 AD and its production and massive consumption were brought by the Industrial Revolution (Hogarth, 1751). While in moderate doses alcohol generates motivational and reinforcing effects, in high doses it can generate aversion, inducing cognitive deficits, motor incoordination and emesis.

There is general agreement that in high concentrations ethanol modifies some neurotransmitter receptors by allosteric binding to their hydrophobic pockets, for example on the gamma-amino butyric acid receptor (GABA-A) (Harris and Allan, 1985; Suzdak et al., 1986; Huidobro-Toro et al., 1987). These hydrophobic pockets are also modifiable, with similar effects, by barbiturates and anesthetics, such as enfluorane, isofluorane and other long chain aliphatic alcohols (Levitan et al., 1988; Pritchett et al., 1989; Mihic et al., 1994, 1997; Krasowski et al., 1998).

Another receptor upon which ethanol generates hypnotic/anesthetic effects is the N-Methyl-D-Aspartate (NMDA) glutamate receptor (Lovinger et al., 1989; Weight et al., 1992). Ethanol binds allosterically to the NMDA receptor (Wright et al., 1996; Wirkner et al., 1999), an effect that leads to the interruption of cognitive processes, consolidation of memory and anesthesia (Robbins and Murphy, 2006). The hydrophobic pockets in the NMDA receptor can also be modified by anesthetics of diverse structures such as halothane, cyclopropane and xenon (Ogata et al., 2006). However, several studies indicate that the hypnotic and anesthetic effects of ethanol do not correlate with its rewarding properties (Riley et al., 1977; Daoust et al., 1987; Elmer et al., 1990).

ACETALDEHYDE AS A MEDIATOR OF THE EFFECTS OF ETHANOL

Crabbe et al. (2006) reviewed the literature on the putative role of 93 genes likely involved in the effects of ethanol. Those involved with greater frequency were alcohol dehydrogenase and aldehyde dehydrogenase. The present review deals primarily with studies that show the effects generated by increasing or reducing acetaldehyde levels and/or the ability of the liver and brain to generate it. The studies were conducted in Wistar-derived rats selectively bred for over 80 generations, which led to two lines of

rats: the UChA (abstainer) and the UChB (bibulous). The studies to be presented dovetail with many other studies described in this issue and potentiate the concept that acetaldehyde is a major contributor of the effects of ethanol, both aversive and rewarding.

Ethanol (CH3-CH2OH) is a small and relatively non-reactive molecule, thus requiring high concentrations to generate its effects. In high concentrations ethanol mainly interacts with hydrophobic pockets in proteins. On the other hand, acetaldehyde (CH3-CHO) is able to bind to amines (e.g., lysine residues) in proteins, generating Schiff bases (CH_3-C=N-CH_2-R), also binding to dopamine generating salsolinol, which has also been studied as a mediator of ethanol effects. Recently, Juricic et al. (2012) confirmed early studies by King et al. (1974) showing that acetaldehyde can, in addition to generating salsolinol, condense non-enzymatically with a carbon vicinal to a hydroxyl group in dopamine, yielding isosalsolinol (**Figure 1**). It was shown that commercial salsolinol (Sigma-Aldrich, sold prior to 2012) used in this field[,] is a mixture of four dopamine-acetaldehyde condensation products: *(R)- and (S)- salsolinol* (85%) and *(R)- and (S)- isosalsolinol* (10–15%).

AVERSIVE EFFECTS OF LIVER-GENERATED ACETALDEHYDE

Among the first studies that described powerful effects of acetaldehyde were those seen in East Asians when consuming ethanol. Studies in the 80's demonstrated that 20–40% of individuals of East Asian origin (e.g., Japan, China, Korea) develop dysphoric effects when consuming alcohol; the effects often include peripheral vasodilatation and overt facial flushing, tachycardia, headache, nausea and emesis (Mizoi et al., 1983). Such individuals carry a point mutation in the gene that codes for the high affinity (low K_m) mitochondrial aldehyde dehydrogenase-2 (*ALDH2**2), a mutation that greatly reduces its affinity for NAD^+, thus generating a virtually inactive dehydrogenase. Subjects carrying the *ALDH2**2 polymorphism develop large increases in blood acetaldehyde (reaching 80–100 μM; over 5-fold that of subjects who carry the wild-type enzyme: *ALDH2**1). It is important to note that these increases are due not only to the inability of liver to oxidize acetaldehyde into acetate, but also the inability of peripheral tissues and vascular tissues to oxidize acetaldehyde; tissues which also express the gene for high affinity mitochondrial aldehyde dehydrogenase. In venous blood of individuals carrying the normal ALDH2*1 enzyme, the levels of acetaldehyde are virtually nil; while levels of the order of 15–20 μM are found when arterial blood is sampled (see Quintanilla et al., 2007). The marked ability of endothelial tissues to metabolize acetaldehyde is of major importance in understanding why a lipophilic metabolite such as acetaldehyde does not cross the tight-junction cells that constitute the blood brain barrier (*vide infra*), and why the effects of acetaldehyde are so different in the periphery vs. the central nervous system.

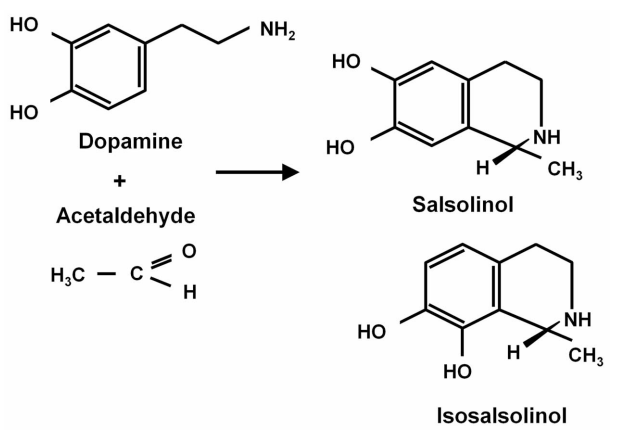


FIGURE 1 | Acetaldehyde condensation with dopamine. Products formed in the non-enzymatic condensation are (R)- and (S)-salsolinol and (R)- and (S)- isosalsolinol (from Juricic et al., 2012).

A number of studies have shown that individuals carrying the *ALDH2**2 allele are protected between 66% (heterozygous *ALDH2**1/*ALDH2**2) and 99% (homozygous *ALDH2**2/*ALDH2**2) against alcoholism (Harada et al., 1982; Thomasson et al., 1991; Higuchi, 1994; Tu and Israel, 1995; Chen et al., 1999; Luczak et al., 2006; Zintzaras et al., 2006). Hence, disulfiram (Antabuse®), a drug that non-specifically inhibits ALDH2, is the most efficient drug in the treatment of alcoholism provided its *daily intake* is secured by another person Chick et al. (1992), see meta-analysis by Jørgensen et al. (2011). Recent studies in animals (Escrig et al., 2012) show that disulfiram, while not showing behavioral effects on its own, reverses the anxiolytic effects of ethanol or shows anxiogenic effects. The administration of a large dose of acetaldehyde (100 mg/Kg) being anxiogenic *per se*.

Animal studies also support the concept that alcohol is a prodrug *vis-a vis* their aversive effects. Rats of the UChA line (virtually Abstainer) display a mutation in the *aldh2* gene (Sapag et al., 2003; see Quintanilla et al., 2006), which codes for an enzyme with a higher K_m for NAD^+ and a lower Vmax that the ALDH2 of heavy drinker animals (UChB; Bibulous). Further, the levels of arterial acetaldehyde display a large "acetaldehyde burst," reaching 40–50 μM (vs. 10–20 μM for controls), which deters their alcohol intake (Quintanilla et al., 2005b). The mutation in the ALDH2 gene accounts for 50–60% of the low ethanol intake of UChA rats (Quintanilla et al., 2005a).

Additional evidence that systemic acetaldehyde is aversive was seen in gene modification studies that inhibited ALDH2 activity and elevated blood acetaldehyde levels. In studies by Ocaranza et al. (2008), UChB (drinker) rats were allowed access to 10% ethanol and water for 60 days (intakes of 7–8 g ethanol/kg/day), and were deprived of ethanol for 3 days. At the time of deprivation animals were injected an adenoviral vector (preferential liver tropism, but no crossing of blood-brain barrier) coding for an antisense RNA against ALDH2 mRNA, which lowered liver ALDH2 activity by 80–90% ($p < 0.001$). When ethanol access was re-allowed, control (empty virus) animals ingested 1.2 g ethanol/kg/60 min (10-fold higher than a naïve UChB) while animals treated with the anti ALDH2 antisense ingested 0.5–0.6 g ethanol/kg/60 min ($p < 0.005$). This inhibitory effect *remained constant* for the 34 days of the study. Acetaldehyde levels of animals that received the antisense against ALDH2 were of the order of 60 vs. 15–20 μM in controls.

In another study (Rivera-Meza et al., 2010), alcohol intake by UChB rats was reduced by 50% by the transfer into the liver (via an adenoviral vector) a rat homolog of the fast human alcohol dehydrogenase ADH1B*2 (ADH-47His) which elevated 6-fold liver ADH activity. In these studies, arterial acetaldehyde levels in the ADH1B*2 transduced animals increased from 20 to 80 μM after the i.p administration of 1.0 g ethanol/kg. These studies, in addition to confirming that increases in acetaldehyde at *physiological levels* generate alcohol aversion in the animals, explain a large number of studies showing that humans carrying the *ADH1B*2* (*ADH-47His*) gene are protected against alcoholism (see meta-analysis by Zintzaras et al., 2006). The lack of understanding of why this enzyme protected against alcoholism stems from the fact that acetaldehyde in humans carrying the *ADH1B*2* was determined in venous blood; being close to zero. As indicated earlier, venous blood, after having perfused the rich ALDH2*1 peripheral tissues are devoid of acetaldehyde.

From the above, genetic and pharmacokinetic evidence indicates that blood acetaldehyde in concentrations of 40–80 μM is aversive to animals and to humans. The mechanism by which ethanol generates vasodilatation, hypotension and nausea could be the release of histamine from mast cells (Shimoda et al., 1996). However, antihistaminic drugs do not increase the intake of ethanol in low drinker UChA rats (Quintanilla and Tampier, unpublished). We would like to point out that it is not clear if the aversive effects of acetaldehyde are generated in the periphery beyond the liver or actually start in nerves terminals that travel from the liver to the CNS, a matter that requires study.

BRAIN ACETALDEHYDE AS A MEDIATOR OF THE REWARDING EFFECTS OF ETHANOL

Alcohol is absorbed from the stomach and intestine and is distributed throughout the body, reaching identical concentrations in the water of all tissues, including the brain. Ethanol is metabolized mainly in the liver by alcohol dehydrogenase ($K_m = 2$ mM) generating acetaldehyde, which is further oxidized to acetate by a ubiquitous high affinity aldehyde dehydrogenase ($K_m < 0.2$ μM). Other enzymes that oxidize ethanol into acetaldehyde are catalase and cytochrome p4502E1 (**Figure 2**). These latter enzymes play a minor role in metabolizing ethanol in the liver (Mezey, 1976; Khanna and Israel, 1980).

An important question in this field is whether liver-generated systemic acetaldehyde (normally not exceeding 20 μM in arterial blood after ethanol intake) can cross the blood brain barrier. Studies indicate that since the capillaries of the blood brain barrier have tight junctions (rather than open pores) acetaldehyde must first enter the ALDH2-rich endothelial cells of the barrier, which clear the acetaldehyde. Thus, under normal conditions of ethanol metabolism, systemic acetaldehyde does not cross the blood brain barrier (Eriksson, 1977; Lindros and Hillbom, 1979; Peterson and Tabakoff, 1979; Stowell et al., 1980). Only when systemic concentrations exceed 100 μM, following the administration of systemic acetaldehyde, acetaldehyde enters the CNS (Tabakoff et al., 1976). High concentrations of brain acetaldehyde can also be attained by the systemic (i.p. or s.c.) administration high doses of acetaldehyde.

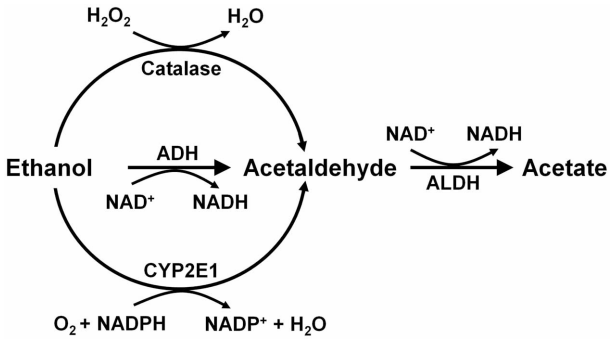


FIGURE 2 | Metabolism of ethanol into acetaldehyde by three enzymatic systems. In the liver alcohol dehydrogenase (ADH) plays a major role, while catalase and cytochrome P450 (CYP2E1) play minor roles. In the brain, ADH (ADH1) is not expressed, but catalase mainly, and CYP2E1 to a minor extent, metabolize ethanol into acetaldehyde. Aldehyde dehydrogenase-2 (ALDH2) is present in virtually all cells (Drawn from Zimatkin et al., 2006).

Alcohol dehydrogenase is not expressed in the brain (see Zimatkin et al., 2006; Deitrich, 2011), however, acetaldehyde can be generated from ethanol by the catalase reaction, and to a minor extent by CYP2E1, both enzymes present in brain (Tampier and Mardones, 1979; Aragon et al., 1992; Gill et al., 1992; Zimatkin et al., 2006). *In vitro* studies indicate that catalase generates 60–70% of brain acetaldehyde while CYP2E1 some 20% (Zimatkin et al., 2006). *In vivo* studies by Zimatkin and Buben (2007) showed that ethanol infusion into the cerebral ventricles can generate acetaldehyde (achieving 60 μM), as detected in the cerebrospinal fluid. However, the concentrations of ethanol infused (85–90 mM) were in the anesthetic range (legal limits in most countries are 6–17 mM). In these studies, the catalase inhibitor aminotriazole reduced acetaldehyde levels in the cisterna magna. While such studies were promising in pointing out a major effect of catalase in acetaldehyde generation, the low specificity of aminotriazole required caution.

The question remains as to whether enough acetaldehyde is generated following a moderate ethanol intake to induce pharmacological effects. Studies in which aminotriazole was administered showed a reduction in voluntary ethanol intake by rats (Aragon and Amit, 1992; Tampier et al., 1995). However, aminotriazole also inhibited the consumption of food and of saccharine solutions (Rotzinger et al., 1994; Tampier et al., 1995), indicating non-specific actions. With a more direct approach, Ledesma and Aragon (2013) showed that reducing brain hydrogen peroxide levels (required by catalase to oxidize ethanol to acetaldehyde) reduced alcohol-induced conditioned place preference. Most early studies in the field have been conducted by either administering large doses of acetaldehyde or by the use of inhibitors or inducers of catalase. The reader is referred to recent reviews in this field (Quertemont et al., 2005; Deitrich, 2011; Correa et al., 2012). Overall, the field generally agrees with the view that acetaldehyde mediates the reinforcing effects of ethanol; however, the methodologies used to achieve such consensus are varied, in some cases employing non-physiological concentrations and routes of administration of ethanol or acetaldehyde or non-specific inhibitors.

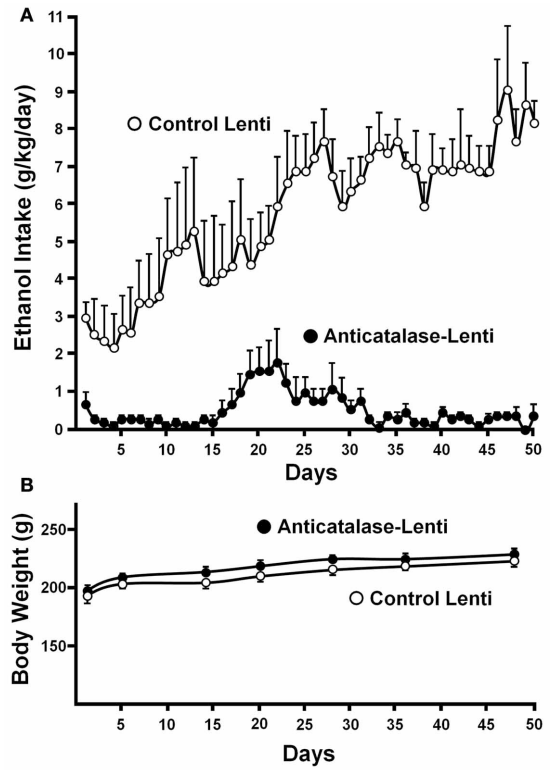


FIGURE 3 | Virtual long-lasting abolition of ethanol intake following the single administration of an anticatalase viral vector into the brain ventral tegmental area (VTA). (A) Ethanol drinker rats (UChB line) were microinjected into the VTA 1.0 microliter (8×10^4 particles) of a lentiviral vector coding for an shRNA against catalase mRNA. Controls received the empty lentiviral vector. Four days after the vector injection animals had 24-h access to 10% ethanol and water. **(B)** Animal weight was not affected by the anticatalase vector. Water intake (not shown) was not modified either (from Karahanian et al., 2011). Replicate studies by Quintanilla et al. (2012) showed that the anticatalase lentiviral vector reduced VTA catalase activity by 70–80%.

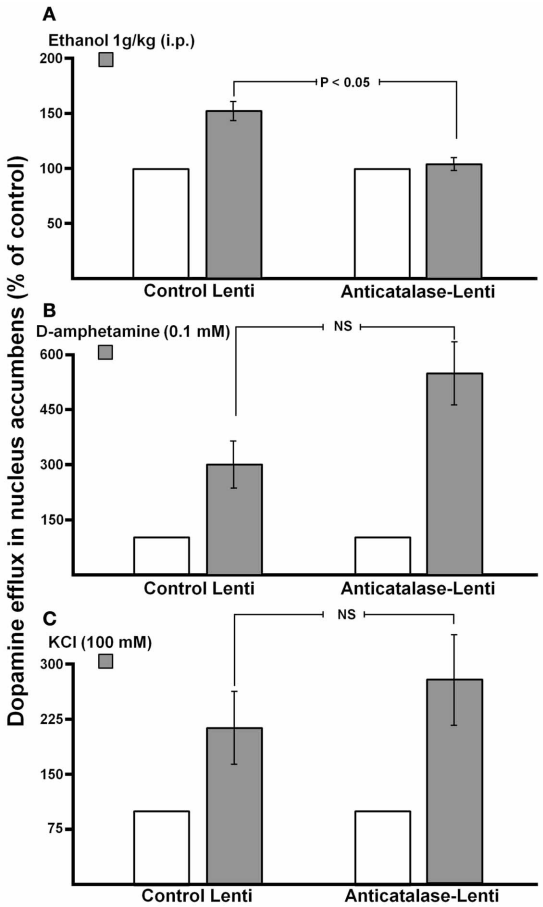


FIGURE 4 | Dopamine levels in the nucleus accumbens following acute ethanol administration. Effect of anticatalase vector administration into the ventral tegmental area on dopamine release monitored in the ipsilateral nucleus accumbens. **(A)** Inhibition by anticatalase-lentiviral vector of dopamine efflux into the microdialysis fluid of nucleus accumbens (shell) induced by the systemic administration of ethanol (1 g/kg i.p.). **(B)** Anticatalase-lentiviral vector does not affect dopamine efflux into the microdialysis fluid of nucleus accumbens (shell) induced by d-amphetamine (0.1 mM) or **(C)** Anticatalase-lentiviral vector does not affect dopamine efflux into the microdialysis fluid of nucleus accumbens (shell) induced by KCl (100 mM) added to the microdialysis fluid. (From Karahanian et al., 2011).

Early studies showed that rats self-administer acetaldehyde intracerebrally (Amit et al., 1977; Brown et al., 1979; Amit and Smith, 1985), indicating a reinforcing effect of this metabolite at the central nervous system level. Rodd et al. (2005) demonstrated that rats selectively bred as alcohol drinkers (strain P of Indianapolis) self-administer both ethanol and acetaldehyde into the brain ventral tegmental area (VTA). Acetaldehyde (6×10^{-6} M) showed reinforcing effects at concentrations that were 1000 smaller than those required for ethanol (17×10^{-3} M) self-administration. The question remained as to whether enough acetaldehyde is generated in the brain to develop rewarding and reinforcing effects *when ethanol is consumed orally*.

Recently, a specific gene blocking technique allowed inhibiting brain catalase synthesis. Karahanian et al. (2011) developed lentiviral vectors coding a shRNA designed to inhibit the synthesis of catalase. Lentiviral vectors permanently integrate into the cell genome the genes they carry. A single stereotaxic administration of an anti-catalase lentiviral vector (anticatalase-lenti) into the VTA, which reduced catalase levels by 70–80% (Quintanilla et al., 2012), virtually abolished the voluntary ethanol consumption (up to 95%) by drinker UChB rats for 40–50 days (Karahanian et al., **Figure 3**). The lentiviral anticatalase shRNA administration also abolished the increased release of dopamine in nucleus accumbens induced by ethanol administration (**Figure 4**). It is noteworthy that rats were not unduly affected (water intake, body weight, behavior) by the administration of the anticatalase lentiviral vector (Karahanian et al., 2011), as in the brain enzymes other than catalase are mainly responsible for the elimination of hydrogen peroxide (Halliwell, 2006), namely glutathione peroxidases and most active peroxiredoxins (Turrens, 2003; Rhee et al., 2005). Overall, the rewarding effects of ethanol appear to be mediated by acetaldehyde generated in the brain by the action of catalase. One can hypothesize that an increased ability of VTA to

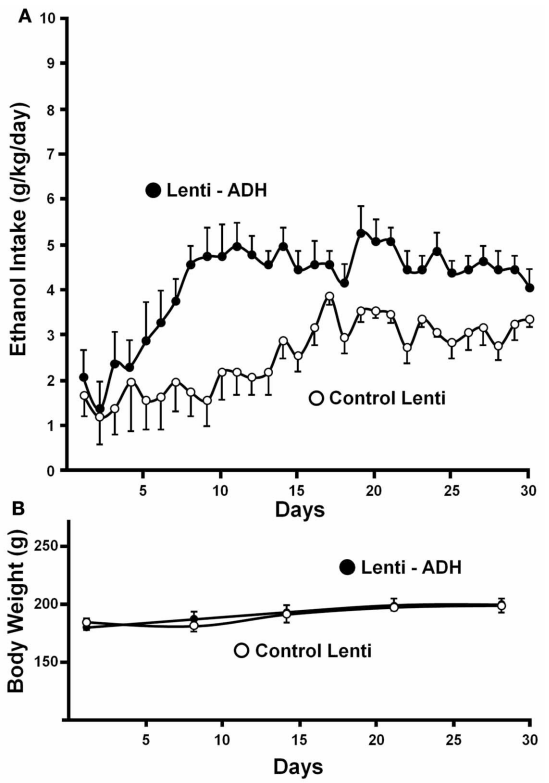


FIGURE 5 | Administration of the alcohol dehydrogenase coding gene (rat liver *adh*) into the VTA increases voluntary alcohol intake in rats (UChB) bred as alcohol drinkers. (A) Four days after the injection of the lentiviral vectors, animals were allowed free availability of 5% (v/v) ethanol and water. Rats significantly ($p < 0.001$) increased their alcohol intake when injected with a lentiviral vector encoding alcohol dehydrogenase (rADH) (Lenti-ADH) into the ventral tegmental area compared to that observed after treatment with an empty lentiviral vector (control-Lenti) **(A)**. **(B)** No differences in body weight were observed along the experiment in Lenti-ADH-treated rats vs. control-Lenti virus-treated rats. Abscissa: days of ethanol availability. (From Karahanian et al., 2011).

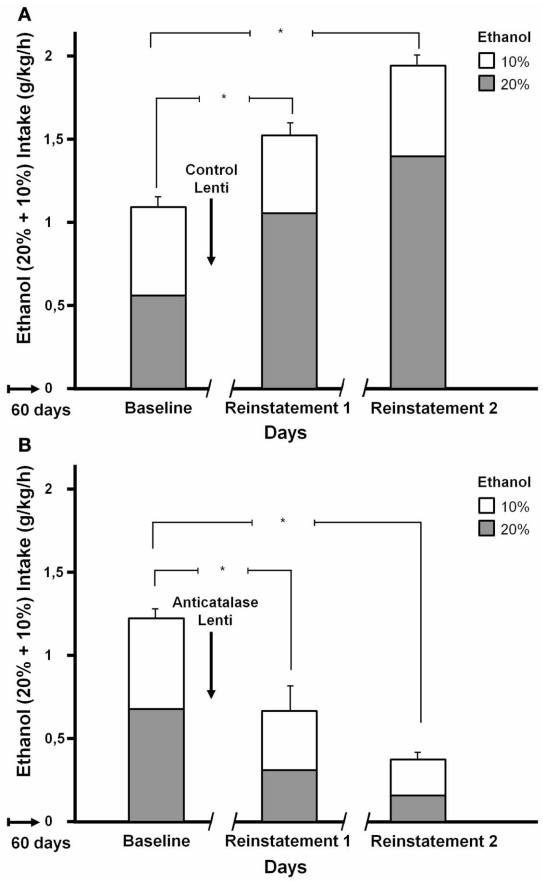


FIGURE 6 | Increased ethanol intake by UChB rats chronically exposed to ethanol, subsequently deprived and ethanol re-exposed. Marked inhibition of intake following the administration of an anticatalase lentiviral vector. Animals with *ad-libitum* access to 10 and 20% ethanol and water for 60 days. Baseline data correspond to the average of ethanol intake restricted to only 1 h a day, for 7 days immediately prior to alcohol deprivation. A single intra-ventral tegmental area injection of an anticatalase-lentiviral vector inhibited the first 1-h ethanol intake after the first and second ethanol deprivation (ADE) periods of 15 days. The total height of each bar represents the sum of ethanol intake (g ethanol/kg/60 min) of the 10% solution (empty bars) plus that of the 20% solution (gray bars). The -/ /- symbol in the *x*-axis represents the 15-day deprivation period. **(A)** Control viral vector. **(B)** Anticatalase viral vector. Arrows indicate the administration of either control lentiviral vector or anticatalase-lentiviral vector prior to the 15 days of deprivation. The first and second re-exposure consumptions were significantly different from baseline. The inhibition induced by anticatalase vector administration was 67% ($p < 0.001$) after the first deprivation period, and 80% ($p < 0.001$) after the second deprivation period. Note also the marked increases in ethanol binge-drinking induced in control vector treated animals after the first and second ethanol intake and deprivation cycles (From Tampier et al., 2013).

generate acetaldehyde, induced by genetic modification, should also demonstrate an increased rewarding effect of ethanol. This was tested by the administration into the VTA of a lentiviral vector coding for liver alcohol dehydrogenase. In these studies, to avoid a ceiling of the rewarding effect of acetaldehyde generated by catalase (when 10% ethanol is available to the animals, ethanol intake approaches the rate of whole body degradation), animals were allowed access to 5% ethanol and water. As can be seen in **Figure 5**, animals transduced with the liver alcohol dehydrogenase (ADH) into the VTA significantly increased their ethanol intake compared to that of animals administered the control vector. The animals administered the vector coding liver ADH or the control vector did not show differences in body weight or behavior.

BRAIN ACETALDEHYDE AS A MEDIATOR OF THE ALCOHOL DEPRIVATION EFFECT (ADE)

Sinclair and Senter (1968, 1977) showed that chronic intake of ethanol by rats, followed by a period of alcohol deprivation and subsequent re-exposure to ethanol leads animals to a marked increase of their ethanol intake above their basal pre-deprivation levels. This effect, termed the "*alcohol deprivation effect*" (ADE), is shown by a marked increase in voluntary intake of ethanol solutions (akin to binge-drinking) over baseline drinking when ethanol is reinstated after the period of alcohol deprivation (Spanagel and Hölter, 1999; Rodd-Henricks et al., 2001). An ADE

can be observed after a short (1–3 days; Sinclair and Li, 1989; Agabio et al., 2000) or a long (up to 60–75 days) deprivation period (Sinclair et al., 1973; Spanagel and Hölter, 1999), but is not observed in non-deprived continuously alcohol-treated animals, suggesting that chronic exposure to ethanol alone is not sufficient to produce such a marked increase in ethanol intake (Spanagel and Hölter, 1999). Examination of the ADE phenomenon has revealed that at least 3–4 weeks of a continuous alcohol-drinking experience are required before deprivation to elicit an ADE (Spanagel and Hölter, 1999).

A number of studies showed that repeated alcohol intake–deprivation–re-administration episodes increase the expression of ADE (see Rodd et al., 2008) and Vengeliene and colleagues (2009) have shown that the motivational and reinforcing effects of ethanol are increased in the ADE condition, as animals experiencing the ADE will increase their work to procure ethanol.

Tampier et al. (2013) asked whether in the ADE condition a greater reinforcing effect of ethanol, leading to binge drinking, is also mediated by acetaldehyde (thus, also by products generated from acetaldehyde). It was postulated that if increases in ethanol intake induced by ADE were mediated by acetaldehyde, inhibition of VTA catalase synthesis by microinjection of an anticatalase lentiviral vector should inhibit ADE binge-drinking. To test this question rats were allowed for 60 days 24-h access to 10 and 20% ethanol solutions and water. On day 61, rats were divided into 2 groups matched for similar 24-h alcohol consumption and preference. One group received an intracerebral administration of the control lentiviral vector and was immediately deprived for 15 consecutive days of both the 10 and 20% ethanol solution, while water was the sole fluid available. The second group was injected into the VTA the anticatalase-Lenti-shRNA. As for the viral control group, these rats were returned to their home cage and deprived for 15 days of both 10 and 20% ethanol solutions. Following the 15 days of ethanol deprivation, re-exposure to free-choice intake of 10 and 20% ethanol and water started at 1 PM (on a normal daily cycle) and lasted for 7 days. Alcohol intake was recorded in all groups on the *first hour* of re-exposure (also for 24 h after alcohol re-exposure each day for 7 days; data not shown see Tampier et al., 2013). Thereafter, rats received a second period of 15 days of ethanol deprivation and further 7 days of ethanol drinking; again ethanol intake on the first hour of re-intake was recorded. **Figure 6** indicates that (1) UChB rats reproduced the ADE binge drinking condition showing large amounts of ethanol consumed on the first hour post ethanol deprivation (achieving an intoxicating 2 g/kg/60 min) (2) subsequent deprivation ADE cycles increased ethanol intake with a greater consumption of the more concentrated ethanol solution (in line with a more reinforcing effect of ethanol after ADE), and (3) the increased in ethanol intake in the ADE condition was strongly inhibited by the anti-catalase vector. After the second deprivation cycle ethanol intake was inhibited by 80%. The above study strongly suggests possible therapeutic avenues in the treatment of alcoholism. It should be noted that administration of viral vectors is used in human therapies (Kaplitt et al., 2007) and are approved by agencies such as the U.S. Food and Drug Administration (FDA). Additional studies are being conducted (Karahanian et al. under review) to determine if an overexpression of rat ALDH2 in the VTA also inhibits the ADE-induced increases in ethanol intake.

CONCLUSIONS

Gene-based specific modifications show that an increased liver generation of acetaldehyde, leading to increased blood acetaldehyde levels, results in aversion to ethanol in animals. Similarly, aversion to ethanol results from an increased acetaldehyde level resulting from the inhibition of liver aldehyde dehydrogenase-2 synthesis. The situation is radically different when acetaldehyde is generated in the brain. When the brain ventral tegmental area is endowed with an increased ability to generate acetaldehyde the reinforcing effects of ethanol are increased, while a highly specific inhibition of catalase synthesis virtually abolishes the reinforcing effects of ethanol as seen by a complete abolition of ethanol intake. Data show two divergent effects of increases in acetaldehyde generation: aversive in the periphery but reinforcing in the brain.

ACKNOWLEDGMENTS

The authors' studies reported were supported by FONDECYT grants # 1095021; #1130012, #1120079 and #1110263 and the Millennium Scientific Initiative P09-015-F. Dr. Mario Rivera-Meza received a FONDECYT postdoctoral fellowship (#3110107).

REFERENCES

Agabio, R., Carais, M. A. M., Lobina, C., Pani, M., Reai, R., Vacca, G., et al. (2000). Development of short-lasting alcohol deprivation effect in Sardinian alcohol-preferring rat. *Alcohol* 21, 59–62. doi: 10.1016/S0741-8329(00)00072-0

Amit, Z., Brown, Z. W., and Rockman, G. E. (1977). Possible involvement of acetaldehyde, norepinephrine and their tetrahydroisoquinoline derivatives in the regulation of ethanol seld-administration. *Drug. Alcohol Depend.* 2, 495–500. doi: 10.1016/0376-8716(77)90049-7

Amit, Z., and Smith, B. R. (1985). A multi-dimensional examination of the positive reinforcing properties of acetaldehyde. *Alcohol* 2, 367–370. doi: 10.1016/0741-8329(85)90077-1

Aragon, C. M., and Amit, Z. (1992). The effect of 3-amino-1 2, 4-triazole on voluntary ethanol consumption: evidence for brain catalase involvement in the mechanism of action. *Neuropharmacology* 31, 709–712. doi: 10.1016/0028-3908(92)90150-N

Aragon, C. M., Rogan, F., and Amit, Z. (1992). Ethanol metabolism in rat brain homogenates by a catalase-H2O2 system. *Biochem. Pharmacol.* 44, 93–98. doi: 10.1016/0006-2952(92)90042-H

Brown, Z. W., Amit, Z., and Rockman, G. E. (1979). Intraventricular self-administration of acetaldehyde, but not ethanol, in naive laboratory rats. *Psychopharmacology* 64, 271–276. doi: 10.1007/BF00427509

Chen, Y. C., Lu, R. B., Peng, G. S., Wang, M. F., Wang, H. K., Ko, H. C., et al. (1999). Alcohol metabolism and cardiovascular response in an alcoholic patient homozygous for the ALDH2*2 variant gene allele. *Alcohol. Clin. Exp. Res* 23, 1853–1860. doi: 10.1111/j.1530-0277.1999.tb04083.x

Chick, J., Gough, K., Kershaw, P., Hore, B., Mehta, B., Ritson, B., et al. (1992). Disulfiram treatment of alcoholism. *Br. J. Psychiatry* 161, 84–89. doi: 10.1192/bjp.161.1.84

Correa, M., Salamone, J. D., Segovia, K. N., Pardo, M., Longoni, R., Spina, L., et al. (2012). Piecing together the puzzle of acetaldehyde as a neuroactive agent. *Neurosci. Biobehav. Rev,* 36, 404–430. doi: 10.1016/j.neubiorev.2011.07.009

Crabbe, J. C., Phillips, T. J., Harris, R. A., Arends, M. A., and Koob, G. F. (2006). Alcohol-related genes: contributions from studies with genetically engineered mice. *Addiction Biol.* 11, 195–269. doi: 10.1111/j.1369-1600.2006.00038.x

Daoust, M., Lhuintre, J. P., Moore, N., Saligaut, C., Flipo, J. L., and Boismare, F. (1987). Is initial sensitivity to ethanol correlated with alcohol preference

in alcohol-drinking and non-drinking rats. *Alcohol Alcohol.* 22, 409–414.

Deitrich, R. (2011). Ethanol as a Prodrug: brain metabolism of ethanolmediates its reinforcing effects – a commentary. *Alcohol. Clin. Exp. Res.*35, 581–583. doi: 10.1111/j.1530-0277.2011.01454.x

Dominy, N. J., Ross, C. F., and Smith, T. D. (2004). Evolution of the special senses in primates: past, present, and future. *Anat. Rec. A Discov. Mol. Cell. Evol. Biol.* 281, 1078–1082. doi: 10.1002/ar.a.20112

Duddley, T. (2000). Evolutionary origins of human alcoholism in primate frugivory. *Q. Rev. Biol.* 75, 3–15. doi: 10.1086/393255

Elmer, G. I., Meish, R. A., Goldberg, S. R., and George, F. R. (1990). Ethanol self-administration in long sleep and short sleep mice indicates reinforcement is not inversely related to neurosensitivity. *J. Pharmacol. Exp. Ther.* 254, 1054–1062.

Eriksson, C. J. (1977). Acetaldehyde metabolism *in vivo* during ethanol oxidation. *Adv. Exp. Med. Biol.* 85A, 319–341.

Escrig, M. A., Pardo, M., Aragon, C. M., and Correa, M. (2012). Anxiogenic and stress-inducing effects of peripherally administered acetaldehyde in mice: similarities with the disulfiram-ethanol reaction. *Pharmacol. Biochem. Behav.* 100, 404–412. doi: 10.1016/j.pbb.2011.10.002

Gill, K., Menez, J. F., Lucas, D., and Deitrich, R. A. (1992). Enzymatic production of acetaldehyde from ethanol in rat brain tissue. *Alcohol. Clin. Exp. Res.* 16, 910–915. doi: 10.1111/j.1530-0277.1992.tb01892.x

Halliwell, B. (2006). Oxidative stress and neurodegeneration: where are we now. *J. Neurochem.* 97, 1634–1658. doi: 10.1111/j.1471-4159.2006.03907.x

Harada, S., Agarwal, D. P., Goedde, H. W., Tagaki, S., and Ishikawa, B. (1982). Possible protective role against alcoholism for aldehyde dehydrogenase isozyme deficiency in Japan. *Lancet* 2, 827. doi: 10.1016/S0140-6736(82)92722-2

Harris, R. A., and Allan, A. M. (1985). Functional coupling of gamma-aminobutyric acid receptors to chloride channels in brain membranes. *Science* 228, 1108–1110. doi: 10.1126/science.2581319

Higuchi, S. (1994). Polymorphisms of ethanol metabolizing enzyme genes and alcoholism. *Alcohol Alcohol. Suppl* 2, 29–34.

Hogarth, W. (1751). "Gin lane," in *Hogarth's Graphic Works,* in Ronald Paulson Yale University Press, 1965.

Huidobro-Toro, J. P., Bleck, V., Allan, A. M., and Harris, R. A. (1987). Neurochemical actions of anesthetic drugs on the gamma-aminobutyric acid receptor-chloride channel complex. *J. Pharmacol. Exp. Ther.* 242, 963–969.

Jørgensen, C. H., Pedersen, B., and Tønnesen, H. (2011). The efficacy of disulfiram for the treatment of alcohol use disorder. *Alcohol. Clin. Exp. Res.* 35, 1749–1758. doi: 10.1111/j.1530-0277.2011.01523.x

Juricic, M. A., Berríos-Cárcamo, P. A., Acevedo, M. L., Israel, Y., Almodóvar, I., Cassels, B. K. (2012). Salsolinol and isosalsolinol: condensation products of acetaldehyde and dopamine. Separation of their enantiomers in the presence of a large excess of dopamine. *J. Pharm. Biomed. Anal.* 63, 170–174. doi: 10.1016/j.jpba.2012.02.002

Kaplitt, M. G., Feigin, A., Tang, C., Fitzsimons, H. L., Mattis, P., Lawlor, P. A., et al. (2007). Safety and tolerability of gene therapy with an adeno-associated virus (AAV) borne GAD gene for Parkinson's disease: an open label, phase I trial. *Lancet* 369, 2097–2105. doi: 10.1016/S0140-6736(07)60982-9

Karahanian, E., Quintanilla, M. E., Tampier, L., Rivera-Meza, M., Bustamante, D., Gonzalez-Lira, V., et al. (2011). Ethanol as a Prodrug: brain metabolism of etanol mediates its reinforcing effects. *Alcohol. Clin. Exp. Res.* 35, 606–612. doi: 10.1111/j.1530-0277.2011.01439.x

Khanna, J. M., and Israel, Y. (1980). Ethanol metabolism. *Int. Rev. Physiol.* 21, 275–315.

King, G. S., Goodwin, B. L., and Sandler, M. (1974). Isosalsolinol formation: a secondary reaction in the Pictet-Spengler condensation, *J. Pharm. Pharmacol.* 26, 476–478. doi: 10.1111/j.2042-7158.1974.tb09323.x

Krasowski, M. D., Koltchine, V. V., Rick, C. E., Ye, Q., Finn, S. E., and Harrison, N. L. (1998). Propofol and other intravenous anesthetics have sites of action on the gamma-aminobutyric acid type A receptor distinct from that for isoflurane. *Mol. Pharmacol.* 53, 530–538.

Ledesma, J. C., and Aragon, C. M. (2013). Acquisition and reconditioning of ethanol-induced conditioned place preference in mice is blocked by the H2O2 scavenger alpha lipoic acid. *Psychopharmacology* 226, 673–685. doi: 10.1007/s00213-012-2831-9

Levitan, E. S., Schofield, P. R., Burt, D. R., Rhee, L. M., Wisden, W., Köhler, M., et al. (1988). Structural and functional basis for GABAA receptor heterogeneity. *Nature* 335, 76–79. doi: 10.1038/335076a0

Lindros, K. O., and Hillbom, M. E. (1979). Acetaldehyde in cerebrospinal fluid: its near-absence in ethanol-intoxicated alcoholics. *Med. Biol.* 57, 246–247.

Lovinger, D. M., White, G., and Wright, F. F. (1989). Ethanol inhibits NMDA-activated ion current in hippocampal neurons. *Science* 243, 1721–1724. doi: 10.1126/science.2467382

Luczak, S. E., Glatt, S. J., and Wall, T. L. (2006). Meta-analyses of ALDH2 and ADH1B with alcohol dependence in Asians. *Psychol. Bull.* 132, 607–621. doi: 10.1037/0033-2909.132.4.607

Mezey, E. (1976). Ethanol metabolism and ethanol-drug interactions. *Biochem. Pharmacol.* 25, 869–875. doi: 10.1016/0006-2952(76)90305-1

Mihic, S. J., McQuilkin, S. J., Eger, E. I. 2nd, Ionescu, P., and Harris, R. A. (1994). Potentiation of gamma-aminobutyric acid type A receptor-mediated chloride currents by novel halogenated compounds correlates with their abilities to induce general anesthesia. *Mol. Pharmacol.* 46, 851–857.

Mihic, S. J., Ye, Q., Wick, M. J., Koltchine, V. V., Krasowski, M. D., Finn, S. E., et al. (1997). Sites of alcohol and volatile anaesthetic action on GABA(A) and glycine receptors. *Nature* 389, 385–389. doi: 10.1038/38738

Mizoi, Y., Tatsuno, Y., Adachi, J., Kigame, M., Fukunaga, T., Hishida, S., et al. (1983). Alcohol sensitivity related to polymorphism of alcohol-metabolizing enzymes in Japanese. *Pharmacol. Biochem. Behav.* 18(Suppl. 1), 127–133. doi: 10.1016/0091-3057(83)90159-4

Ocaranza, P., Quintanilla, M. E., Tampier, L., Karahanian, E., Sapag, A., and Israel, Y. (2008). Gene therapy reduces ethanol intake in an animal model of alcohol dependence. *Alcohol. Clin. Exp. Res.* 32, 52–57. doi: 10.1111/j.1530-0277.2007.00553.x

Ogata, J., Shiraishi, M., Namba, T., Smothers, C. T., Woodward, J. J., and Harris, R. A. (2006). Effects of anesthetics on mutant N-methyl-D-aspartate receptors expressed in Xenopus oocytes. *J. Pharmacol. Exp. Ther.* 318, 434–443. doi: 10.1124/jpet.106.101691

Oster, S. M., Toalston, J. E., Kuc, K. A., Pommer, T. J., Murphy, J. M., Lumeng, L., et al. (2006). Effect of multiple alcohol deprivations on operant ethanol self-administration by high-ethanol drinking replicate rat lines. *Alcohol* 38, 155–164. doi: 10.1016/j.alcohol.2006.06.001

Peterson, D. R., and Tabakoff, B. (1979). Characterization of brain acetaldehyde oxidizing systems in the mouse. *Drug Alcohol Depend.* 4, 137–144. doi: 10.1016/0376-8716(79)90054-1

Pritchett, D. B., Lüddens, H., and Seeburg, P. H. (1989). Type I and type II GABAA-benzodiazepine receptors produced in transfected cells. *Science* 245, 1389–1392. doi: 10.1126/science.2551039

Quertemont, E., Tambour, S., and Tirelli, E. (2005). The role of acetaldehyde in the neurobehavioral effects of ethanol: a comprehensive review of animal studies. *Prog. Neurobiol.* 75, 247–274. doi: 10.1016/j.pneurobio.2005.03.003

Quintanilla, M. E., Israel, Y., Sapag, A., and Tampier, L. (2006). The UChA and UChB rat lines: metabolic and genetic differences influencing ethanol intake. *Addiction Biol.* 11, 310–323. doi: 10.1111/j.1369-1600.2006.00030.x

Quintanilla, M. E., Tampier, L., Karahanian, E., Rivera-Meza, M., Herrera-Marschitz, M., and Israel, Y. (2012). Reward and relapse: complete gene-induced dissociation in an animal model of alcohol dependence. *Alcohol. Clin. Exp. Res.* 36, 517–522. doi: 10.1111/j.1530-0277.2011.01606.x

Quintanilla, M. E., Tampier, L., Sapag, A., Gerdzen, Z., and Israel, Y. (2007). Sex differences, alcohol dehydrogenase, acetaldehyde burst, and aversion to ethanol in the rat: a systems perspective. *Am. J. Physiol. Endocrinol. Metab.* 293, E531–E537. doi: 10.1152/ajpendo.00187.2007

Quintanilla, M. E., Tampier, L., Valle-Prieto, A., Sapag, A., and Israel, Y. (2005a). Complex I regulates mutant mitochondrial aldehyde dehydrogenase activity and voluntary ethanol consumption in rats. *FASEB J.* 19, 36–42. doi: 10.1096/fj.04-2172com

Quintanilla, M. E., Tampier, L., Sapag., and Israel, Y. (2005b). Polymorphisms in the mitochondrial aldehyde dehydrogenase gene (Aldh2) determine peak blood acetaldehyde levels and voluntary ethanol consumption in rats. *Pharmacogenet. Genomics* 15, 427–431. doi: 10.1097/01213011-200506000-00009

Ratcliff, W. C., Denison, R. F., Borrelo, M., and Travisano, M. (2012).

Experimental evolution of multicellularity. *Proc. Natl. Acad. Sci. U.S.A.* 109, 1595–1600. doi: 10.1073/pnas.1115323109

Rhee, S. G., Chae, H. Z., and Kim, K. (2005). Peroxiredoxins: a historical overview and speculative preview of novel mechanisms and emerging concepts in cell signaling. *Free Radic. Biol. Med.* 38, 1543–1552. doi: 10.1016/j.freeradbiomed.2005.02.026

Riley, E. P., Worsham, E. D., Lester, D., and Freed, E. X. (1977). Selective breeding of rats for differences in reactivity to alcohol. An approach to an animal model of alcoholism. II. Behavioral measures. *J. Stud. Alcohol* 38, 1705–1717.

Rivera-Meza, M., Quintanilla, M. E., Tampier, L., Mura, C. V., Sapag, A., and Israel, Y. (2010). Mechanism of protection against alcoholism by an alcohol dehydrogenase polymorphism: development of an animal model. *FASEB J.* 24, 266–274. doi: 10.1096/fj.09-132563

Robbins, T. W., and Murphy, E. R. (2006). Behavioural pharmacology: 40+ years of progress, with a focus on glutamate receptors and cognition. *Trends Pharmacol. Sci.* 27, 141–148. doi: 10.1016/j.tips.2006.01.009

Rodd, Z. A., Bell, R. L., Kuc, K. A., Murphy, J. M., Lumeng, L., and McBride, W. J. (2008). Effects of concurrent access to multiple ethanol concentrations and repeated deprivations on alcohol intake of high-alcohol-drinking (HAD) rats. *Addiction Biol.* 14, 152–164. doi: 10.1111/j.1369-1600.2008.00140.x

Rodd, Z. A., Bell, R. L., Zhang, Y., Murphy, J. M., Goldstain, A., Zafarroni, A., et al. (2005). Regional heterogeneity for the intracranial self-administration of ethanol and acetaldehyde within the ventral tegmental area of alcohol-preferring (P) rats: involvement of dopamine and serotonin. *Neuropsychopharmacology* 30, 330–338. doi: 10.1038/sj.npp.1300561

Rodd-Henricks, Z. A., Bell, R. L., Kuc, K. A., Murphy, J. M., McBride, W. J., Lumeng, L., et al. (2001). Effect of concurrent access to multiple ethanol concentrations and repeated deprivations on alcohol intake of alcohol-preferring rats. *Alcohol. Clin. Exp. Res.* 25, 1140–1150. doi: 10.1111/j.1530-0277.2001.tb02328.x

Rotzinger, S., Smith, B. R., and Amit, Z. (1994). Catalase inhibition attenuates the acquisition of ethanol and saccharin-quinine consumption in laboratory rats. *Behav. Pharmacol.* 5, 203–209. doi: 10.1097/00008877-199404000-00012

Sapag, A., Tampier, L., Valle-Prieto, A., Quintanilla, M. E., Moncada, M. E., and Israel, Y. (2003). Mutations in mitochondrial aldehyde dehydrogenase (ALDH2) change cofactor affinity and segregate with voluntary alcohol consumption in rats. *Pharmacogenetics* 13, 509–515. doi: 10.1097/00008571-200308000-00009

Shimoda, T., Kohno, S., Takao, A., Fujiwara, C., Matsuse, H., Sakai, H., et al. (1996). Investigation of the mechanism of alcohol-induced bronchial asthma. *J. Allergy Clin. Immuno.* 97, 74–84. doi: 10.1016/S0091-6749(96)70285-3

Sinclair, J. D., and Li, T.-K. (1989). Long and short alcohol deprivation effects on AA and P alcohol-preferring rats. *Alcohol* 6, 505–509. doi: 10.1016/0741-8329(89)90059-1

Sinclair, J. D., and Senter, R. J. (1968). Development of an alcohol-deprivation effect in rats. *Q. J. Stud. Alcoho*l 29, 863–867.

Sinclair, J. D., and Senter, R. J. (1977). Increased preference for ethanol in rats following deprivation *Psychon. Sci.* 8, 11–12.

Sinclair, J. D., Walker, S., and Jordan, W. (1973). Behavioral and physiological changes associated with various durations of alcohol deprivation in rats. *Q. J. Stud. Alcohol* 34, 544–757.

Spanagel, R., and Hölter, S. M. (1999). Long-term alcohol self-administration with repeated alcohol deprivation phases: an animal model of alcoholism. *Alcohol Alcohol.* 34, 231–243. doi: 10.1093/alcalc/34.2.231

Stowell, A., Hillbom, M., Salaspuro, M., and Lindros, K. O. (1980). Low acetaldehyde levels in blood, breath and cerebrospinal fluid of intoxicated humans as assayed by improved methods. *Adv. Exp. Med. Biol.* 132, 635–645.

Suzdak, P. D., Glowa, J. R., Crawley, J. N., Schwartz, R. D., Skolnick, P., and Paul, S. M. (1986). A selective imidazobenzodiazepine antagonist of ethanol in the rat. *Science* 234, 1243–1247. doi: 10.1126/science.3022383

Tabakoff, B., Anderson, R, A., and Ritzmann, R. F. (1976). Brain acetaldehyde after ethanol administration. *Biochem. Pharmacol.* 25, 1305–1309. doi: 10.1016/0006-2952(76)90094-0

Tampier, L., and Mardones, J. (1979). Catalase mediated oxidation of ethanol by rat brain homogenates. *IRCS Med. Sci.* 7, 389.

Tampier, L., Quintanilla, M. E., Karahanian, E., Rivera-Meza, M., Herrera-Marschitz, M., and Israel, Y. (2013). The alcohol deprivation effect:marked inhibition by anticatalase gene administration into the ventral tegmental area in rats *Alcohol Clin. Exp. Res.* doi: 10.1111/acer.12101. [Epub ahead of print].

Tampier, L., Quintanilla, M. E., and Mardones, J. (1995). Effects of aminotriazole on ethanol, water, and food intake and on brain catalase in UChA and UChB rats. *Alcohol* 12, 341–344. doi: 10.1016/0741-8329(95)00014-I

Thomasson, H. R., Edenberg, H. J., Crabb, D. W., Mai, X. L., Jerome, R. E., Li, T. K., et al. (1991). Alcohol and aldehyde dehydrogenase genotypes and alcoholism in Chinese men. *Am. J. Hum. Genet.* 48, 677–681.

Tu, G. C., and Israel, Y. (1995). Alcohol consumption by Orientals in North America is predicted largely by a single gene. *Behav. Genet.* 25, 59–65. doi: 10.1007/BF02197242

Turrens, J. F. (2003). Mitochondrial formation of reactive oxygen species. *J. Physiol.* 552, 335–344. doi: 10.1113/jphysiol.2003.049478

Vengeliene, V., Celerier, E., Chaskiel, L., Penzo, F., and Spanagel, R. (2009). Compulsive alcohol drinking in rodents. *Addiction Biol.* 14, 384–396. doi: 10.1111/j.1369-1600.2009.00177.x

Weight, F. F., Aguayo, L. G., White, G., Lovinger, D. M., and Peoples, R. W. (1992). GABA- and glutamate-gated ion channels as molecular sites of alcohol and anesthetic action. *Adv. Biochem. Psychopharmacol.* 47, 335–347.

Wirkner, K., Poelchen, W., Köles, L., Múlberg, K., Scheibler, P., Allgaier, C., et al. (1999). Ethanol-induced inhibition of NMDA receptor channels. *Neurochem. Int.* 35, 153–162. doi: 10.1016/S0197-0186(99)00057-1

Wright, J. M., Peoples, R. W., and Weight, F. F. (1996). Single-channel and whole-cell analysis of ethanol inhibition of NMDA-activated currents in cultured mouse cortical and hippocampal neurons. *Brain Res.* 738, 249–256. doi: 10.1016/S0006-8993(96)00780-9

Zimatkin, S. M., and Buben, A. L. (2007). Ethanol oxidation in the living brain. *Alcohol Alcohol.* 42, 529–532. doi: 10.1093/alcalc/agm059

Zimatkin, S. M., Pronko, S. P., Vasiliou, V., Gonzalez, F. J., and Deitrich, R. A. (2006).Enzymatic mechanisms of ethanol oxidation in the brain. *Alcohol. Clin. Exp. Res.* 30, 1500–1505. doi: 10.1111/j.1530-0277.2006.00181.x

Zintzaras, E., Stefanidis, I., Santos, M., and Vidal, F. (2006). Do alcohol-metabolizing enzyme gene polymorphisms increase the risk of alcoholism and alcoholic liver disease? *Hepatology* 43, 352–361. doi: 10.1002/hep.21023

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 06 May 2013; accepted: 18 June 2013; published online: 08 July 2013.
Citation: Israel Y, Rivera-Meza M, Karahanian E, Quintanilla ME, Tampier L, Morales P and Herrera-Marschitz M (2013) Gene specific modifications unravel ethanol and acetaldehyde actions. Front. Behav. Neurosci. 7:80. doi: 10.3389/fnbeh.2013.00080

frontiers in
BEHAVIORAL NEUROSCIENCE

ORIGINAL RESEARCH ARTICLE
published: 24 May 2013
doi: 10.3389/fnbeh.2013.00048

c-Fos immunoreactivity in prefrontal, basal ganglia and limbic areas of the rat brain after central and peripheral administration of ethanol and its metabolite acetaldehyde

Kristen N. Segovia[1], *Regina Vontell*[1], *Laura López-Cruz*[2], *John D. Salamone*[1] *and Mercè Correa*[1,2]*

[1] Department of Psychology, University of Connecticut, Storrs, CT, USA
[2] Departament de Psicobiologia, Universitat Jaume I, Castelló, Spain

Edited by:
Elio Acquas, Università di Cagliari, Italy

Reviewed by:
Elio Acquas, Università di Cagliari, Italy
Alessandra T. Peana, Università degli Studi di Sassari, Italy

***Correspondence:**
Mercè Correa, Departament de Psicobiologia, Universitat Jaume I, Avda. Sos Baynat s/n, 12071 Castelló, Spain.
e-mail: correa@psb.uji.es

Considerable evidence indicates that the metabolite of ethanol (EtOH), acetaldehyde, is biologically active. Acetaldehyde can be formed from EtOH peripherally mainly by alcohol dehydrogenase (ADH), and also centrally by catalase. EtOH and acetaldehyde show differences in their behavioral effects depending upon the route of administration. In terms of their effects on motor activity and motivated behaviors, when administered peripherally acetaldehyde tends to be more potent than EtOH but shows very similar potency administered centrally. Since dopamine (DA) rich areas have an important role in regulating both motor activity and motivation, the present studies were undertaken to compare the effects of central (intraventricular, ICV) and peripheral (intraperitoneal, IP) administration of EtOH and acetaldehyde on a cellular marker of brain activity, c-Fos immunoreactivity, in DA innervated areas. Male Sprague-Dawley rats received an IP injection of vehicle, EtOH (0.5 or 2.5 g/kg) or acetaldehyde (0.1 or 0.5 g/kg) or an ICV injection of vehicle, EtOH or acetaldehyde (2.8 or 14.0 μmoles). IP administration of EtOH minimally induced c-Fos in some regions of the prefrontal cortex and basal ganglia, mainly at the low dose (0.5 g/kg), while IP acetaldehyde induced c-Fos in virtually all the structures studied at both doses. Acetaldehyde administered centrally increased c-Fos in all areas studied, a pattern that was very similar to EtOH. Thus, IP administered acetaldehyde was more efficacious than EtOH at inducing c-Fos expression. However, the general pattern of c-Fos induction promoted by ICV EtOH and acetaldehyde was similar. These results are consistent with the pattern observed in behavioral studies in which both substances produced the same magnitude of effect when injected centrally, and produced differences in potency after peripheral administration.

Keywords: alcohol, metabolism, early gene, nucleus accumbens, dopamine

INTRODUCTION

Ethanol (EtOH) is converted into acetaldehyde in many organs by the enzyme alcohol dehydrogenase (ADH) (Cohen et al., 1980). Acetaldehyde is then metabolized to acetate by aldehyde dehydrogenase (ALDH) (Svanas and Weiner, 1985; Deng and Deitrich, 2008). EtOH crosses the blood brain barrier and is found in peripheral organs as well as in the brain (Eriksson and Sippel, 1977; Deitrich, 1987; Zimatkin, 1991). However, acetaldehyde cannot easily cross into the brain because of the abundance of ALDH in capillary endothelium and surrounding astrocytes of the blood brain barrier (Sippel, 1974; Westcott et al., 1980; Zimatkin, 1991). Only when blood acetaldehyde levels are raised after ALDH blockade, significant amounts of acetaldehyde cross into the brain. In addition, an alternative source of acetaldehyde in the brain is the local intracerebral metabolism of EtOH by the enzyme catalase (Cohen et al., 1980; Aragon et al., 1992; Correa et al., 2012).

EtOH and acetaldehyde do not always have the same pattern of effects on behavior (for a review see Correa et al., 2012). For instance, in rats they produce similar effects on motor activities such as locomotion, and on motivated behaviors such as lever pressing for food in different reinforcement conditions; both exert suppressant effects when peripherally administered (Chuck et al., 2006; McLaughlin et al., 2008), and activating effects when administered in the brain (Arizzi et al., 2003; Correa et al., 2003a,b, 2009a; Arizzi-LaFrance et al., 2006; McLaughlin et al., 2008; Pastor and Aragon, 2008). Yet while the relative

Abbreviations: AcbC, accumbens core; AcbSh, accumbens shell; ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; aCSF, artificial cerebrospinal fluid; ac, anterior commissure; BLA, basolateral amygdala; CA1, CA1 of the hippocampus; CeA, central amygdala; Cg1, cingulate cortex 1; cc, corpus callosum; DG, dentate gyrus; DA, dopamine; DLS, dorsolateral striatum; DMS, dorsomedial striatum; EtOH, ethanol; ERK, extracellular regulated kinase; VLS, ventrolateral striatum; IL, infralimbic cortex; IP, intraperitoneal; ICV, intraventricular; LS, lateral septum; O, orbitofrontal cortex; HPV, paraventricular nuclei of the hypothalamus; PVTh, paraventricular nuclei of the thalamus; PrL, prelimbic cortex; Tu, lateral tubercle; VP, ventral pallidum.

efficacy and potency of both substances is very similar after central administration, they are very different after peripheral administration to both rats and mice (Correa et al., 2004, 2009b; Tambour et al., 2005; Chuck et al., 2006; McLaughlin et al., 2008; Closon et al., 2009; Escrig et al., 2012); peripherally acetaldehyde seems always more potent than EtOH.

The induction of Fos/Jun family transcription factors has been widely used as a tool to show neuronal activation in response to a wide range of stimuli (Curran and Morgan, 1995). EtOH exposure through different routes of administration induces early-gene protein expression in several brain regions (Chang et al., 1995; Ogilvie et al., 1998; Bachtell et al., 1999, 2002; Thiele et al., 2000; Knapp et al., 2001; Crankshaw et al., 2003; Canales, 2004), and such expression reflects specific activation of intracellular pathways (Curran and Morgan, 1995; Thiele et al., 2000; Ibba et al., 2009). For instance, c-Fos protein expression is modulated after dopamine (DA) receptor signaling in neurons receiving DA input (Moratalla et al., 1992; Nguyen et al., 1992; Farrar et al., 2010; Pardo et al., 2012, 2013; Segovia et al., 2012). EtOH, as well as acetaldehyde, have been demonstrated to regulate DA release in some of these areas (Di Chiara and Imperato, 1986; Acquas et al., 1993; Melis et al., 2007; Bustamante et al., 2008; Enrico et al., 2009; Sirca et al., 2011). However, very few studies have assessed the effect of acetaldehyde on c-Fos protein expression. Thus, expression of c-Fos mRNA after intravenous administration of a low dose of acetaldehyde was only induced in the paraventricular nuclei of the thalamus (PVTh) (Cao et al., 2007). In another study, blood acetaldehyde accumulated after intraperitoneal (IP) coadministration of EtOH and cyanamide (an ALDH inhibitor; Kinoshita et al., 2002), resulted in a significant increase in c-Fos mRNA in the paraventricular nuclei of the hypothalamus (HPV) (Kinoshita et al., 2002). Thus, it seems that peripheral acetaldehyde accumulation, by direct administration or by blockade of its degradation, results in c-Fos mRNA increases in some brain nuclei (Kinoshita et al., 2002; Cao et al., 2007). However, inhibition of brain catalase activity with aminotriazole did not alter EtOH evoked dose-dependent increases in c-Fos inmunoreactivity in several brain regions (Canales, 2004). This lack of effect after the blockade of centrally generated acetaldehyde could lead to the suggestion that in the brain, only EtOH triggers this neuronal marker. However, no study thus far has investigated the effect of acetaldehyde increases in the brain on c-Fos immunoreactivity.

In the present study we assessed the pattern of c-Fos expression after peripheral (IP) or central (intraventricular, ICV) EtOH and acetaldehyde administration. We analyzed a broad range of prefrontal, basal ganglia and limbic system areas, most of which receive a substantial DA innervation (Fallon and Moore, 1978; Fields, 2007; Ikemoto, 2007), and we used doses of both substances that have been demonstrated to modulate several motor activities and motivated behaviors (Arizzi et al., 2003; Correa et al., 2003a,b; Arizzi-LaFrance et al., 2006; Chuck et al., 2006; McLaughlin et al., 2008) regulated by DA. Peripherally we also administered higher doses of acetaldehyde than the ones used in behavioral studies in order to make additional direct comparisons between EtOH and acetaldehyde.

METHODS

SUBJECTS

Male Sprague-Dawley rats (290–320 g; $N = 45$) (Harlan Sprague-Dawley, Indianapolis, IN) were housed in a colony maintained at 23°C with a 12 L: 12 D cycle (lights on at 7 h). Water and food were available *ad libitum* in the home cages. In order to minimize the possible effects of receiving a novel potentially stressful injection, rats were handled for 5 days prior to drug administration. For the ICV experiment, the handling was done after recovery from surgery. All animals received humane care in compliance with the protocols approved by the University of Connecticut Institutional Animal Care and Use Committee, and the studies have been conducted according to National Institute of Health Guide for the care and use of animals, National Academy Press (1996) and the EC Directive 86/609/EEC.

DRUGS

EtOH [100%, 200 proof, USP (United States Pharmacopea); AAPER Alcohol and Chemical Co.], acetaldehyde (Fisher Scientific) were dissolved in physiological saline (0.9% w/v) for IP administration and in artificial cerebrospinal fluid (aCSF) for the ICV administration. The aCSF was prepared by mixing sodium chloride, potassium chloride and calcium chloride (147.2 mM NaCl, 2.4 mM CaCl2, 4.0 mM KCl) in purified water. For IP injections, the stock solutions from which the different doses were obtained were: EtOH 20% v/v and acetaldehyde 2% v/v. The doses were 0.5 and 2.5 g/kg of EtOH or 0.1 and 0.5 g/kg acetaldehyde. The two IP doses of EtOH and the lower dose of acetaldehyde were selected based on previous behavioral studies (Chuck et al., 2006; McLaughlin et al., 2008) and the higher dose of acetaldehyde (0.5 g/kg) was selected in order to compare it with the same dose of EtOH. For the ICV studies, EtOH and acetaldehyde were injected at doses of 2.8 and 14.0 μmoles (solutions were 16% and 80% v/v, respectively), in 1.0 μl total volume (EtOH: 129 or 644 μg; Acetaldehyde: 123 or 617 μg). These doses are in the range that had previously produced significant effects in behavioral studies (Arizzi et al., 2003; Correa et al., 2003a,b; Arizzi-LaFrance et al., 2006; McLaughlin et al., 2008). The vehicle control procedure consisted of injections of 1.0 ml of aCSF.

For the surgery, rats were anesthetized with a solution (1.0 ml/kg, IP) that contained ketamine and xylazine (10 ml of 100 mg/ml ketamine plus 0.75 ml of 20 mg/ml xylazine) (Phoenix Pharmaceutical, Inc. St. Joseph, Mo).

SURGICAL PROCEDURE

For ICV injections, rats were implanted with unilateral guide cannulae (10 mm length 23 ga.). The stereotaxic coordinates for lateral ventricle cannulation were as follows: AP −0.5 mm (from bregma), DL ± 1.3 mm lateral (from midline), and DV −3.0 mm ventral (from the surface of the skull). The incisor bar on the stereotax was set to 0.0 mm above the interaural line. All animals were singly housed after surgery, and were allowed 10 days of recovery. Stainless steel stylets were kept in the guide cannulae to maintain their integrity. Injections were made via 30 ga. stainless steel injectors extending 1.5 mm below the guide cannulae. The injectors were attached to 10.0 ml Hamilton syringes by PE-10 tubing, and were driven by a Harvard Apparatus syringe

pump (0.5 ml / min, 1 ml total volume). After the infusions injectors were left in place for 1 min to allow for diffusion of the drug, after which the injectors were removed, stylets were replaced, and animals were placed back into their home cages. The placements of the injectors were verified histologically.

TISSUE PROCESSING AND c-Fos IMMUNOHISTOCHEMISTRY

Animals were anesthetized with CO_2 and perfused transcardially with 0.9% physiological saline followed by 3.7% paraformaldehyde, 120 min after drug administration. The brains were removed and post-fixed in formalin for 2 days. Thereafter, the brains were cut into three coronal sections, ranging from 3–5 mm in thickness prior to being placed into tissue processing cassettes for paraffin. The tissue cassettes were rinsed in water followed by an EtOH rinse prior to immersion fixation. Paraffin embedded coronal sections were cut (5–7 μm) on a microtome (Leitz Wetzlar, Spencer Scientific Co., New Hampshire) and immediately placed in a 40°C water bath for mounting tissue on Plus slides (Erie Scientific Co, New Hampshire) and allowed to air dry for 24 h. Thereafter, the tissue slides underwent dehydration by a series of three separate washes in citrosolve (2 × 7 min), 100% EtOH (2 × 7 min), and 95% EtOH (2 × 7 min). The slides were rinsed in distilled water and incubated in a 0.3% H_2O_2 solution to block endogenous peroxidase activity. The slides were washed (3 × 5 min) in 0.1 M phosphate buffer (PBS) (Dulbecco's phosphate buffered saline; pH 7.4; Sigma Chemical Co) followed by a high pH antigen retrieval (DAKO, Denmark) incubation for 15 min. The slides were then allowed to cool and washed in PBS prior to incubation in the primary antiserum. c-Fos was visualized with a rabbit polyclonal anti-cFos (1:5000, Calbiochem, Germany) for 24 h at room temperature. Following the primary antibody incubation, the sections were washed in PBS (3 × 5 min) and incubated in the secondary, anti-rabbit Horseraddish Peroxidase (HRP)-conjugate envision plus (DAKO, Denmark) for 2 h at room temperature. Thereafter, sections were washed and rinsed for 1–3 min in 3,3′ diaminobenzidine chromagen (DAB) (brown). The sections were then rinsed in distilled water before the hydration series of rinses in 95% EtOH (2 × 7 min), EtOH (2 × 7 min), and citrosolve (2 × 7 min). The slides were cover-slipped using Cytoseal 60 (Thermo Scientific) as a mounting medium and then examined by light microscopy.

QUANTIFICATION OF c-Fos-LABELED CELL DENSITY

Tissue sections were imaged by optic microscopy (Nikon Eclipse E600; Melville, NY) and photographed using SPOT software (Diagnostic Instruments, Inc.). Selected areas of the brain were outlined at low resolution (10×) using known landmarks (see **Figure 1** for schematic depictions of regions quantified), and c-Fos-positive cells were identified and quantified at a higher resolution (20×) (0.125 mm^2/field) by light thresholding. A counting grid (10 × 10) was superimposed on each photomicrograph after background correction. The total density of c-Fos cells were counted by a trained observer, who was unaware of the experimental conditions in a minimum of three adjacent coronal sections. The average value was used for statistical analysis. This manual counting method was validated by comparing results quantified with a modified automated ImageJ software program (v. 1.42, National Institutes of Health sponsored image analysis program) in a total of 20 pictures from different brain areas. The correlation in the scores between both methods was $r = 0.89$ ($p < 0.01$).

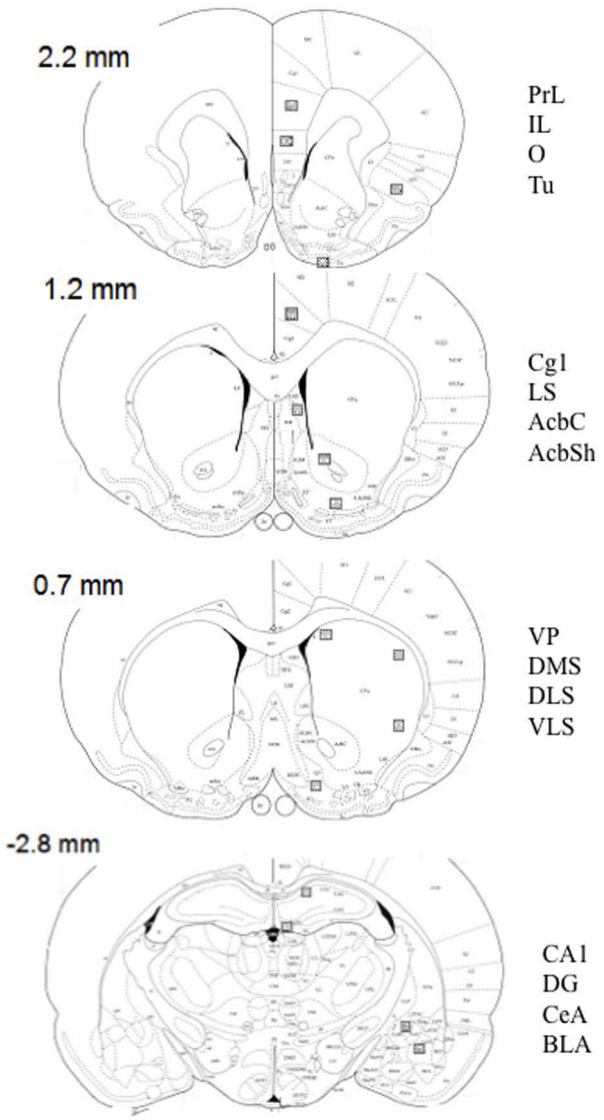


FIGURE 1 | Schematic diagrams of relevant coronal levels and specific brain regions depicted in Tables 1–6, and Figures 4, 5 [based on the atlas of Paxinos and Watson (1998)]. The squares indicate the placement of optical dissectors for counting c-Fos positive cells. Numbers represent bregma at every coronal level. AcbC, accumbens core; AcbSh, accumbens shell; BLA, basolateral amygdala; CA1, CA1 of the hippocampus; CeA, central amygdala; Cg1, cingulate cortex 1; DG, dentate gyrus; DLS, dorsolateral striatum; DMS, dorsomedial striatum; VLS, ventrolateral striatum; IL, infralimbic cortex; LS, lateral septum; O, orbitofrontal cortex; PrL, prelimbic cortex; Tu, lateral tubercle; VP, ventral pallidum.

STATISTICAL ANALYSIS

For each defined brain region and route of administration, cell counts (c-Fos-positive cells per mm^2) were compared across

treatment groups by means of one way between-groups simple ANOVA with five levels, followed by non-orthogonal planned comparisons using the overall error term, comparing vehicle to the other doses (Keppel, 1991). Significance was set at $p < 0.05$. These analyses were conducted using a computerized statistical package (SPSS). **Figures 3**, **5** are a representation of these data as a percentage of change in relation to the corresponding vehicle group for every brain structure. No additional statistical analyses were performed.

RESULTS

Figure 1 shows schematic depictions of brain regions selected. Name and abbreviations are listed in the figure legend.

EXPERIMENT 1. EFFECTS OF PERIPHERAL ADMINISTRATION OF EtOH AND ACETALDEHYDE ON c-Fos IMMUNOREACTIVITY IN PREFRONTAL CORTEX (PFC), BASAL GANGLIA AND LIMBIC AREAS

The overall one-way ANOVA yielded statistical significance for all the brain areas tested but BLA and DG. These results are depicted in **Tables 1**–**3**, respectively. The significance levels for the post hoc analyses are shown in these tables only.

Thus, in the PFC the ANOVA results were as follows; Cg1 [$F_{(4,\ 18)} = 5.64$, $p < 0.01$], PrL [$F_{(4,\ 17)} = 24.61$, $p < 0.01$], IL [$F_{(4,\ 17)} = 16.83$, $p < 0.01$], and O [$F_{(4,\ 17)} = 5.55$, $p < 0.01$]. The planned comparisons indicated that EtOH only induced c-Fos expression in the PrL and O cortices and only at the lowest dose (0.5 g/kg), while both doses of acetaldehyde significantly

Table 1 | Effect of acute IP administration of EtOH or acetaldehyde on c-Fos expression in PFC areas of rat brain.

Brain area	Vehicle	EtOH 0.5 g/kg	EtOH 2.5 g/kg	Acetal 0.1 g/kg	Acetal 0.5 g/kg
CELL COUNTS (per mm^2) ± SEM					
Cg1	418.6 ± 47.5	618.4 ± 70.4	678.9 ± 130.3	835.5 ± 129.7*	1156.2 ± 125.9$^{**+\#\#}$
PrL	267.0 ± 22.8	440.8 ± 53.1*	303.0 ± 14.8	546 ± 60.8**	881.0 ± 49.6$^{**++\#\#}$
IL	258.0 ± 13.7	372.0 ± 17.7	320.0 ± 16.5	508.8 ± 78.4**	787.0 ± 61.9$^{**++\#\#}$
O	318.0 ± 23.6	589.6 ± 86.3**	345 ± 25.9^{++}	560.0 ± 63.7**	620 ± 38.3**

Data are the mean number of c-Fos-positive cells (+SEM) per mm^2 in the regions listed. ($p < 0.05$, **$p < 0.01$ different from vehicle for the same brain region, $^{+}p < 0.05$, $^{++}p < 0.01$ different from the lower dose of the same drug, $^{\#\#}p < 0.01$ different from the same dose of EtOH).*

Table 2 | Effect of acute IP administration of EtOH or acetaldehyde on c-Fos expression in basal ganglia areas of rat brain.

Brain area	Vehicle	EtOH 0.5 g/kg	EtOH 2.5 g/kg	Acetal 0.1 g/kg	Acetal 0.5 g/kg
CELL COUNTS (per mm^2) ± SEM					
AcbC	260.0 ± 66.5	486.8 ± 95.7*	264.8 ± 25.2^{+}	693.7 ± 103.8**	858.4 ± 22.8$^{**\#\#}$
AcbSh	242.6 ± 51.0	349.5 ± 19.7	325.6 ± 57.8	459.0 ± 55.9**	633.2 ± 71.3$^{**+\#\#}$
VLS	229.3 ± 9.6	456.5 ± 30.8*	547.3 ± 95.3**	594.1 ± 82.6**	648.6 ± 53.5$^{**\#}$
DLS	258.0 ± 17.2	449.3 ± 19.6**	335.4 ± 19.7	569.6 ± 60.1**	534.0 ± 50.1**
DMS	218.0 ± 29.5	462.4 ± 30.2**	405.8 ± 35.6*	559.3 ± 91.2**	562.0 ± 56.9**
VP	151.0 ± 47.0	296.8 ± 23.3*	304.0 ± 58.8*	328.4 ± 26.1*	417.5 ± 70.8$^{**\#\#}$

Data are the mean number of c-Fos-positive cells (+SEM) per mm^2 in the regions listed. ($p < 0.05$, **$p < 0.01$ different from vehicle for the same brain region, $^{+}p < 0.05$ different from the lower dose of the same drug, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ different from the same dose of EtOH).*

Table 3 | Effect of acute IP administration of EtOH or acetaldehyde on c-Fos expression in limbic areas of rat brain.

Brain area	Vehicle	EtOH 0.5 g/kg	EtOH 2.5 g/kg	Acetal 0.1 g/kg	Acetal 0.5 g/kg
CELL COUNTS (per mm^2) ± SEM					
Tu	118.0 ± 8.1	284.0 ± 28.1**	244.0 ± 17.5*	307.0 ± 36.5**	337.0 ± 61.7**
LS	124.0 ± 9.3	183.2 ± 18.5	194.4 ± 13.0*	263.0 ± 26.9**	398.0 ± 41.5$^{**++\#\#}$
BLA	66.0 ± 3.8	115.2 ± 29.1	44.8 ± 13.5	89.0 ± 14.2	135.0 ± 24.2
CeA	79.0 ± 16.9	118.4 ± 18.6	34.4 ± 16.9^{+}	99.0 ± 20.9	172.0 ± 23.2^{**++}
CA1	74.0 ± 7.3	89.6 ± 7.2	114.4 ± 33.6	79.0 ± 7.7	182 ± 31.4$^{**++\#\#}$
DG	105.0 ± 15.3	128 ± 18.5	88.0 ± 32.4	90.4 ± 32.8	186.0 ± 38.9

Data are the mean number of c-Fos-positive cells (+SEM) per mm^2 in the regions listed. ($p < 0.05$, **$p < 0.01$ different from vehicle for the same brain region, $^{+}p < 0.05$, $^{++}p < 0.01$ different from the lower dose of the same drug, $^{\#\#}p < 0.01$ different from the same dose of EtOH).*

induced c-Fos in all the cortical areas analyzed. Moreover, acetaldehyde was more efficacious than EtOH in all the cortical areas but O, since 0.5 g/kg acetaldehyde was statistically different from 0.5 g/kg EtOH.

In the basal ganglia structures the overall one-way ANOVA's for all the different regions were significant. The *F* values were as follows: AcbC [$F_{(4, 17)} = 12.87$, $p < 0.01$], AcbSh [$F_{(4, 17)} = 6.96$, $p < 0.01$], VLS [$F_{(4, 17)} = 6.28$, $p < 0.01$], DLS [$F_{(4, 17)} = 10.94$, $p < 0.01$], DMS [$F_{(4, 17)} = 8.48$, $p < 0.01$], and VP [$F_{(4, 17)} = 4.11$, $p < 0.05$]. The planned comparisons indicated that EtOH produced a significant increase at both doses in the VLS, DMS, and VP, while in the AcbC and in the DLS only the low dose induced c-Fos. Surprisingly, none of the EtOH doses induced significantly c-Fos in the AcbSh. Acetaldehyde produced significant induction of c-Fos in all the structures at both doses and it was significantly more efficacious than EtOH at inducing c-Fos in all the ventral areas of the striatum and in the VP, but not in the dorsal areas of striatum (DLS and DMS). **Figure 2** shows representative microphotographs of PFC and Acb areas.

As pointed out above, in the limbic areas the one-way ANOVAs were not significant in the BLA and in the DG. However, in the other areas the ANOVAs were significant; Tu [$F_{(4, 17)} = 5.89$, $p < 0.01$], LS [$F_{(4, 17)} = 8.14$, $p < 0.01$], CeA [$F_{(4, 17)} = 11.48$, $p < 0.01$], and CA1 [$F_{(4, 17)} = 3.66$, $p < 0.05$]. EtOH produced a significant effect only in the Tu and in the LS, and these two areas were also more sensitive to the effect of acetaldehyde (i.e., both doses produced an increase).

Overall, it seems that, among the EtOH groups, while 0.5 g/kg increased c-Fos expression (although it was not always statistically significant), 2.5 g/kg EtOH induced c-Fos only in some areas of the striatum and limbic system, but it did not produce a larger increase than the lower dose, thus possibly indicating a biphasic effect of EtOH on c-Fos expression (see for example PrL and O cortex, AcbC, and CeA). As for the acetaldehyde groups, in general both doses increased c-Fos immunoreactivity at higher levels than the EtOH groups, especially in cortical structures and in both Acb subregions. Acetaldehyde was more efficacious than EtOH at inducing c-Fos immunoreactivity, since EtOH at 0.5 g/kg significantly induced c-Fos in 8 of the 16 areas, while 0.5 g/kg acetaldehyde did so in 14 areas. Moreover, 0.5 g/kg acetaldehyde was significantly different from 0.5 g/kg EtOH in 9 of the 16 areas. The percentage change that this dose produced in relation to vehicle for both drugs in all the structures studied is graphically shown in **Figure 3**.

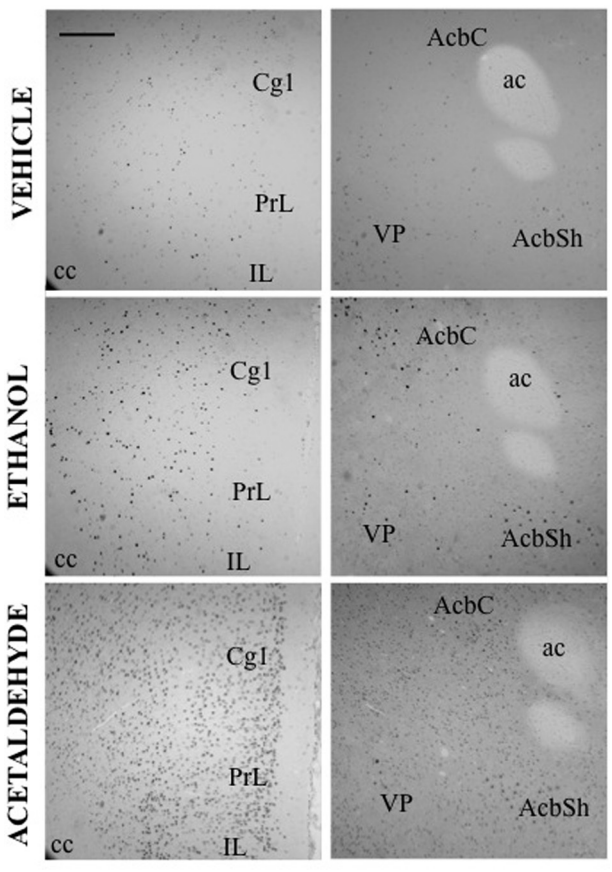


FIGURE 2 | c-Fos induction after IP administration of saline, ethanol (0.5 g/kg) and acetaldehyde (0.5 g/kg). Low power images (10×) showing PFC (left column) and Acb (right column). Scale bar = 100 μm. ac, anterior commissure; cc, corpus callosum.

EXPERIMENT 2. EFFECTS OF CENTRAL ADMINISTRATION OF EtOH AND ACETALDEHYDE ON c-Fos IMMUNOREACTIVITY IN PFC, BASAL GANGLIA AND LIMBIC AREAS

The effects of ICV administration of EtOH or acetaldehyde on c-Fos immunoreactivity are shown in **Tables 4–6** for PFC, basal ganglia and limbic areas, respectively. The results of the one-way ANOVA for every brain area showed that the treatment produced significant effects for all the areas studied. Thus in the PFC; Cg1 [$F_{(4, 18)} = 11.28$, $p < 0.01$], PrL [$F_{(4, 19)} = 10.88$, $p < 0.01$], IL [$F_{(4, 19)} = 15.41$, $p < 0.01$], and O [$F_{(4, 19)} = 19.34$, $p < 0.01$]. The planned comparisons indicated that EtOH induced c-Fos only at the low dose (2.8 μmoles) and only in Cg1 and O. However, acetaldehyde produced significant effects in all structures and at both doses. When comparing the low dose (2.8 μmoles) of EtOH with the same dose of acetaldehyde, it was found that only in the Cg1 were there no differences between the two drugs, although the higher dose (14 μmoles) of acetaldehyde induced c-Fos significantly compared with the effect of the high dose of EtOH. In the rest of PFC areas both doses of acetaldehyde produced an increase compared to the corresponding dose of EtOH.

The differences between ETOH and acetaldehyde were not so pronounced in the basal ganglia or limbic structures. The one-way ANOVA for the different structures indicated that the treatment had a significant overall effect in all of them. The *F* values were as follows: AcbC [$F_{(4, 18)} = 5.61$, $p < 0.01$], in the AcbSh [$F_{(4, 18)} = 3.24, p < 0.05$], in the VLS [$F_{(4, 17)} = 3.12, p < 0.05$], in the DLS [$F_{(4, 18)} = 5.16$, $p < 0.01$], in the DMS [$F_{(4, 18)} = 7.45$, $p < 0.01$], and in the VP [$F_{(4, 17)} = 7.26$, $p < 0.01$]. The planned comparisons indicated that the low dose of EtOH significantly induced c-Fos in both subregions of the Acb and also in the projection area VP. However, the high dose of EtOH produced an increase only in the VP. The low dose of acetaldehyde

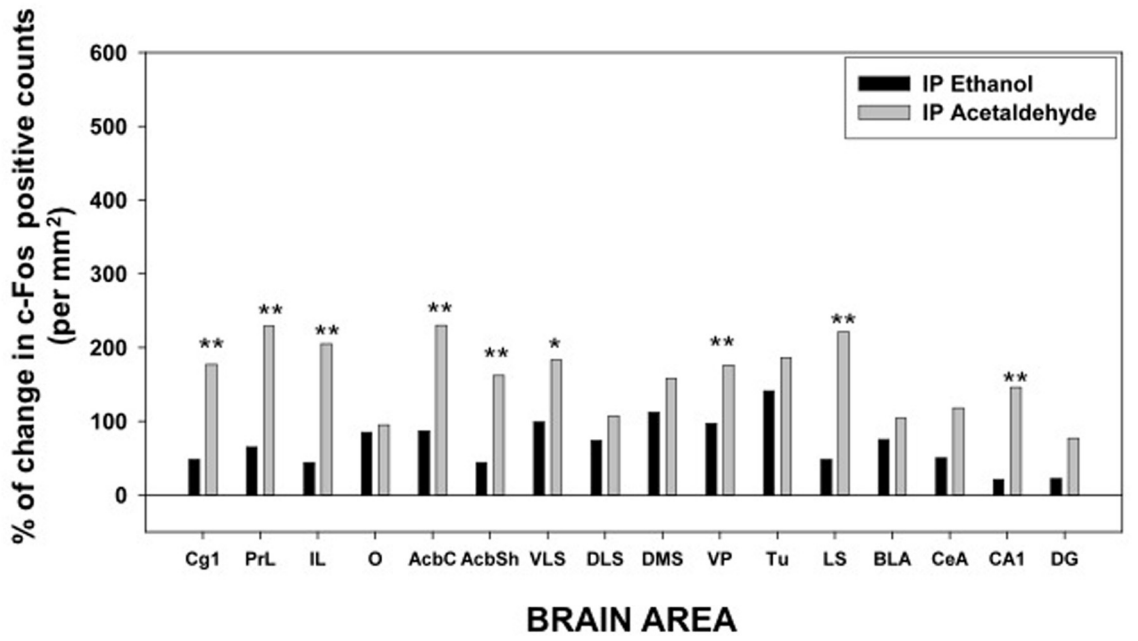


FIGURE 3 | Percentage of change in c-Fos counts after IP administration of ethanol and acetaldehyde (0.5 g/kg) in relation to the saline vehicle group for every brain structure. $^{*}p < 0.05$, $^{**}p < 0.01$ different from EtOH in that brain area.

Table 4 | Effect of acute ICV administration of EtOH or acetaldehyde on c-Fos expression in PFC areas of rat brain.

Brain area	Vehicle	EtOH 2.8 μmoles	EtOH 14 μmoles	Acetal 2.8 μmoles	Acetal 14 μmoles
CELL COUNTS (per mm^2) ± SEM					
Cg1	307.0 ± 48.5	675.0 ± 167.5*	356.9 ± 28.2	884.9 ± 169.3**	1299.4 ± 131.8$^{**+\#\#}$
PrL	241.6 ± 20.0	295.2 ± 17.9	257.6 ± 26.0	521.6 ± 43.8$^{**\#\#}$	556.0 ± 99.9$^{**\#\#}$
IL	244.0 ± 36.0	281.6 ± 28.3	287.2 ± 31.5	539.5 ± 53.6$^{**\#\#}$	533.0 ± 19.3$^{**\#\#}$
O	318.4 ± 45.3	565.6 ± 50.5**	308.0 ± 42.0	610.4 ± 37.2**	764.0 ± 28.9$^{**+\#\#}$

Data are the mean number of c-Fos-positive cells (+SEM) per mm^2 in the regions listed. ($^{*}p < 0.05$, $^{**}p < 0.01$ different from vehicle for the same brain region, $^{+}p < 0.05$ different from the lower dose of the same drug, $^{\#\#}p < 0.01$ different from the same dose of EtOH).

Table 5 | Effect of acute ICV administration of EtOH or acetaldehyde on c-Fos expression in basal ganglia areas of rat brain.

Brain area	Vehicle	EtOH 2.8 μmoles	EtOH 14 μmoles	Acetal 2.8 μmoles	Acetal 14 μmoles
CELL COUNTS (per mm^2) ± SEM					
AcbC	78.5 ± 31.5	409.0 ± 96.6*	371.0 ± 69.3	504.3 ± 162.1**	823.0 ± 150.7$^{**\#\#}$
AcbSh	108.4 ± 39.4	377.5 ± 120.9*	257.6 ± 60.7	398.5 ± 110.2*	503.8 ± 72.6$^{**\#}$
VLS	372.4 ± 21.7	489.8 ± 72.4	507.6 ± 47.7	616.3 ± 62.1**	468.4 ± 48.9
DLS	331.0 ± 15.7	409.4 ± 41.1	426.4 ± 18.1	435.3 ± 75.8	633.2 ± 48.4$^{**++\#\#}$
DMS	389.6 ± 26.1	366.5 ± 50.4	388.6 ± 48.8	500.1 ± 56.7$^{**\#}$	691.0 ± 49.9$^{**+\#\#}$
VP	208.8 ± 9.1	354.0 ± 20.4**	360.9 ± 15.1**	390.0 ± 40.0**	307.0 ± 36.2^{*+}

Data are the mean number of c-Fos-positive cells (±SEM) per mm^2 in the regions listed. ($^{*}p < 0.05$; $^{**}p < 0.01$ different from vehicle for the same brain region, $^{+}p < 0.05$; $^{++}p < 0.01$ different from the lower dose of the same drug, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ different from the same dose of EtOH).

induced c-Fos in the ventral areas of the striatum (including both Acb subregions and VLS) and in the VP, but not in the dorsal striatum. However there were no differences between EtOH and acetaldehyde at this behaviorally relevant dose. Differences between EtOH and acetaldehyde emerged only at the highest dose in both subregions of the Acb and in both areas of the dorsal striatum. See representative microphotographs of PFC and Acb areas in **Figure 4**.

Table 6 | Effect of acute ICV administration of EtOH or acetaldehyde on c-Fos expression in limbic areas of rat brain.

Brain area	Vehicle	EtOH 2.8 μmoles	EtOH 14 μmoles	Acetal 2.8 μmoles	Acetal 14 μmoles
CELL COUNTS (per mm^2) ± SEM					
Tu	214.4 ± 27.2	369.6 ± 55.7*	241.6 ± 34.4^{+}	425.6 ± 27.9**	509.0 ± 46.6**$^{\#\#}$
LS	224.8 ± 28.6	248.0 ± 22.0	214.4 ± 13.0	325.6 ± 29.7*$^{\#}$	446.0 ± 31.4**$^{++\#\#}$
BLA	72.0 ± 6.8	144.0 ± 20.2*	196.0 ± 13.2**	187.0 ± 20.0*	146.0 ± 40.0**
CeA	60.8 ± 13.7	142.4 ± 27.1	238.4 ± 20.8**$^{+}$	193.6 ± 32.2**	125.0 ± 53.5$^{\#}$
CA1	91.2 ± 8.6	196.8 ± 12.2**	227.2 ± 13.1**	160.8 ± 31.2	259.0 ± 57.0**$^{+}$
DG	89.6 ± 8.5	141.6 ± 14.8	243.2 ± 14.6**$^{++}$	199.2 ± 29.0**	168.0 ± 52.4*

Data are the mean number of c-Fos-positive cells (±SEM) per mm^2 in the regions listed. ($p < 0.05$, **$p < 0.01$ different from vehicle for the same brain region, $^{+}p < 0.05$, $^{++}p < 0.01$ different from the lower dose of the same drug, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ different from the same dose of EtOH).*

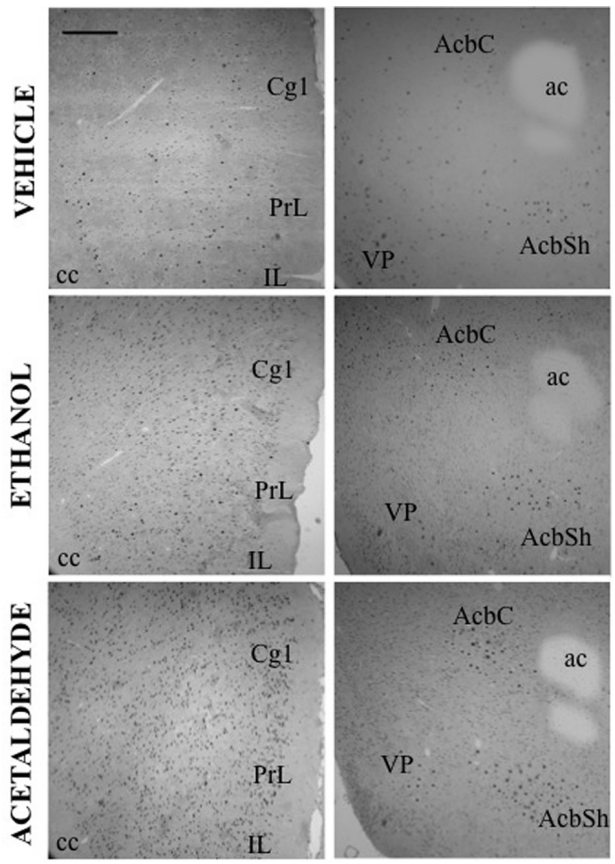


FIGURE 4 | c-Fos induction after ICV administration of vehicle, ethanol (2.8 μmoles) and acetaldehyde (2.8 μmoles). Low power images (10×) showing PFC (left column) and Acb (right column). Scale bar = 100 mm.

The one-way ANOVA for the limbic regions also demonstrated a significant overall effect of the treatment in all areas studied. The F values were as follows: Tu [$F_{(4, 19)} = 9.39$, $p < 0.01$], LS [$F_{(4, 19)} = 13.01$, $p < 0.01$], BLA [$F_{(4, 19)} = 5.72$, $p < 0.01$], CeA [$F_{(4, 19)} = 5.23$, $p < 0.01$], CA1 [$F_{(4, 19)} = 5.60$, $p < 0.01$], and DG [$F_{(4, 19)} = 5.16$, $p < 0.01$]. The planned comparisons demonstrated that the low dose of EtOH produced significant increases only in three of the areas (Tu, BLA, and CA1), while the higher dose did so in both areas of the amygdala and of the hippocampus. Acetaldehyde produced a more robust increase, since at the low dose all areas but CA1 showed increased c-Fos immunoreactivity compared to vehicle. At the high dose all areas but CeA showed increased c-Fos counts. Significant differences between both drugs were only seen in the Tu and in the LS at both doses, while in the CeA the high dose of EtOH produced an increase in c-Fos that was significantly different from acetaldehyde, which at the highest dose did not significantly induced c-Fos compared to vehicle. The percentage change that 2.8 μmoles of EtOH and acetaldehyde produced in relation to vehicle is graphically shown in **Figure 5**.

DISCUSSION

The purpose of the present study was twofold: first, to compare the pattern of c-Fos induction after EtOH and its metabolite acetaldehyde were administered by two routes of administration that have been demonstrated to reveal differences in the potency between both drugs, and second, we chose to study brain areas with DArgic innervations because both drugs have demonstrated to have effects on motor activity and motivated behaviors regulated by DA. Thus, comparisons between behaviorally relevant doses of both compounds after both routes of administration revealed the impact of each drug on different areas of the brain.

EFFECTS OF PERIPHERAL ADMINISTRATION OF ETHANOL AND ACETALDEHYDE

Overall, control values (saline or aCSF treatments) reflected comparable levels of c-Fos across the different routes of administration for all 16 brain regions quantified. In no case did EtOH or acetaldehyde significantly reduce basal c-Fos levels. Peripheral administration was the route that generated higher differences between EtOH and acetaldehyde at the same dose. However, ICV administration showed more contrasting effects between brain areas, though less so between drugs. These results reflect the same pattern of results found in behavioral studies (Arizzi et al., 2003; Correa et al., 2003a,b; Arizzi-LaFrance et al., 2006; Chuck et al., 2006; McLaughlin et al., 2008).

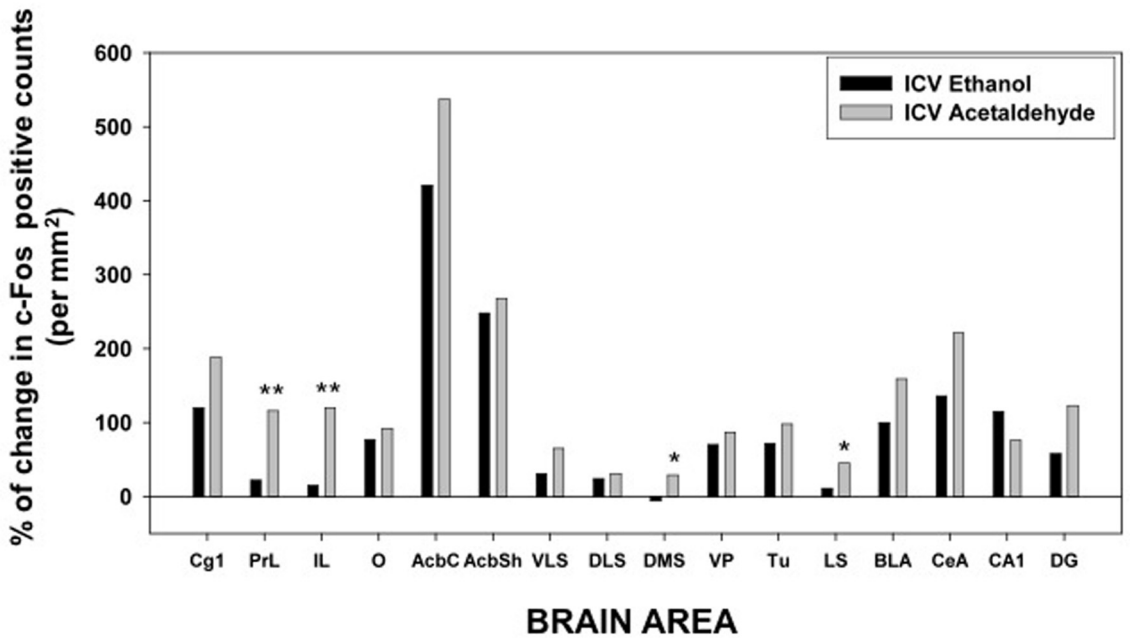


FIGURE 5 | Percentage of change in c-Fos counts after ICV administration of ethanol and acetaldehyde (2.8 μmoles) in relation to the aCSF vehicle group for every brain structure. $^{*}p < 0.05$, $^{**}p < 0.01$ different from EtOH in that brain area.

Thus, as it can be seen in **Figure 3**, with the exception of the O, PFC areas were significantly more responsive to peripherally administered acetaldehyde than to EtOH (see pictures in **Figure 2**). In **Figure 3** it can also be appreciated that the magnitude of change in c-Fos counts from vehicle at this dose was significantly bigger for acetaldehyde in ventral basal ganglia structures. However, these differences were less robust in limbic structures, in which only LS and CA1 revealed a significantly larger impact of acetaldehyde. Very few previous studies have addressed the impact of peripheral acetaldehyde, either locally formed or exogenously administered, on c-Fos immunoreactivity. Expression of *c-Fos* mRNA in the HPV was increased after peripheral accumulation of acetaldehyde by blocking ALDH activity with cyanamide and administering a dose of EtOH (1 g/kg) that did not have this effect on its own (Kinoshita et al., 2002). Kinoshita et al. (2002) did not explore any other brain area, thus further comparisons with the present results are not possible. So far, there is only one previous study of direct peripheral administration of acetaldehyde and c-Fos expression (Cao et al., 2007). In that study a small dose of acetaldehyde (0.032 g/kg) was administered also to Sprague-Dawley rats using intravenous injections as the peripheral route of administration. In agreement with the results of the 0.1 g/kg in the present study, 0.032 g/kg did not produce a significant increase in the CeA, but in contrast to our results, it did not induce c-Fos in the AcbSh (Cao et al., 2007). That dose was only able to increase c-Fos mRNA in the PVTh, possibly because this dorsal thalamic region is minimally protected by the blood brain barrier (Ueno et al., 2000; Cao et al., 2007). Moreover, the dose of acetaldehyde used in that study seems to be very low, since it did not affect ambulation or anxiety parameters (Cao et al., 2007). A dose of acetaldehyde of 0.1 g/kg IP, like the lowest one used in the present study, was demonstrated to induce anxiety and reduce locomotion in mice (Tambour et al., 2005; Escrig et al., 2012), and to slow lever pressing in rats (McLaughlin et al., 2008), although in this last study, higher doses of acetaldehyde (0.2 g/kg) were necessary to produce a significant suppression of total lever pressing. For EtOH, the required doses to slow lever pressing performance and to suppress total amount of lever pressing were 0.8 and 1.6 g/kg (McLaughlin et al., 2008), indicating that the two drugs also show differences in potency in terms of their behavioral effects.

EFFECTS AFTER CENTRAL ADMINISTRATION OF ETHANOL AND ACETALDEHYDE

After central administration, only 4 brain areas showed significant differences between EtOH and acetaldehyde at the lowest dose of both drugs (2.8 μmoles): two cortical structures, PrL and IL (see pictures in **Figure 4**), DMS and LS. The highest dose (14 μmoles) of EtOH and acetaldehyde was the one that lead to major differences between drugs after ICV administration (11 out of 16 structures). Interestingly, EtOH seems to show a biphasic effect at this dose since in many structures EtOH did not differ from vehicle. Acetaldehyde at the high dose had a very different pattern because, in all but two structures, there was still a significant increase in expression of c-Fos compared to vehicle, and in several cases these changes were even bigger than the ones produced by the lowest dose of acetaldehyde. Similar high doses have been used in previous studies (Arizzi et al., 2003; Crankshaw et al., 2003) in which a dose of

17.6 μmoles of EtOH and acetaldehyde did not produce a significant change in operant responding for food compared to vehicle in a task that required minimal rates of responding, while acetate, a metabolite of acetaldehyde, did suppress behavior at these dose. Moreover, a dose of around 17 μmoles of EtOH administered ICV did not produce conditioned taste aversion but it induced conditioned taste preference to a sweet solution (Crankshaw et al., 2003). These data indicate that although it is a high dose, the present 14 μmoles dose does not affect behavioral outcomes such as sustained attention, lever pressing, eating, or taste related learning (Arizzi et al., 2003; Crankshaw et al., 2003).

Two studies so far have measured c-Fos expression after ICV EtOH administration in some areas of the brain (Crankshaw et al., 2003; Larkin et al., 2010). Both of them have used higher doses (790 μg and 4 mg; Crankshaw et al., 2003; Larkin et al., 2010) than the high dose used in the present study (14 μmoles = 644 μg). The pattern of results found by those studies was different from the present ones: positive increases in Cg cortex, (Larkin et al., 2010), AcbSh and LS (Crankshaw et al., 2003), and no increase in the CeA (Crankshaw et al., 2003; Larkin et al., 2010). No previous study has assessed the involvement of acetaldehyde in c-Fos expression after central administration. However, catalase activity inhibition by aminotriazole (thus, blockade of brain acetaldehyde formation), did not affect c-Fos expression in Acb and CeA, after peripheral EtOH administration (Canales, 2004). Those results point to an independent effect of EtOH on this cellular parameter of activity.

IMPACT OF ETHANOL AND ACETALDEHYDE ON Acb-DA RELATED FUNCTIONS

Among all the structures studied in the present work, the Acb warrants additional examination. AcbC was revealed as the more sensitive area to c-Fos induction after EtOH or acetaldehyde with both routes of administration (see pictures in **Figures 2**, **4**). After peripheral administration, EtOH 0.5 g/kg produced a significant induction of c-Fos immunoreactivity in AcbC, although not in AcbSh. These results are somehow consistent with previous studies from other laboratories. For example, after IP administration of 1.0 or 2.5 g/kg of EtOH, also in Sprague-Dawley rats, there was an increase on c-Fos expression in the general area of the Acb (Canales, 2004). However, although in the present study the increase in the AcbC was seen after the administration of a smaller dose (0.5 g/kg), we did not find a significant increase at 2.5 g/kg, thus possibly pointing to a typical biphasic effect of EtOH in this type of cellular markers in the Acb (Ibba et al., 2009). In other studies c-Fos expression after 2.5 g/kg IP of EtOH, did not produce an effect on AcbC but it did significantly induced c-Fos in AcbSh (47% increase) (Knapp et al., 2001). In the present study 2.5 g/kg EtOH tended to induce c-Fos in the AcbSh to a very similar magnitude (34%), although this difference was not statistically significant. On the other hand, after central administration of 2.8 μmoles (129 μg) of EtOH the increases in c-Fos expression produced in both Acb subregions (421% for the AcbC and 248% for AcbSh) reached statistical significance (see **Figure 5**). In the only study of c-Fos expression after ICV EtOH administration that analyzed the Acb area (Crankshaw et al., 2003), it was found a significant increase (127%) in the AcbSh after a single injection of 790 μg of EtOH. In our study, the highest dose (644 μg) of EtOH did not produce a statistically significant increase, although the percentage of increase from vehicle was 375% for the AcbC and 138% for the AcbSh, an increase very similar to the above mentioned study. The effects of acetaldehyde in the Acb showed a significant increase at both doses and after both routes of administration in both subregions (see pictures in **Figures 2**, **4**). Both doses of acetaldehyde increased c-Fos expression significantly in AcbC (166 and 230%) and in AcbSh (89 and 161%). Moreover, 0.5 g/kg acetaldehyde produced a significantly higher increase than the same dose of EtOH both in AcbC and in AcbSh (see **Figure 3**). After central administration of acetaldehyde the pattern of effects was the same as for peripheral administration. There was a difference in efficacy at the highest dose; 14 μmoles of acetaldehyde, both in the AcbC and in the AcbSh, increased c-Fos expression while 14 μmoles of EtOH did not produce a significant change. However, there was no difference in efficacy between EtOH and acetaldehyde at a more behaviorally relevant dose, 2.8 μmoles (see **Figure 5**), as it was the case in food reinforced lever pressing (Arizzi et al., 2003; McLaughlin et al., 2008).

EtOH- and acetaldehyde-induced changes in c-Fos expression of DA target areas may be mediated by modulation of DA release and DA receptor activation. Acetaldehyde has been demonstrated to induce neuronal firing of DArgic neurons in the ventral tegmental area (Foddai et al., 2004; Diana et al., 2008) and to stimulate DA transmission in the Acb (Melis et al., 2007; Enrico et al., 2009; Sirca et al., 2011; Deehan et al., 2013). Extracellular regulated kinase (ERK) activation has been suggested as a postsynaptic correlate of activated DA transmission (Acquas et al., 2007), and acetaldehyde has been reported to elicit ERK phosphorylation in the rat Acb and extended amygdala, via DA D1 receptors (Vinci et al., 2010; Peana et al., 2011). Thus, peripheral intragrastric administration of EtOH (0.5–2.0 g/kg) increased pERK in the AcbC and AcbS in a biphasic dose response way (Ibba et al., 2009). A much lower dose of acetaldehyde (0.02 g/kg) induced ERK phosphorylation also in AcbC, AcbSh (Vinci et al., 2010; Peana et al., 2011). This is also important because ERK seems to be necessary for the induction of c-Fos expression after alcohol administration (Bachtell et al., 2002).

CONCLUSION

From the present study we can conclude that EtOH and acetaldehyde produce a general pattern of c-Fos induction in PFC, basal ganglia, and limbic areas, most of which have a substantial DA innervation (Fields, 2007; Ikemoto, 2007), at doses that are able to affect motor activity and motivated behaviors (Arizzi et al., 2003; Correa et al., 2003a,b; Arizzi-LaFrance et al., 2006; Chuck et al., 2006; McLaughlin et al., 2008). Peripherally administered acetaldehyde is more potent than EtOH at suppressing motor behaviors (McLaughlin et al., 2008), and the same potency pattern can be seen in relation to c-Fos expression (present paper). The present results are also in accordance with the behavioral data on locomotion and lever pressing after central administration. Both drugs do not show big differences in efficacy or potency

when injected in the ventricles (Arizzi et al., 2003; Correa et al., 2003a; Arizzi-LaFrance et al., 2006; McLaughlin et al., 2008). Thus, c-Fos can be used as a general marker of neural activity in DA terminal areas, one which reflects the impact of EtOH and acetaldehyde after different routes of administration on motor and motivational functions.

ACKNOWLEDGMENTS

This research was supported by a grant to Mercè Correa from Plan Nacional de Drogas (2010I024), Spain, and a grant to John D. Salamone from NIH/NIMH (MH078023-01A1). Laura López-Cruz was funded by a fellowship from Ministerio de Educación FPU (AP2010-3793), Spain.

REFERENCES

Acquas, E., Meloni, M., and Di Chiara, G. (1993). Blockade of delta-opioid receptors in the nucleus accumbens prevents ethanol-induced stimulation of dopamine release. *Eur. J. Pharmacol.* 230, 239–241.

Acquas, E., Pisanu, A., Spiga, S., Plumitallo, A., Zernig, G., and Di Chiara, G. (2007). Differential effects of intravenous R, S-(+/−)-3, 4 methylenedioxymethamphetamine (MDMA, Ecstasy) and its S(+)- and R(-)-enantiomers on dopamine transmission and extracellular signal regulated kinase phosphorylation (pERK) in the rat nucleus accumbens shell and core. *J. Neurochem.* 102, 121–132.

Aragon, C. M., Rogan, F., and Amit, Z. (1992). Ethanol metabolism in rat brain homogenates by acatalase-H2O2 system. *Biochem. Pharmacol.* 44, 93–98.

Arizzi, M. N., Correa, M., Betz, A. J., Wisniecki, A., and Salamone, J. D. (2003). Behavioral effects of intraventricular injections of low doses of ethanol, acetaldehyde, and acetate in rats: studies with low and high rate operant schedules. *Behav. Brain Res.* 147, 203–210.

Arizzi-LaFrance, M. N., Correa, M., Aragon, C. M., and Salamone, J. D. (2006). Motor stimulant effects of ethanol injected into the substantia nigra pars reticulata: importance of catalase-mediated metabolism and the role of acetaldehyde. *Neuropsychpharmacology* 31, 997–1008.

Bachtell, R. K., Tsivkovskaia, N. O., and Ryabinin, A. E. (2002). Alcohol-induced c-Fos expression in the Edinger-Westphal nucleus: pharmacological and signal transduction mechanisms. *J. Pharmacol. Exp. Ther.* 302, 516–524.

Bachtell, R. K., Wang, Y. M., Freeman, P., Risinger, F. O., and Ryabinin, A. E. (1999). Alcohol drinking produces brain region-selective changes in expression of inducible transcription factors. *Brain Res.* 847, 157–165.

Bustamante, D., Quintanilla, M. E., Tampier, L., Gonzalez-Lira, V., Israel, Y., and Herrera-Marschitz, M. (2008). Ethanol induces stronger dopamine release in nucleus accumbens (shell) of alcohol-preferring (bibulous) than in alcohol-avoiding (abstainer) rats. *Eur. J. Pharmacol.* 591, 153–158.

Canales, J. J. (2004). Catalase-independent early-gene expression in rat brain following acute ethanol exposure. *Brain Res.* 1016, 96–101.

Cao, J., Belluzzi, J. D., Loughlin, S. E., Keyler, D. E., Pentel, P. R., and Leslie, F. M. (2007). Acetaldehyde, a major constituent of tobacco smoke, enhances behavioral, endocrine, and neuronal responses to nicotine in adolescent and adult rats. *Neuropsychopharmacology* 32, 2025–2035.

Chang, S. L., Patel, N. A., and Romero, A. A. (1995). Activation and desensitization of Fos immunoreactivity in the rat brain following ethanol administration. *Brain Res.* 679, 89–98.

Chuck, T. L., McLaughlin, P. J., Arizzi-LaFrance, M. N., Salamone, J. D., and Correa, M. (2006). Comparison between multiple behavioral effects of peripheral ethanol administration in rats: sedation, ataxia, and bradykinesia. *Life Sci.* 79, 154–161.

Closon, C., Didone, V., Tirelli, E., and Quertemont, E. (2009). Acetaldehyde and the hypothermic effects of ethanol in mice. *Alcohol. Clin. Exp. Res.* 33, 2005–2014.

Cohen, G., Sinet, P. M., and Heikkila, R. (1980). Ethanol oxidation by rat brain *in vivo*. *Alcohol. Clin. Exp. Res.* 4, 366–370.

Correa, M., Arizzi, M. N., Betz, A., Mingote, S., and Salamone, J. D. (2003a). Open-field locomotor effects in rats after intraventricular injections of ethanol and the ethanol metabolites acetaldehyde and acetate. *Brain Res. Bull.* 62, 197–202.

Correa, M., Arizzi, M. N., Betz, A., Mingote, S., and Salamone, J. D. (2003b). Locomotor stimulant effects of intraventricular injections of low doses of ethanol in rats: acute and repeated administration. *Psychopharmacology* 170, 368–375.

Correa, M., Arizzi, M. N., and Salamone, J. D. (2009a). Infusions of acetaldehyde into the arcuate nucleus of the hypothalamus induce motor activity in rats. *Life Sci.* 84, 321–327.

Correa, M., Viaggi, C., Escrig, M. A., Pascual, M., Guerri, C., Vaglini, F., et al. (2009b). Ethanol intake and ethanol-induced locomotion and locomotor sensitization in Cyp2e1 knockout mice. *Pharmacogenet. Genomics* 19, 217–225.

Correa, M., Salamone, J. D., Segovia, K. N., Pardo, M., Longoni, R., Spina, L., et al. (2012). Piecing together the puzzle of acetaldehyde as a neuroactive agent. *Neurosci. Biobehav. Rev.* 36, 404–430.

Correa, M., Sanchis-Segura, C., Pastor, R., and Aragon, C. M. (2004). Ethanol intake and motor sensitization: the role of brain catalase activity in mice with different genotypes. *Physiol. Behav.* 82, 231–240.

Crankshaw, D. L., Briggs, J. E., Olszewski, P. K., Shi, Q., Grace, M. K., Billington, C. J., et al. (2003). Effects of intracerebroventricular ethanol on ingestive behavior and induction of c-Fos immunoreactivity in selected brain regions. *Physiol. Behav.* 79, 113–120.

Curran, T., and Morgan, J. I. (1995). Fos: an immediate-early transcription factor in neurons. *J. Neurobiol.* 26, 403–412.

Deehan, G. A. Jr., Engleman, E. A., Ding, Z. M., McBride, W. J., and Rodd, Z. A. (2013). Microinjections of acetaldehyde or salsolinol into the posterior ventral tegmental area increase dopamine release in the nucleus accumbens shell. *Alcohol. Clin. Exp. Res.* 37, 722–729.

Deitrich, R. A. (1987). Specificity of the action of ethanol in the central nervous system: behavioral effects. *Alcohol Alcohol. Suppl.* 1, 133–138.

Deng, X., and Deitrich, R. A. (2008). Putative role of brain acetaldehyde in ethanol addiction. *Curr. Drug Abuse Rev.* 1, 3–8.

Diana, M., Peana, A. T., Sirca, D., Lintas, A., Melis, M., and Enrico, P. (2008). Crucial role of acetaldehyde in alcohol activation of the mesolimbic dopamine system. *Ann. N.Y. Acad. Sci.* 1139, 307–317.

Di Chiara, G., and Imperato, A. (1986). Preferential stimulation of dopamine release in the nucleus accumbens of freely moving rats by ethanol. *J. Pharmacol. Exp. Ther.* 239, 219–228.

Enrico, P., Sirca, D., Mereu, M., Peana, A. T., Lintas, A., Golosio, A., et al. (2009). Acetaldehyde sequestering prevents ethanol-induced stimulation of mesolimbic dopamine transmission. *Drug Alcohol Depend.* 100, 265–271.

Eriksson, C. J., and Sippel, H. W. (1977). The distribution and metabolism of acetaldehyde in rats during ethanol oxidation-I. The distribution of acetaldehyde in liver, brain, blood and breath. *Biochem. Pharmacol.* 26, 241–247.

Escrig, M. A., Pardo, M., Aragon, C. M., and Correa, M. (2012). Anxiogenic and stress-inducing effects of peripherally administered acetaldehyde in mice: similarities with the disulfiram-ethanol reaction. *Pharmacol. Biochem. Behav.* 100, 404–412.

Fallon, J. H., and Moore, R. Y. (1978). Catecholamine innervation of the basal forebrain. *J. Comp. Neurol.* 180, 545–580.

Farrar, A. M., Segovia, K. N., Randall, P. A., Nunes, E. J., Collins, L. E., Stopper, C. M., et al. (2010). Nucleus accumbens and effort-related functions: behavioral and neural markers of the interactions between adenosine A2A and dopamine D2 receptors. *Neuroscience* 166, 1056–1067.

Fields, H. L. (2007). Understanding how opioids contribute to reward and analgesia. *Reg. Anesth. Pain Med.* 32, 242–246.

Foddai, M., Dosia, G., Spiga, S., and Diana, M. (2004). Acetaldehyde increases dopaminergic neuronal activity in the VTA. *Neuropsychopharmacology* 29, 530–536.

Ibba, F., Vinci, S., Spiga, S., Peana, A. T., Assaretti, A. R., Spina, L., et al. (2009). Ethanol-induced extracellular signal regulated kinase: role of dopamine D1 receptors. *Alcohol. Clin. Exp. Res.* 33, 858–867.

Ikemoto, S. (2007). Dopamine reward circuitry: two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex. *Brain Res. Rev.* 56, 27–78.

Keppel, G. (1991). *Design and Analysis: A Researcher's Handbook.* Englewood Cliffs, NJ: Prentice-Hall.

Kinoshita, H., Jessop, D. S., Roberts, D. J., Ameno, K., Ijiri, I., Hishida, S., et al. (2002). Effects of acetaldehyde on c-Fos mRNA induction in the paraventricular nucleus following ethanol administration. *Alcohol Alcohol.* 37, 432–435.

Knapp, D. J., Braun, C. J., Duncan, G. E., Qian, Y., Fernandes, A., Crews, F. T., et al. (2001). Regional specificity of ethanol and NMDA action in brain revealed with FOS-like immunohistochemistry and differential routes of drug administration. *Alcohol. Clin. Exp. Res.* 25, 1662–1672.

Larkin, J. W., Binks, S. L., Li, Y., and Selvage, D. (2010). The role of oestradiol in sexually dimorphic hypothalamic-pituitary-adrenal axis responses to intracerebroventricular ethanol administration in the rat. *J. Neuroendocrinol.* 22, 24–32.

McLaughlin, P. J., Chuck, T. L., Arizzi-LaFrance, M. N., Salamone, J. D., and Correa, M. (2008). Central vs. peripheral administration of ethanol, acetaldehyde and acetate in rats: effects on lever pressing and response initiation. *Pharmacol. Biochem. Behav.* 89, 304–313.

Melis, M., Enrico, P., Peana, A. T., and Diana, M. (2007). Acetaldehyde mediates alcohol activation of the mesolimbic dopamine system. *Eur. J. Neurosci.* 26, 2824–2833.

Moratalla, R., Robertson, H. A., and Graybiel, A. M. (1992). Dynamic regulation of NGFI-A (zif268, egr1) gene expression in the striatum. *J. Neurosci.* 12, 2609–2622.

Nguyen, T. V., Kosofsky, B. E., Birnbaum, R., Cohen, B. M., and Hyman, S. E. (1992). Differential expression of c-fos and zif268 in rat striatum after haloperidol, clozapine, and amphetamine. *Proc. Natl. Acad. Sci. U.S.A.* 89, 4270–4274.

Ogilvie, K. M., Lee, S., and Rivier, C. (1998). Divergence in the expression of molecular markers of neuronal activation in the parvocellular paraventricular nucleus of the hypothalamus evoked by alcohol administration via different routes. *J. Neurosci.* 18, 4344–4352.

Pardo, M., Lopez-Cruz, L., Valverde, O., Ledent, C., Baqi, Y., Müller, C. E., et al. (2012). Adenosine A2A receptor antagonism and genetic deletion attenuate the effects of dopamine D2 antagonism on effort-based decision making in mice. *Neuropharmacology* 62, 2068–2077.

Pardo, M., Lopez-Cruz, L., Valverde, O., Ledent, C., Baqi, Y., Müller, C. E., et al. (2013). Effect of subtype-selective adenosine receptor antagonists on basal or haloperidol-regulated striatal function: studies of exploratory locomotion and c-Fos immunoreactivity in outbred and A2AR KO mice. *Behav. Brain Res.* 247, 217–226.

Pastor, R., and Aragon, C. M. (2008). Ethanol injected into the hypothalamic arcuate nucleus induces behavioral stimulation in rats: an effect prevented by catalase inhibition and naltrexone. *Behav. Pharmacol.* 19, 698–705.

Paxinos, G., and Watson, C. (1998). *The Rat Brian: in Stereotaxic Coordinates.* San Diego, CA: Academic Press.

Peana, A. T., Muggironi, G., Fois, G. R., Zinellu, M., Vinci, S., and Acquas, E. (2011). Effect of opioid receptor blockade on acetaldehyde self-administration and erk phosphorylation in the rat nucleus accumbens. *Alcohol* 45, 773–783.

Segovia, K. N., Correa, M., Lennington, J. B., Conover, J. C., and Salamone, J. D. (2012). Changes in nucleus accumbens and neostriatal c-Fos and DARPP-32 immunoreactivity during different stages of food-reinforced instrumental training. *Eur. J. Neurosci.* 35, 1354–1367.

Sippel, H. W. (1974). The acetaldehyde content in rat brain during ethanol metabolism. *J. Neurochem.* 23, 451–452.

Sirca, D., Enrico, P., Mereu, M., Peana, A. T., and Diana, M. (2011). L-cysteine prevents ethanol-induced stimulation of mesolimbic dopamine transmission. *Alcohol. Clin. Exp. Res.* 35, 862–869.

Svanas, G. W., and Weiner, H. (1985). Aldehyde dehydrogenase activity as the rate-limiting factor for acetaldehyde metabolism in rat liver. *Arch. Biochem. Biophys.* 236, 36–46.

Tambour, S., Didone, V., Tirelli, E., and Quertemont, E. (2005). Dissociation between the locomotor and anxiolytic effects of acetaldehyde in the elevated plus-maze: evidence that acetaldehyde is not involved in the anxiolytic effects of ethanol in mice. *Eur. Neuropsychopharmacol.* 15, 655–662.

Thiele, T. E., Cubero, I., Van Dijk, G., Mediavilla, C., and Bernstein, I. L. (2000). Ethanol-induced c-Fos expression in catecholamine- and neuropeptide Y-producing neurons in rat brainstem. *Alcohol. Clin. Exp. Res.* 24, 802–809.

Ueno, M., Akiguchi, I., Hosokawa, M., Kotani, H., Kanenishi, K., and Sakamoto, H. (2000). Blood-brain barrier permeability in the periventricular areas of the normal mouse brain. *Acta Neuropathol.* 99, 385–392.

Vinci, S., Ibba, F., Longoni, R., Spina, L., Spiga, S., and Acquas, E. (2010). Acetaldehyde elicits ERK phosphorylation in the rat nucleus accumbens and extended amygdala. *Synapse* 64, 916–927.

Westcott, J. Y., Weiner, H., Shultz, J., and Myers, R. D. (1980). *In vivo* acetaldehyde in the brain of the rat treated with ethanol. *Biochem. Pharmacol.* 29, 411–417.

Zimatkin, S. M. (1991). Histochemical study of aldehyde dehydrogenase in the rat CNS. *J. Neurochem.* 56, 1–11.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 28 March 2013; paper pending published: 08 April 2013; accepted: 05 May 2013; published online: 24 May 2013.

Citation: Segovia KN, Vontell R, López-Cruz L, Salamone JD and Correa M (2013) c-Fos immunoreactivity in prefrontal, basal ganglia and limbic areas of the rat brain after central and peripheral administration of ethanol and its metabolite acetaldehyde. Front. Behav. Neurosci. 7:48. doi: 10.3389/fnbeh.2013.00048

frontiers in
BEHAVIORAL NEUROSCIENCE

ORIGINAL RESEARCH ARTICLE
published: 14 June 2013
doi: 10.3389/fnbeh.2013.00058

Acetaldehyde-ethanol interactions on calcium-activated potassium (BK) channels in pituitary tumor (GH3) cells

Astrid G. Handlechner[1†], Anton Hermann[1], Roman Fuchs[2] and Thomas M. Weiger[1]*

[1] *Division of Cellular and Molecular Neurobiology, Department of Cell Biology, University of Salzburg, Salzburg, Austria*
[2] *Neurosignaling Unit, Department of Organismic Biology, University of Salzburg, Salzburg, Austria*

Edited by:
Merce Correa, Universitat Jaume, Spain

Reviewed by:
Alejandro Dopico, The University of Tennessee Health Science Center, USA
Marco Diana, University of Sassari, Italy

****Correspondence:***
Thomas M. Weiger, Division of Cellular and Molecular Neurobiology, Department of Cell Biology, University of Salzburg, Hellbrunnerstraße 34, 5020 Salzburg, Austria
e-mail: thomas.weiger@sbg.ac.at
† Recipient of a DOC-fFORTE—fellowship of the Austrian Academy of Science.

Background: In the central nervous system ethanol (EtOH) is metabolized to acetaldehyde (ACA) primarily by the oxidative enzyme catalase. Evidence suggests that ACA is responsible for at least some of the effects on the brain that have been attributed to EtOH. Various types of ion channels which are involved in electrical signaling are targets of EtOH like maxi calcium-activated potassium (BK) channels. BK channels exhibit various functions like action potential repolarization, blood pressure regulation, hormone secretion, or transmitter release. In most neuronal and neuroendocrine preparations at physiological intracellular calcium levels, EtOH increases BK channel activity. The simultaneous presence of ACA and EtOH reflects the physiological situation after drinking and may result in synergistic as well as antagonistic actions compared to a single application of either drug. The action of ACA on electrical activity has yet not been fully established.

Methods: GH3 pituitary tumor cells were used for outside-out and inside-out patch-clamp recordings of BK activity in excised patches. Unitary current amplitude, open probability and channel mean open time of BK channels were measured.

Results: Extracellular EtOH raised BK channel activity. In the presence of intracellular ACA this increment of BK activity was suppressed in a dose- as well as calcium-dependent manner. Mean channel open time was significantly reduced by internal ACA, whereas BK channel amplitudes were not affected. The EtOH counteracting effect of ACA was found to depend on succession of application. EtOH was prevented from activating BK channels by pre-exposure of membrane patches to ACA. In contrast BK activation by a hypotonic solution was not affected by internal ACA.

Conclusions: Our data suggest an inhibitory impact of ACA on BK activation by EtOH. ACA appears to interact specifically with EtOH at BK channels since intracellular ACA had no effect when BK channels were activated by hypotonicity.

Keywords: ethanol, acetaldehyde, BK channels, GH3 pituitary tumor cells, patch-clamp technique

INTRODUCTION

Evidence suggests that acetaldehyde (ACA) is responsible for at least some of the effects on the brain that have been attributed to ethanol (EtOH) (Quertemont et al., 2005a). Peripheral accumulation of ACA in the blood accounts for aversion by producing unpleasant physical symptoms (Eriksson, 2001). This is different in the brain where ACA is supposed to be responsible for some rewarding and reinforcing effects of EtOH (Rodd-Henricks et al., 2002; Quertemont et al., 2005b; Karahanian et al., 2011). Importantly, EtOH oxidation has been found within the living brain revealing catalase to be the predominant enzyme responsible for ACA accumulation (Zimatkin et al., 1998; Zimatkin and Buben, 2007). The question, whether effective amounts of ACA derived from peripheral EtOH metabolism can pass through the blood-brain-barrier has, however, not been answered conclusively (Correa et al., 2011).

ACA has been shown in a few studies to modulate ion channels. For instance the action potential activity of dopaminergic neurons in the mesolimbic system is increased due to its action on IA (A-type) and Ih (hyperpolarization-activated inward) K^+ currents (Foddai et al., 2004; Melis et al., 2007). In contrast a decrease in activity was reported for voltage-gated L-type calcium channels in neuronal cells (Bergamaschi et al., 1988) and in smooth muscle cells (Morales et al., 1997).

GH3 cells, isolated from rat pituitary tumors, are excitable neuroendocrine cells which produce growth hormone and prolactin (Tashjian et al., 1970). Further, they express calcium-activated maxi potassium channels (also referred as BK, Maxi-K_{Ca}, KCNMA1, KCa1.1, or Slo1 channels) and are used in numerous studies as model cells to study BK channel properties (for review see: Weiger and Hermann, 2009; Hermann et al., 2012a). Our study focused on these channels, which are abundantly expressed throughout the body and exhibit various functions like action potential repolarization, regulation of blood pressure, hormone secretion or transmitter release (recently reviewed in Hermann et al., 2012a). BK channel activity is initiated by

depolarization and enhanced by the simultaneous increase in free intracellular calcium (Ca^{2+}) concentration (McManus, 1991). According to this property BK channels represent a link between the intracellular second messenger system and the electrical state of the cell membrane. BK channel activity can be altered by a wide variety of modulatory factors, including changes in pH (Church et al., 1998), redox potential (DiChiara and Reinhart, 1997), protein kinases or phosphatases (Reinhart et al., 1991; Reinhart and Levitan, 1995), interactions with auxiliary beta (β) subunits (Weiger et al., 2000), or gasotransmitters (Hermann et al., 2012b). BK channels are also involved in behavioral processes like clock controlled behavior (Montgomery et al., 2013), in behavioral responses to EtOH (Davies et al., 2003), or react to social stress with a change in expression patterns (Chatterjee et al., 2009).

BK channels as integral membrane proteins are prominent cellular targets for EtOH, which is well documented to increase BK channel activity via a Ca^{2+} and protein kinase C (PKC) dependent mechanism in a dose dependent manner. This potentiation is based on the increment of channel open probability (Po) and mean channel open time (MCOT) and thus is related to channel gating, whereas ion conductance and selectivity are not affected (Dopico et al., 1996; Jakab et al., 1997). The EtOH-mediated activation of BK channels leads to hyperpolarization of the membrane potential disposing the cell to reduce hormone secretion and transmitter release (Dopico et al., 1999). Furthermore, BK channels show fast adaptation to EtOH appearing in the form of a rapidly reduced sensitivity to acute EtOH exposure within a few minutes. This molecular tolerance is intrinsic to the channel and can be overcome by the association with a β4-subunit, an assembly often found in the brain (Martin et al., 2008). EtOH as well as hypotonicity (Hypo) induce cell swelling and both increase BK channel activity. Additionally, EtOH induced cell swelling comes along with an increment of the intracellular Ca^{2+} concentration (Jakab et al., 2006). With regard to physiological effects, EtOH modulation of BK channels influences neuronal excitability, cerebrovascular tone, brain function, and behavior (Brodie et al., 2007; Liu et al., 2008).

Basic knowledge about neurochemical mechanisms and molecular targets of ACA is poor. Little is known about the action of ACA on ion channels, including BK channels. Although EtOH and ACA are present simultaneously in brain after drinking usually each of these chemicals is investigated separately in experimental settings. In our study both drugs were applied individually, simultaneously or successively in order to reveal possible interactions.

MATERIALS AND METHODS

CELL CULTURE

GH3 pituitary tumor cells were cultured in MEM-Eagle (Minimum Essential Medium, Sigma, Vienna, Austria), enriched with 7% fetal bovine serum and with 3% horse serum (sera from Invitrogen, Vienna, Austria). Cells were grown in tissue culture flasks at 37°C, 95% humidity and 5% CO_2 and fed two times a week. For electrophysiological recordings cells were seeded on PDL (poly-D-lysine, Sigma, Vienna, Austria) coated glass cover slips and used after 2–5 days for experiments. Cell passages 10–60 (internal count) were used in this study.

SOLUTIONS

All chemicals were from Sigma (Vienna, Austria). Bath solution (mM): 145 NaCl, 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 HEPES, 10 glucose; EtOH solution isoosmolar (mM): 130 NaCl, 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 HEPES, 10 glucose, 30 EtOH; 30% hypotonic solution (mM): 110 NaCl, 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 HEPES, 10 glucose; standard pipette solution (mM): 140 KCl, 2 $MgCl_2$, 0.88 $CaCl_2$, 1 EGTA (which results in 1.2 μM free Ca^{2+}), 20 HEPES, 20 glucose, 1 ATP. For solutions with 3 μM and 10 μM free internal Ca^{2+} 1 mM HEDTA was used as Ca^{2+} buffer; 0.043 mM total Ca^{2+} ($CaCl_2$) result in 3 μM free Ca^{2+}, and 0.125 mM total Ca^{2+} result in 10 μM free Ca^{2+}. Free internal Ca^{2+} concentrations were calculated with the Webmaxc extended calculator: http://www.stanford.edu/~cpatton/webmaxc/webmaxcE.htm. pH-values of all solutions were adjusted to 7.2. Osmolarities of solutions were controlled with a manually operated Micro-Osmometer (Type OM 806, Löser, Berlin, Germany) and adjusted to 315–325 mOsm. Within an experimental setting the difference in osmolarities did not exceed 5 mOsm.

ACA (Sigma, Vienna, Austria) was diluted into the standard bath solution (extracellular side of the cell membrane) to result in final bath concentrations of 300 μM, 1 mM, 3 mM, or 10 mM, into EtOH containing solutions (applied to the extracellular side of the cell membrane) to give a final concentration of 100 μM or into the standard pipette solution (intracellular side of the cell membrane) to produce final pipette concentrations of 30 nM, 100 nM, 300 nM, 1 μM, 30 μM, 100 μM, or 300 μM. Actual concentrations of ACA in solutions used for perfusion to the extracellular side were tested with an ACA-assay-kit (Megazyme, Bray, Ireland) according the manufactures guidelines. Since up to 20% of ACA concentrations were found to evaporate during 30 min at room temperature the effective concentrations were between 80–100 μM, usually close to 90 μM within the experimental time course. ACA containing solutions were discarded after 30 min. ACA and other equipment such as pipettes and pipette tips were kept in the fridge. Stock solutions were kept on ice and sealed with parafilm. All ACA containing solutions for perfusion were prepared immediately before use. Pipette solutions containing ACA held in the filling syringe were kept on ice and filled into the electrodes stored at room temperature. Then the electrodes were slightly warmed up by rubbing between the fingertips for at least 15 s and subsequently used for recordings. In order to rule out an impact of temperature itself on channel activity this procedure was also applied to control measurements.

ELECTROPHYSIOLOGY

Recordings were performed at room temperature (20–23°C). Single channel recordings were obtained in the outside-out and inside-out mode as described previously by Sitdikova et al. (2010). Cell free patches were clamped to a holding potential of +30 mV for outside-out and −30 mV for inside-out patches which is caused by the sign inversion technically necessary in inside out patches to receive a +30 mV depolarization at the internal side of the membrane. All recordings were started with control perfusion (bath or pipette solution) over a period of 1 min in order to exclude false results introduced by sheer forces due to

the flow of the perfusate. Microelectrodes were vertically pulled from borosilicate glass capillaries (GB150F-10, Science Products, Hofheim, Germany) for outside-out patches and from Garner Glass, Type 7052 (Claremont, California, USA) for inside-out patches. Patch pipettes used had usually tip resistances of 5–8 MegaOhm. Test solutions were applied via a gravity-driven perfusion system (ALA Scientific Instruments Westbury, New York, USA). For rapid solution exchange (about 300–500 ms) membrane patches were held in a stream of the experimental solution from a second pipette. Analog signals were amplified with an Axopatch 200B amplifier (Axon Instruments/ Molecular Devices, Sunnyvale, California, USA) and converted to digital signals by an Axon Instruments 1322A Digidata interface. Recordings were taken with a low pass Bessel filter at a frequency of 5 kHz and filtered offline at 1 kHz before further analyses using Axon pClamp10 software (Clampfit, Axon Instruments). Channel *Po* was expressed as $P_{open} = NPo/n$, where $NPo = [(t_o)/(t_o + t_c)]$, Po = open probability for one channel, t_o = sum of open times, t_c = sum of closed times, N = actual number of channels in the patch, and n = maximum number of individual channels observed in the patch at +30 mV. Channel mean open time and unitary current amplitudes were measured using Clampfit software (Axon Instruments).

Table 1 | Effect of extracellular ACA (ACAe) on BK channel open probability (*Po*) compared to control conditions (con) at 1.2 μM $[Ca^{2+}]$i and after wash out (w. o.).

[ACA]e	*Po* con	*Po* ACAe	*Po* w. o.
300 μM ($n = 7$)	0.056 ± 0.015	0.059 ± 0.017	0.060 ± 0.020
1 mM ($n = 9$)	0.046 ± 0.017	0.048 ± 0.019	0.041 ± 0.010
3 mM ($n = 6$)	0.039 ± 0.005	0.043 ± 0.005	0.041 ± 0.005
10 mM ($n = 6$)	0.079 ± 0.043	0.072 ± 0.039	0.066 ± 0.035

Extracellular ACA in different concentrations ([ACA]e) did not affect BK channel open probability.

Table 2 | Effect of 30 mM EtOH on BK channel open probability (*Po*) compared to control conditions (con) at different levels of $[Ca^{2+}]$i.

$[Ca^{2+}]$i	*Po* con	*Po* EtOH	*Po* w. o.
1.2 μM ($n = 23$)	0.065 ± 0.011	0.097 ± 0.012***	0.069 ± 0.011
3 μM ($n = 9$)	0.102 ± 0.023	0.120 ± 0.026**	0.101 ± 0.032
10 μM ($n = 8$)	0.246 ± 0.072	0.238 ± 0.063	0.207 ± 0.074

*The increasing effect was fully reversible after wash out (w. o.). Paired Student's t-test: ***$p < 0.001$, **$p < 0.01$.*

STATISTICS

Measurements were replicated several times with different membrane patches. The number of recordings (i.e., "replicates" or n) per experiment is mentioned in the text or in the legends of the graphs. Each recording or n represents a single patch of an individual cell. Data are shown as arithmetic mean ± standard error of mean (SEM). Since original data partially exhibit non-normality and heteroscedasticity, the respective data sets were subjected to appropriate transformations [logit-transformation for *Po* and log-transformation for MCOT] before parametric statistical testing was applied. For statistical analyses the following parametric tests were then performed on the transformed data: Paired or unpaired Student's *t*-test, One-way or Repeated Measures ANOVA followed by Bonfferoni-corrected *post-hoc* tests. Statistic significance was assumed at a *p*-value of <0.05. Dose-response-relation was fitted with GraphPad Prism (GraphPad Software Inc., San Diego, USA) to the following sigmoidal dose-response-equation: $Y = 1/(1 + 10\hat{}((\text{LogEC50-X}) \times \text{HillSlope}))$. X is the logarithm of concentration, Y is the response, EC50 is the half maximal effective concentration.

RESULTS

EXTRACELLULAR ACA

Extracellular ACA did not affect BK channel properties irrespective of the concentration applied [at free internal Ca^{2+} concentrations ($[Ca^{2+}]$i) of 1.2 μM]. Data of all experiments were analyzed with regard to ACA mediated alterations in BK channel *Po* (**Table 1**), mean channel amplitude and MCOT (data not shown).

INTRACELLULAR ACA

In single channel recordings from excised inside-out patches, ACA (100 μM) was applied to the intracellular side of the membrane. The effect of ACA was tested at ($[Ca^{2+}]$i) of 1.2 μM ($n = 10$), 3 μM ($n = 6$), and 10 μM ($n = 9$). BK channel *Po* and single channel amplitudes were not affected by internal ACA irrespective of the $[Ca^{2+}]$i (data not shown). However, mean open time of BK channels was significantly reduced at 1.2 μM $[Ca^{2+}]$i (control: 1.931 ± 0.507 ms; ACA: 1.721 ± 0.546 ms*, Paired Student's *t*-test: *$p < 0.05$), but not at 3 μM or 10 μM $[Ca^{2+}]$i.

EFFECT OF ETHANOL ON BK CHANNELS

The effect of EtOH was tested at different $[Ca^{2+}]$i of 1.2 μM, 3 μM, and 10 μM. Application of 30 mM isoosmolar EtOH increased BK channel *Po* significantly at low, but not at high $[Ca^{2+}]$i (**Table 2**; also see **Figures 1**, **2B**/left panels, respectively). The activation remained constant for the time of EtOH application (1 min) and was not transient as described previously by Jakab et al. (1997). Channel amplitudes and MCOTs were not affected (data not shown). The activating effect was fully reversible by reperfusion with bath solution.

COMBINED EFFECT OF EtOH AND ACA

Extracellular ACA application

In outside-out single channel recordings EtOH (30 mM) was applied simultaneously in combination with ACA (100 μM) to the extracellular side of cell membrane at 1.2 μM $[Ca^{2+}]$i. These experiments were done in order to reveal possible interactions of EtOH and ACA on BK channels at the outer surface of the membrane. BK channel *Po* was significantly increased by extracellular application of EtOH and ACA in combination. The EtOH-mediated increment of BK channel activity was highly significant irrespective of the presence of external ACA (**Figure 1**).

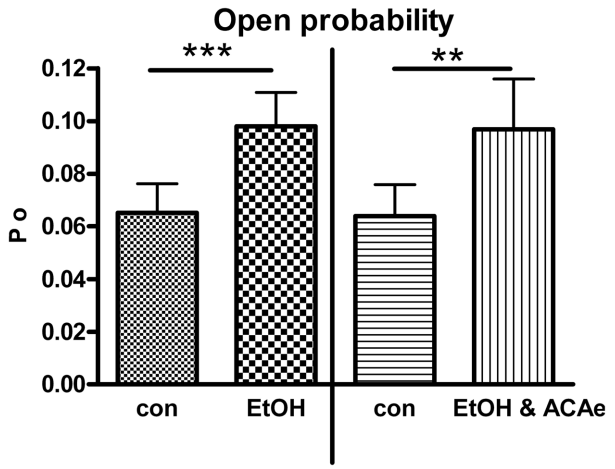


FIGURE 1 | Combined extracellular EtOH/ACA application. Effect of 30 mM EtOH in absence (left panel, Paired Student's *t*-test: $^{***}p < 0.001$, $n = 23$) and in presence of ACA at the extracellular side (ACAe, right panel, Paired Student's *t*-test: $^{**}p < 0.01$, $n = 16$). In both cases open probability (*Po*) was significantly increased compared to control (con).

Hence, external ACA was not able to affect EtOH action on BK channel activity. Amplitude and MCOT were not modified (data not shown).

Intracellular ACA application

In this experimental setting 30 mM isoosmolar EtOH was applied to outside-out patches via perfusion from the extracellular side. ACA was applied to the intracellular side of the membrane in a concentration of 100 μM by addition to the pipette solution. Therefore, internal ACA was present during the entire time course of the experiment. **Figure 2A** shows original traces from outside-out recordings under different experimental conditions corresponding to the bars in graph 2B. The increase of BK channel *Po* by extracellular EtOH, as shown in **Figure 2B** (left panel) was totally abolished by the simultaneous presence of 100 μM ACA at the intracellular side at 1.2 μM $[Ca^{2+}]$i (**Figure 2B**/right panel). Furthermore, MCOT was significantly diminished in the presence of internal ACA (**Figure 2C**), but mean channel amplitudes were not affected.

The suppression of the EtOH-mediated increment of BK channel activity by internal ACA was dose dependent. Experiments were performed at 1.2 μM $[Ca^{2+}]$i and intracellular ACA concentrations were increased from 30 nM ($n = 10$), 100 nM ($n = 6$), 300 nM ($n = 6$), 1 μM ($n = 6$), 30 μM ($n = 7$), 100 μM ($n = 13$) to 300 μM ($n = 6$). **Figure 3** shows a dose–response relationship with an EC50 at 403 nM ACA and a Hill coefficient (n_H) of −1.738. The augmentation of BK channel *Po* at 30 mM isoosmolar EtOH as reference was set to 1.

EtOH AND ACA APPLICATION IN VARIABLE SUCCESSION

In further experiments we tested if there is any difference in the interaction of EtOH and ACA in dependency of succession

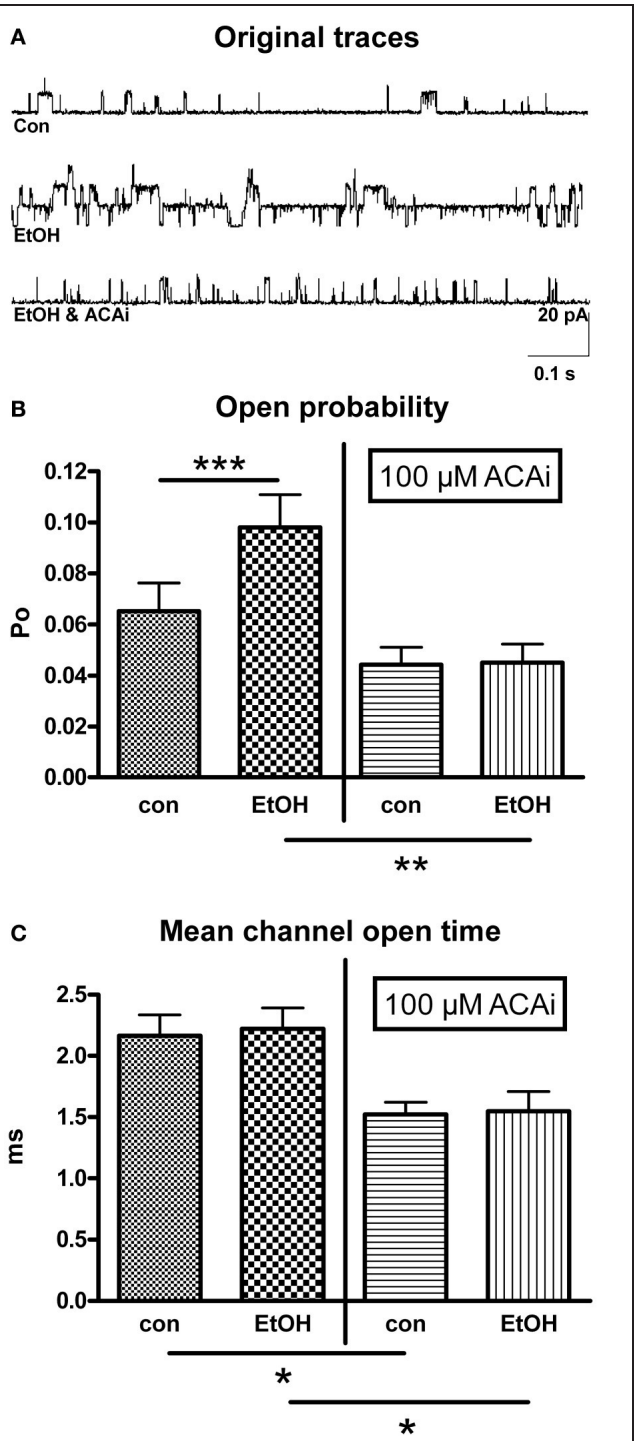


FIGURE 2 | Effect of EtOH in absence and presence of internal ACA (ACAi). (A) Representative original traces from outside-out patches under control (con) conditions, during perfusion with EtOH alone and under the influence of EtOH and ACAi. **(B)** Open probability (*Po*) was significantly increased ($n = 23$, Paired Student's *t*-test: $^{***}p < 0.001$) by 30 mM EtOH compared to control (con, left panel). This increment was totally abolished in the presence of 100 μM ACAi (right panel, $n = 13$, Unpaired Student's *t*-test: $^{**}p < 0.01$). **(C)** Mean channel open time was significantly reduced in the presence of 100 μM ACAi (Unpaired Student's *t*-test: $^{*}p < 0.05$) under control conditions as well as under EtOH.

of the drugs. Our results indicate a "first come, first serve" effect. In inside-out experiments 30 mM EtOH and/or 100 μM ACA were applied to the intracellular side of the membrane in varying sequences. Recordings were performed at 1.2 μM $[Ca^{2+}]i$. Original data of the individual experiments as well as the sequence of application are listed in **Tables 3**, **4** and **Figure 4**, respectively.

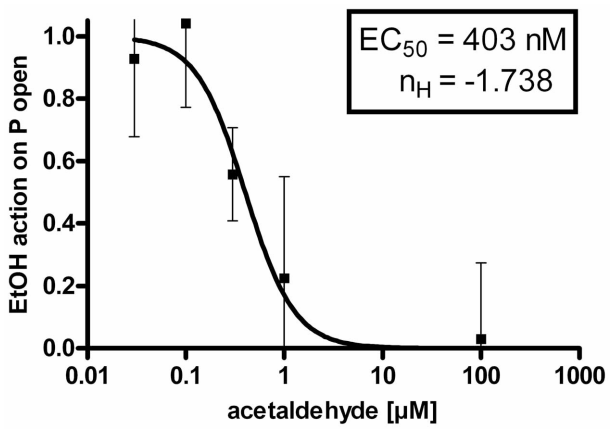


FIGURE 3 | Dose-response-relationship: The EtOH effect on BK channel open probability (P open) was progressively inhibited by increasing concentrations of ACA at the intracellular side of the membrane (EC_{50} = half maximal effective concentration = 403 ± 108 nM, n_H = Hill coefficient = −1.738 ± 0.731, semilogarithmic graph).

Table 3 | Sequence (↓) of application from top to bottom: con–EtOH–ACA–EtOH and ACA together.

Application sequence	*Po*
con	0.165 ± 0.065
EtOH (30 mM)	0.214 ± 0.086*
ACA (100 μM)	0.191 ± 0.076
EtOH (30 mM) and ACA (100 μM)	0.164 ± 0.066

*Only the application of EtOH alone resulted in a significant increase of BK channel open probability (Po), (n = 5, Repeated Measures ANOVA followed by Bonferroni's Multiple Comparison Test: *p < 0.05) compared to control (con).*

Table 4 | Sequence (↓) of application from top to bottom: con–ACA–EtOH.

Application sequence	*Po*	MCOT
con	0.258 ± 0.084	1.297 ± 0.253
ACA (100 μM)	0.267 ± 0.092	1.075 ± 0.235**
EtOH (30 mM)	0.260 ± 0.084	1.354 ± 0.291

*BK channel open probability (Po) was affected neither by ACA nor by EtOH (n = 6, Repeated Measures ANOVA followed by Bonferroni's Multiple Comparison Test), but mean channel open time (MCOT) was significantly lower under ACA treatment compared to MCOT under EtOH (ACA vs. EtOH, **p < 0.01, Paired t-test).*

ACA SUCCEEDS AN EtOH APPLICATION

As known from previous experiments (see also **Table 2**) BK channel *Po* was increased by EtOH when applied subsequent to a control solution. The following ACA reduced EtOH action. During ensuing application of EtOH and ACA in combination BK channel activity recovered to control level. **Table 3** displays the sequence of application in direction of the arrow. The data show that the EtOH effect on BK channel *Po* is prevented following an ACA application and in presence of internal ACA, respectively. Channel amplitudes and mean open times were not affected (data not shown).

EtOH SUCCEEDS ACA APPLICATION

In this experimental setting the order was reversed, i.e., EtOH was applied following an ACA application. Both substances were applied separately. Internal ACA did not change BK channel *Po* significantly (as already described above), but surprisingly the action of a following EtOH application was inhibited regardless of the absence of ACA (**Table 4**). This indicates that the prevention of an EtOH-mediated increment on the *Po* was a lasting effect that occurred also after removal of ACA within the experimental time of 30 s after switching from ACA to EtOH. Channel amplitudes were influenced neither by EtOH nor by ACA, but MCOT was significantly lower under the impact of ACA compared to EtOH conditions.

PERMANENT PRESENCE OF EtOH

BK channel *Po* was significantly increased by the first EtOH application. In the presence of ACA this EtOH-mediated increment was progressively reduced and abolished, even when ACA was removed. A second application of EtOH alone was not able to activate BK channels anymore (**Figure 4A**). In control experiments we could show that EtOH is well able, however, to cause BK channel activation a second time, after a 1 min wash out with control solution (**Figure 4B**). Inhibition of another EtOH action did not arise without preceding ACA application. In both experimental settings channel amplitudes and MCOTs were not affected (data not shown).

EFFECT OF ACA ON HYPOTONICITY INDUCED BK CHANNEL ACTIVATION

Beside EtOH, Hypo is also well known to mediate BK channel activation (Jakab et al., 2006). To investigate whether ACA specifically modulated EtOH-induced BK channel activation or, rather, ACA modulatory action extended to other BK channel activators, we tested the effect of internal ACA on Hypo-induced BK channel activation in outside-out recordings. A 30% hypotonic solution increased BK channel activity significantly. Internal ACA (100 μM) was not able to modify this increasing effect (**Figure 5**).

DISCUSSION

ACA is supposed to be responsible for some of the pharmacological and neurobehavioral effects which so far have been assigned to EtOH (Quertemont et al., 2005a,b). We focused our investigation on the direct ACA-mediated effects on BK channels as well as on the interference of ACA and EtOH. EtOH increases

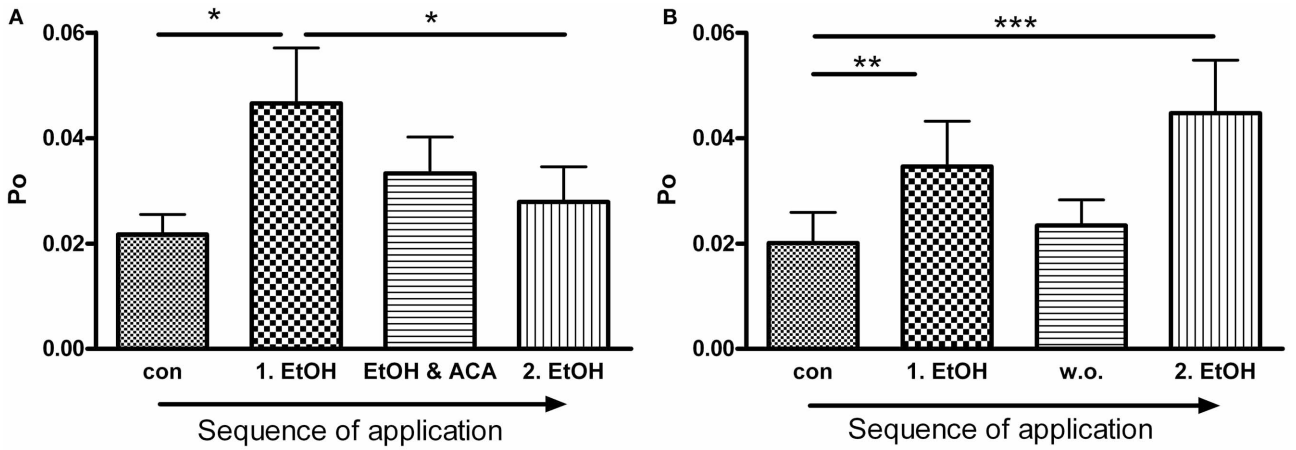


FIGURE 4 | ACA inhibits further EtOH action: (A) BK channel open probability (*Po*) was significantly increased by the first EtOH application. The following simultaneous treatment with ACA and EtOH reduced the EtOH induced increment and prevented activation by a 2nd separate EtOH (2.EtOH) application ($n = 11$, con vs. 1.EtOH $^*p < 0.05$, 1.EtOH vs. 2.EtOH $^*p < 0.05$, Repeated Measures ANOVA followed by Bonferroni's Multiple Comparison Test). **(B)** In control experiments a 2nd separate EtOH (2.EtOH) application after perfusion with control solution (1 min, wash out, w. o.) increased BK channel activity significantly. Without a preceding ACA application EtOH mediated activation was not impeded (outside-out patches, $n = 9$, con vs. 1.EtOH $^{**}p < 0.01$, con vs. 2.EtOH $^{***}p < 0.001$, Repeated Measures ANOVA followed by Bonferroni's Multiple Comparison Test).

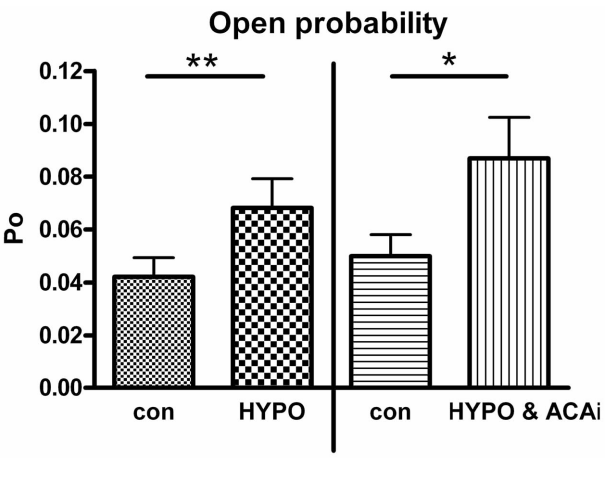


FIGURE 5 | Effect of internal ACA on hypotonicity induced BK channel activation. BK channel open probability was significantly increased by a hypotonic solution at 1.2 μM $[Ca^{2+}]i$ (left panel, Paired Student's *t*-test: $^{**}p < 0.01$, $n = 4$). Internal ACA (ACAi) did not affect this hypotonicity induced activation (right panel, Paired Student's *t*-test: $^*p < 0.05$, $n = 12$).

BK channel *Po* (Dopico et al., 1996; Jakab et al., 1997). ACA, as the first metabolic EtOH product, occurs concurrently with its progenitor under physiological conditions and hence we hypothesized that the simultaneous application of both substances may cause interactions.

Extracellular application of ACA and EtOH represents a physiological situation which occurs when these molecules diffuse from the vascular system to the cells within the brain. EtOH as an amphiphilic molecule is able to penetrate the blood brain barrier (BBB) (Mendelson et al., 1990), to pass the lipid phase of cell membranes and to diffuse within the cytosol. ACA should at least partly be able to pass the BBB by diffusion (Quertemont et al., 2005a; Correa et al., 2011). However, since aldehyde dehydrogenase (ALDH) is highly effective in endothelial cells of the BBB, the amount of ACA in the brain produced by peripheral EtOH metabolism is rather small (Deitrich, 1987; Zimatkin, 1991). Hence, ACA concentrations derived from blood circulation are thought to be insufficient to cause central effects within the brain (Zimatkin et al., 2006; Zimatkin and Buben, 2007). Recent research indicates that both central EtOH degradation by catalase and peripherally produced ACA contribute to ACA accumulation in the brain (Jamal et al., 2007). We therefore tested if extracellular application of ACA influences BK channels. Concentrations of up to 10 mM did not affect BK channel properties. We also applied EtOH and ACA simultaneously to the extracellular side of the channels, but ACA did not alter the augmented channel activity induced by the action of EtOH. Since internally applied ACA, as discussed below, reduced BK channel mean open time significantly, these experiments support the idea that ACA is not able to cross the cell membrane in the short time range of a few minutes.

After alcohol consumption ACA and EtOH are present together in the body. However, the concurrent application of EtOH and ACA has not been investigated previously. Concerning the simultaneous existence of EtOH and ACA in the brain as a consequence of EtOH degradation it was shown that EtOH oxidation occurs in the living brain. A study by Zimatkin et al. (2006) confirmed that catalase, an enzyme which predominantly occurs in peroxisomes, plays a major role in the brain EtOH metabolism. The finding of ACA accumulation within cells (Zimatkin et al., 2006; Zimatkin and Buben, 2007) led to the conclusion that ACA can achieve some of its effects from the intracellular side of the membrane. Our experiments show that

intracellular ACA prevents the EtOH mediated increment of BK channel activity. This inhibition of the EtOH action on the BK channels was dose-dependent. The inhibitory impact of ACA on EtOH-induced BK channel activation did not change single channel conductance which indicates that ACA does not interfere with potassium ions passing through the channel, neither does EtOH affect this ion passage (Brodie et al., 2007; Treistman and Martin, 2009). MCOT was reduced in the presence of internal ACA which points to an interaction of ACA with the channel gating process. The reduction of MCOT by ACA was observed when applied together with EtOH, but also when ACA was applied separately, suggesting a direct, non-EtOH dependent interaction of ACA with BK channels. In all our studies EtOH was unable to activate BK channels after a preceding internal ACA application, and most notably, the prevention of EtOH activation was a lasting effect which persisted after ACA removal, i.e., the continued presence of ACA was not mandatory. On the other hand, if EtOH was applied previous to ACA, it was not able to sustain its effect on BK channels. The EtOH-mediated increment of BK channel *Po* was rapidly reduced and finally abolished in spite of the continued presence of EtOH. In this respect EtOH and ACA appear to obey a "first come, first serve" rule, since ACA was able to counteract the action of acute EtOH on BK channels in a lasting way when applied first.

BK channels are known to play a key role in behavioral tolerance to EtOH, since BK loss-of-function mutants of *C. elegans* are resistant to EtOH (Davies et al., 2003). Furthermore, Cowmeadow et al. (2005) could show in *D. melanogaster* that EtOH tolerance was only observed when BK channels were expressed. In BK null flies the capacity for tolerance was eliminated. Tolerance develops as a consequence of prolonged or repeated drug consumption. This raises the question whether ACA may contribute to the mechanism(s) causing tolerance. In fact, BK channels display tolerance to EtOH-mediated effects after short- or long-term exposure which is manifested by a decrease in BK channel potentiation under continuous or repeated EtOH exposure (Jakab et al., 1997; Pietrzykowski et al., 2004; Yuan et al., 2008). This so-called molecular tolerance is intrinsic to BK channel alpha (α)-subunits and appears in the form of reduced sensitivity to EtOH within a few minutes due to a decrease in Ca^{2+} sensitivity during persistent exposure (Feinberg-Zadek et al., 2008). In presence of the accessory and modulatory β4-subunit tolerance disappears (Martin et al., 2008). The lipid environment is an additional crucial factor modulating intrinsic tolerance of BK channel α-subunits (Yuan et al., 2008). Physiologically activation of BK channels by EtOH alters action potential discharge activity and neurotransmitter release. Since the cell tries to countervail against these alterations in order to keep the system in balance, these perturbations on the molecular level may have powerful influence on behavioral tolerance and addiction (Treistman and Martin, 2009). As the reduction of sensitivity to EtOH is a considerable component of tolerance ACA could be involved in this process. It could be argued that the inhibitory impact of internal ACA on EtOH related augmentation of BK channel activity reflects a kind of 'protective' effect under acute EtOH exposure, maintaining neuronal activity and excitability. This is interesting with regard to the continued ACA action which is preserved also after its removal.

Our study shows that internal ACA reduces MCOT under control conditions as well as in presence of EtOH. This reduction of MCOT did not result in alterations of *Po*, which can be explained by more frequent channel openings. We interpret this result as evidence of an interaction of ACA with the BK channel gating mechanism. It remains to be investigated if this is a direct effect where ACA interacts with some site of the channel protein, or is an indirect effect via some signaling pathways, such as phosphorylation. There is evidence that the EtOH-related activation of BK channels is due to the stimulation of PKC (Jakab et al., 1997) indicating that phosphorylation is an efficient modulatory factor in this process (Liu et al., 2006). Therefore, the inhibition of the EtOH-related effect via ACA could be caused by prevention of PKC-mediated phosphorylation. The mechanisms of ACA engagement in PKC phosphorylation processes need further investigation.

Since the functional efficiency of some PKC species relies on intracellular Ca^{2+} availability ACA could achieve its counteracting effect on EtOH-induced BK channel activation by engaging with the Ca^{2+} influx into the cytosol. These considerations agree with the finding that ACA inhibits voltage-dependent Ca^{2+} channels. The inhibition of L-type Ca^{2+} channels by ACA was demonstrated both in neurons (Bergamaschi et al., 1988) and smooth muscle cells (Morales et al., 1997). Liu et al. (2008) postulate that EtOH may simply act as an adjuvant of activating Ca^{2+} by selectively facilitating Ca^{2+}-driven gating, but without triggering alterations in protein conformation of BK channels or rearrangement of subunits. In addition, EtOH was shown to fail its activating action on BK channels in the absence of Ca^{2+}. In fact, EtOH activation of BK channels depends on the amount of internally present Ca^{2+}, displaying potentiation only at low but not at high Ca^{2+} concentrations (Dopico et al., 1998). Our results in this study confirm these findings. The effectiveness of the Ca^{2+} action depends on the high affinity sensors within the intracellular BK channel tail of the α-subunit, namely the calcium bowl and the RCK1 (regulatory domain of K conductance). However, the RCK 1 domain is sufficient to promote inhibition at high Ca^{2+} levels. Hence, very high internal Ca^{2+} concentrations have a toxic impact on the physiological state of the cell, since inhibition of BK channels implies a lack of protection from excitotoxicity (Liu et al., 2008). The findings of our study demonstrate that internal ACA at high nanomolar concentrations is able to counteract BK channel potentiation by EtOH at low Ca^{2+} levels. At high Ca^{2+} levels ACA did not exhibit any decreasing effect on BK channel activity or MCOT, indicating that ACA is not able to override the impact of high Ca^{2+}.

Beside EtOH, Hypo is another mechanism which leads to an increase of BK channel activity (Jakab et al., 2006). The presence of intracellular ACA did not prevent the activation of BK channels by Hypo. These findings suggest that EtOH and Hypo affect BK channels by different mechanisms and implicate a specific interaction of ACA and EtOH on BK channels.

The reasons for the stunning absence of basic knowledge concerning effects of ACA on ion channels may be due to its chemical and physical characteristics. ACA is highly volatile at

room temperature which complicates the application of ACA in experiments performed especially *in vitro*. A further problem is that ACA concentrations of both blood and brain are difficult to quantify since the techniques to measure ACA levels by brain micro-dialysis *in vivo* is limited. Moreover, *in vivo* administered ACA is rapidly converted to EtOH by alcohol dehydrogenase (ADH) in the liver and to acetate by ALDH in the liver and in the brain. In addition the question whether significant ACA concentrations accumulate in the brain after alcohol ingestion is still a topic of controversial discussions (Deng and Deitrich, 2008; Correa et al., 2011). In consideration of these experimental and methodical restrictions it is not surprising that research on ACA is difficult and may still lead to inconsistent results.

In summary, our study supports the notion that ACA is a key player in the context of EtOH action. ACA achieves its immediate effects on BK channels only from the intracellular side of the membrane. Furthermore, ACA does not interfere with BK channel activation by Hypo. This evidence suggests that EtOH, ACA, and Hypo affect BK channels via different mechanisms. The inhibitory impact of ACA on the EtOH mediated increase of BK channel activity implicates that ACA has to be carefully taken into account if EtOH effects are studied. ACA and EtOH should be treated as an entity in the context of the EtOH action, whose compound effects may be more dramatic than those of the individual drugs.

ACKNOWLEDGMENTS

We gratefully acknowledge Karin Oberascher for technical support and Verena Kainz for assistance in cell culture. This work was supported by the Stiftungs und Förderungsgesellschaft der Paris-Lodron-Universität Salzburg and by a DOC-fFORTE—fellowship of the Austrian Academy of Science (grant no 23212) awarded to Astrid Handlechner.

REFERENCES

Bergamaschi, S., Govoni, S., Rius, R. A., and Trabucchi, M. (1988). Acute ethanol and acetaldehyde administration produce similar effects on L-type calcium channels in rat brain. *Alcohol* 5, 337–340. doi: 10.1016/0741-8329(88)90076-6

Brodie, M. S., Scholz, A., Weiger, T. M., and Dopico, A. M. (2007). Ethanol interactions with calcium-dependent potassium channels. *Alcohol. Clin. Exp. Res.* 31, 1625–1632. (Review). doi: 10.1111/j.1530-0277.2007.00469.x

Chatterjee, O., Taylor, L. A., Ahmed, S., Nagaraj, S., Hall, J. J., Finckbeiner, S. M., et al. (2009). Social stress alters expression of large conductance calcium-activated potassium channel subunits in mouse adrenal medulla and pituitary glands. *J. Neuoendocrinol.* 21, 167–176. doi: 10.1111/j.1365-2826.2009.01823.x

Church, J., Baxter, K. A., and McLarnon, J. G. (1998). pH modulation of Ca^{2+} responses and a Ca^{2+}-dependent K^+ channel in cultured rat hippocampal neurons. *J. Physiol.* 511(Pt 1), 119–132. doi: 10.1111/j.1469-7793.1998.119bi.x

Correa, M., Salamone, J. D., Segovia, K. N., Pardo, M., Longoni, R., Spina, L., et al. (2011). Piecing together the puzzle of acetaldehyde as a neuroactive agent. *Neurosci. Biobehav. Rev.* 36, 404–430. doi: 10.1016/j.neubiorev.2011.07.009

Cowmeadow, R. B., Krishnan, H. R., and Atkinson, N. S. (2005). The slowpoke gene is necessary for rapid ethanol tolerance in Drosophila. *Alcohol. Clin. Exp. Res.* 29, 1777–1786. doi: 10.1097/01.alc.0000183232.56788.62

Davies, A. G., Pierce-Shimomura, J. T., Kim, H., Vanhoven, M. K., Thiele, T. R., Bonci, A., et al. (2003). A central role of the BK potassium channel in behavioral responses to ethanol in *C. elegans*. *Cell* 115, 655–666. doi: 10.1016/S0092-8674(03)00979-6

Deitrich, R. A. (1987). Specificity of the action of ethanol in the central nervous system: behavioral effects. *Alcohol Alcohol. Suppl.* 1, 133–138.

Deng, X. S., and Deitrich, R. A. (2008). Putative role of brain acetaldehyde in ethanol addiction. *Curr. Drug Abuse Rev.* 1, 3–8. doi: 10.2174/1874473710801010003

DiChiara, T. J., and Reinhart, P. H. (1997). Redox modulation of hslo Ca^{2+}-activated K^+ channels. *J. Neurosci.* 17, 4942–4955.

Dopico, A. M., Anantharam, V., and Treistman, S. N. (1998). Ethanol increases the activity of Ca(++)-dependent K+ (mslo) channels: functional interaction with cytosolic Ca++. *J. Pharmacol. Exp. Ther.* 284, 258–268.

Dopico, A. M., Chu, B., Lemos, J. R., and Treistman, S. N. (1999). Alcohol modulation of calcium-activated potassium channels. *Neurochem. Int.* 35, 103–106. doi: 10.1016/S0197-0186(99)00051-0

Dopico, A. M., Lemos, J. R., and Treistman, S. N. (1996). Ethanol increases the activity of large conductance, Ca^{2+}-activated K^+ channels in isolated neurohypophysial terminals. *Mol. Pharmacol.* 49, 40–48.

Eriksson, C. J. (2001). The role of acetaldehyde in the actions of alcohol (update 2000). *Alcohol. Clin. Exp. Res.* 25(5 Suppl. ISBRA), 15S–32S. doi: 10.1111/j.1530-0277.2001.tb02369.x

Feinberg-Zadek, P. L., Martin, G., and Treistman, S. N. (2008). BK channel subunit composition modulates molecular tolerance to ethanol. *Alcohol. Clin. Exp. Res.* 32, 1207–1216. doi: 10.1111/j.1530-0277.2008.00704.x

Foddai, M., Dosia, G., Spiga, S., and Diana, M. (2004). Acetaldehyde increases dopaminergic neuronal activity in the VTA. *Neuropsychopharmacology* 29, 530–536. doi: 10.1038/sj.npp.1300326

Hermann, A., Sitdikova, G. F., and Weiger, T. M. (2012a). "BK Channels—Focus on polyamines, ethanol/acetaldehyde and hydrogen sulfide (H2S)," in *Patch Clamp Technique*, ed F. S. Kaneez (Rijeka: InTech), 109–142.

Hermann, A., Sitdikova, G. F., and Weiger, T. M. (2012b). "Modulated by gasotransmitters: BK channels," in *Gasotransmitters: Physiology and Pathophysiology*, eds A. Hermann, G. F. Sitdikova, and T. M. Weiger (Berlin, Heidelberg, New York: Springer), 163–201.

Jakab, M., Schmidt, S., Grundbichler, M., Paulmichl, M., Hermann, A., Weiger, T., et al. (2006). Hypotonicity and ethanol modulate BK channel activity and chloride currents in GH4/C1 pituitary tumour cells. *Acta Physiol. (Oxf.)* 187, 51–59. doi: 10.1111/j.1748-1716.2006.01544.x

Jakab, M., Weiger, T. M., and Hermann, A. (1997). Ethanol activates maxi Ca^{2+}-activated K^+ channels of clonal pituitary (GH3) cells. *J. Membr. Biol.* 157, 237–245. doi: 10.1007/PL00005895

Jamal, M., Ameno, K., Uekita, I., Kumihashi, M., Wang, W., and Ijiri, I. (2007). Catalase mediates acetaldehyde formation in the striatum of free-moving rats. *Neurotoxicol.* 28, 1245–1248. doi: 10.1016/j.neuro.2007.05.002

Karahanian, E., Quintanilla, M. E., Tampier, L., Rivera-Meza, M., Bustamante, D., Gonzalez-Lira, V., et al. (2011). Ethanol as a prodrug: brain metabolism of ethanol mediates its reinforcing effects. *Alcohol. Clin. Exp. Res.* 35, 606–612. doi: 10.1111/j.1530-0277.2011.01439.x

Liu, J., Asuncion-Chin, M., Liu, P., and Dopico, A. M. (2006). CaM kinase II phosphorylation of slo Thr107 regulates activity and ethanol responses of BK channels. *Nat. Neurosci.* 9, 41–49. doi: 10.1038/nn1602

Liu, J., Vaithianathan, T., Manivannan, K., Parrill, A., and Dopico, A. M. (2008). Ethanol modulates BKCa channels by acting as an adjuvant of calcium. *Mol. Pharmacol.* 74, 628–640. doi: 10.1124/mol.108.048694

Martin, G. E., Hendrickson, L. M., Penta, K. L., Friesen, R. M., Pietrzykowski, A. Z., Tapper, A. R., et al. (2008). Identification of a BK channel auxiliary protein controlling molecular and behavioral tolerance to alcohol. *Proc. Natl. Acad. Sci. U.S.A.* 105, 17543–17548. doi: 10.1073/pnas.0801068105

McManus, O. B. (1991). Calcium-activated potassium channels: regulation by calcium. *J. Bioenerg. Biomembr.* 23, 537–560.

Melis, M., Enrico, P., Peana, A. T., and Diana, M. (2007). Acetaldehyde mediates alcohol activation of the mesolimbic dopamine system. *Eur. J. Neurosci.* 26, 2824–2833. doi: 10.1111/j.1460-9568.2007.05887.x

Mendelson, J., Woods, B. T., Chiu, T. M., Mello, N. K., Lukas, S. E., Teoh, S. K., et al. (1990). Measurement of brain ethanol concentrations in humans with *in vivo* proton magnetic resonance spectroscopy. *NIDA Res. Monogr.* 105, 68–74.

Montgomery, J. R., Whitt, J. P., Wright, B. N., Lai, M. H., and Meredith, A. L. (2013). Mis-expression of the BK K(+) channel disrupts suprachiasmatic nucleus circuit rhythmicity and alters clock-controlled behavior. *Am. J. Physiol. Cell Physiol.* 304, C299–C311. doi: 10.1152/ajpcell.00302.2012

Morales, J. A., Ram, J. L., Song, J., and Brown, R. A. (1997). Acetaldehyde inhibits current through voltage-dependent calcium channels. *Toxicol. Appl. Pharmacol.* 143, 70–74. doi: 10.1006/taap.1996.8072

Pietrzykowski, A. Z., Martin, G. E., Puig, S. I., Knott, T. K., Lemos, J. R., and Treistman, S. N. (2004). Alcohol tolerance in large-conductance, calcium-activated potassium channels of CNS terminals is intrinsic and includes two components: decreased ethanol potentiation and decreased channel density. *J Neurosci.* 24, 8322–8332. doi: 10.1523/JNEUROSCI.1536-04.2004

Quertemont, E., Tambour, S., and Tirelli, E. (2005a). The role of acetaldehyde in the neurobehavioral effects of ethanol: a comprehensive review of animal studies. *Progress Neurobiol.* 75, 247–274. doi: 10.1016/j.pneurobio.2005.03.003

Quertemont, E., Eriksson, C. J., Zimatkin, S. M., Pronko, P. S., Diana, M., Pisano, M., et al. (2005b). Is ethanol a pro-drug? Acetaldehyde contribution to brain ethanol effects. *Alcohol. Clin. Exp. Res.* 29, 1514–1521.

Reinhart, P. H., Chung, S., Martin, B. L., Brautigan, D. L., and Levitan, I. B. (1991). Modulation of calcium-activated potassium channels from rat brain by protein kinase A and phosphatase 2A. *J. Neurosci.* 11, 61627–61635.

Reinhart, P. H., and Levitan, I. B. (1995). Kinase and phosphatase activities intimately associated with a reconstituted calcium-dependent potassium channel. *J. Neurosci.* 15, 4572–4579.

Rodd-Henricks, Z. A., Melendez, R. I., Zaffaroni, A., Goldstein, A., McBride, W. J., and Li, T. K. (2002). The reinforcing effects of acetaldehyde in the posterior ventral tegmental area of alcohol-preferring rats. *Pharmacol. Biochem. Behav.* 72, 55–64. doi: 10.1016/S0091-3057(01)00733-X

Sitdikova, G. F., Weiger, T. M., and Hermann, A. (2010). Hydrogen sulfide increases calcium-activated potassium (BK) channel activity of rat pituitary tumor cells. *Pflüg. Arch.* 459, 389–397. doi: 10.1007/s00424-009-0737-0

Tashjian, A. H. Jr., Bancroft, F. C., and Levine, L. (1970). Production of both prolactin and growth hormone by clonal strains of rat pituitary tumor cells. Differential effects of hydrocortisone and tissue extracts. *J. Cell Biol.* 47, 61–70.

Treistman, S. N., and Martin, G. E. (2009). BK Channels: mediators and models for alcohol tolerance. *Trends Neurosci.* 32, 629–637. doi: 10.1016/j.tins.2009.08.001

Weiger, T. M., and Hermann, A. (2009). "Modulation of potassium channels by polyamines," in *Biological Aspects of Biogenic Amines, Polyamines and Conjugates*, ed G. Dandrifosse (Kerala: Transworld Research Network), 185–199.

Weiger, T. M., Holmqvist, M. H., Levitan, I. B., Clark, F. T., Sprague, S., Huang, W. J., et al. (2000). A novel nervous system beta subunit that downregulates human large conductance calcium-dependent potassium channels. *J. Neurosci.* 20, 3563–3570.

Yuan, C., O'Connell, R. J., Wilson, A., Pietrzykowski, A. Z., and Treistman, S. N. (2008). Acute alcohol tolerance is intrinsic to the BKCa protein, but is modulated by the lipid environment. *J Biol. Chem.* 283, 5090–5098. doi: 10.1074/jbc.M708214200

Zimatkin, S. M. (1991). Histochemical study of aldehyde dehydrogenase in the rat CNS. *J. Neurochem.* 56, 1–11. doi: 10.1111/j.1471-4159.1991.tb02555.x

Zimatkin, S. M., and Buben, A. L. (2007). Ethanol oxidation in the living brain. *Alcohol Alcohol.* 42, 529–532. doi: 10.1093/alcalc/agm059

Zimatkin, S. M., Liopo, A. V., and Deitrich, R. A. (1998). Distribution and kinetics of ethanol metabolism in rat brain. *Alcohol. Clin. Exp. Res.* 22, 1623–1627. doi: 10.1111/j.1530-0277.1998.tb03958.x

Zimatkin, S. M., Pronko, S. P., Vasiliou, V., Gonzalez, F. J., and Deitrich, R. A. (2006). Enzymatic mechanisms of ethanol oxidation in the brain. *Alcohol. Clin. Exp. Res.* 30, 1500–1505. doi: 10.1111/j.1530-0277.2006.00181.x

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 19 March 2013; accepted: 17 May 2013; published online: 14 June 2013.

Citation: Handlechner AG, Hermann A, Fuchs R and Weiger TM (2013) Acetaldehyde-ethanol interactions on calcium-activated potassium (BK) channels in pituitary tumor (GH3) cells. Front. Behav. Neurosci. 7:58. doi: 10.3389/fnbeh.2013.00058

frontiers in
BEHAVIORAL NEUROSCIENCE

REVIEW ARTICLE
published: 24 May 2013
doi: 10.3389/fnbeh.2013.00052

Salsolinol modulation of dopamine neurons

Guiqin Xie[1,2], Krešimir Krnjević[3] and Jiang-Hong Ye[1]*

[1] *Department of Anesthesiology, Pharmacology, and Physiology, New Jersey Medical School, University of Medicine and Dentistry of New Jersey, Newark, NJ, USA*
[2] *Department of Physiology, Nanjing Medical University, Nanjing, China*
[3] *Department of Physiology, McGill University, Montreal, QC, Canada*

Edited by:
Merce Correa, University Jaume I, Spain

Reviewed by:
Mark S. Brodie, University of Illinois at Chicago, USA
Luis Granero, Universitat de Valencia, Spain

****Correspondence:***
Jiang-Hong Ye, Department of Anesthesiology, Pharmacology, and Physiology, New Jersey Medical School, University of Medicine and Dentistry of New Jersey, Newark, NJ 07103, USA.
e-mail: ye@umdnj.edu

Salsolinol, a tetrahydroisoquinoline present in the human and rat brains, is the condensation product of dopamine and acetaldehyde, the first metabolite of ethanol. Previous evidence obtained *in vivo* links salsolinol with the mesolimbic dopaminergic (DA) system: salsolinol is self-administered into the posterior of the ventral tegmental area (pVTA) of rats; intra-VTA administration of salsolinol induces a strong conditional place preference and increases dopamine release in the nucleus accumbens (NAc). However, the underlying neuronal mechanisms are unclear. Here we present an overview of some of the recent research on this topic. Electrophysiological studies reveal that DA neurons in the pVTA are a target of salsolinol. In acute brain slices from rats, salsolinol increases the excitability and accelerates the ongoing firing of dopamine neurons in the pVTA. Intriguingly, this action of salsolinol involves multiple pre- and post-synaptic mechanisms, including: (1) depolarizing dopamine neurons; (2) by activating μ opioid receptors on the GABAergic inputs to dopamine neurons – which decreases GABAergic activity – dopamine neurons are disinhibited; and (3) enhancing presynaptic glutamatergic transmission onto dopamine neurons via activation of dopamine type 1 receptors, probably situated on the glutamatergic terminals. These novel mechanisms may contribute to the rewarding/reinforcing properties of salsolinol observed *in vivo*.

Keywords: reward, addictive property, electrophysiology, brain slices, mu opioid receptors, GABAergic transmission, glutamatergic transmissions, dopaminergic neurons

INTRODUCTION

Alcohol/Ethanol is one of the most widely used drugs and the global burden of alcoholism is immense, with an estimated 3.8% (Rehm et al., 2009) or 3.2% (Spanagel et al., 2010) of world-wide deaths attributed to alcohol consumption. Accumulating evidence suggests that some addictive properties of alcohol are generated by its metabolites, such as acetaldehyde and its derivatives, notably salsolinol (SAL, 1-methyl-1,2,3,4-tetrahydro-6,7-dihydroxy-isoquinolines) (Deng and Deitrich, 2008). SAL is formed from dopamine: either by non-enzymatic Pictet-Spengler condensation with acetaldehyde, yielding racemic (R/S)-SAL, or by combination with pyruvic acid, followed by enzymatic decarboxylation and reduction, producing (R)-SAL. In the brains of mammals racemic (R/S)-SAL is formed by the Pictet-Spengler condensation (Rommelspacher et al., 1995; Haber et al., 1996). In the human brain, enantio-selective (R)-SAL can be synthesized from dopamine and acetaldehyde by (R)-SAL synthase (Naoi et al., 1998). (R/S)-SAL is present in biological fluids such as urine, plasma, cerebrospinal fluid and postmortem brains of both alcoholics and non-alcoholics (Sjöquist et al., 1982b; Haber et al., 1996). The effects of alcohol consumption on SAL concentrations in the biological fluids and the brain have been extensively reviewed by Hipolito et al. (2012).

Many studies have attempted to establish a correlation between alcohol ingestion and the increase of SAL levels in brain tissues. According to the majority, chronic alcohol exposure produces an increase in SAL levels in several brain regions such as the striatum, hypothalamus and limbic regions (Sjöquist et al., 1982a,b; Myers et al., 1985; Matsubara et al., 1987; Rojkovicova et al., 2008). The increase varied remarkably ranging from 0.08 (Starkey et al., 2006) to 7.59 pg/mg (Rojkovicova et al., 2008). With one exception (Haber et al., 1996), previous human studies found that, after acute (Faraj et al., 1989; Rommelspacher et al., 1995) or chronic (Faraj et al., 1989) alcohol drinking, SAL levels rise in plasma (and presumably brain). Recent evidence that SAL in the brain accounts for some aspects of alcohol's addictive properties have been elegantly reviewed (Hipolito et al., 2012; Deehan et al., 2013a,b); but still little is known about how SAL acts at the cellular level.

The midbrain ventral tegmental area (VTA) has been extensively studied as a target for the central effects of alcohol (Morikawa and Morrisett, 2010), its first metabolite acetaldehyde (Melis et al., 2009; Karahanian et al., 2011), as well as its metabolic derivative SAL (Hipolito et al., 2012). The majority of neurons in the VTA are either dopaminergic (DA) or GABAergic (Lacey et al., 1989; Yung et al., 1991; Johnson and North, 1992b; Chieng et al., 2011), with only a few glutamatergic neurons (Nair-Roberts et al., 2008). The VTA DA neurons project mainly to the nucleus accumbens (NAc) and prefrontal cortex (PFC) (Oades and Halliday, 1987). Dopamine is involved in self-administration of most drugs of abuse, and drugs abused by humans increase dopamine output in target regions of the brain (Di Chiara and Imperato, 1988; Volkow et al., 2007). Addictive substances of different types modulate

DA neuron activity and dopamine release from the VTA (Lüscher and Malenka, 2011). Recently, we employed patch clamp in combination with pharmacological techniques to investigate SAL's immediate effects on VTA DA neurons in rat brain slices (Xie and Ye, 2012; Xie et al., 2012). We found that SAL (0.01–1 μM) significantly stimulates the activity of DA neurons. In this review, we will focus on the multiple underlying cellular mechanisms, in order to clarify how SAL modulates neuronal excitability in the VTA.

SAL's PSYCHOACTIVE EFFECTS IN THE MESOLIMBIC DOPAMINE SYSTEM *In vivo*

Research from several laboratories has led to the idea that SAL participates in ethanol's psychoactive effects in rodents through its own rewarding properties. Early animal studies revealed that SAL promotes alcohol drinking (Duncan and Deitrich, 1980; Myers et al., 1982). Recent data from several groups support the notion that SAL is responsible for some addiction-related psychoactive behaviors related to the mesolimbic dopamine system. Indeed, in Wistar rats, microinjections of SAL (5, 25 μM) into the NAc core increase local dopamine extracellular levels (measured by HPLC), whereas the same doses of SAL injected into the NAc shell significantly reduced the dopamine levels in that subregion (Hipolito et al., 2009). However, microinjection of SAL into the posterior VTA increased DA levels in the ipsilateral accumbens shell by 41% (Hipolito et al., 2009). Recently, Deehan et al. (2013a,b) also reported that SAL stimulates dopamine release in the posterior ventral tegmental area (pVTA). In this study, the effects of SAL on dopamine release were dose-dependent, in an inverted U-shape manner, with 0.3 μM SAL producing a peak dopamine efflux (to 300% of baseline) and higher concentrations (3 μM) a significantly lower response (Deehan et al., 2013a,b). In parallel with these neurochemical findings, SAL elicited some behavioral effects. Direct injection of only 30 pmol SAL into the pVTA resulted in behavioral sensitization and induced strong motor activity in rats (Hipolito et al., 2010). Moreover, significant place preference was induced by SAL, given either intraperitoneally (ip) (Matsuzawa et al., 2000) or by local microinjection into the pVTA (Hipolito et al., 2011). Rodd and colleagues found that rats readily self-administered SAL into the NAc shell (Rodd et al., 2003, 2008) and pVTA (Rodd et al., 2008). Such reinforcing actions seem to depend on activation of DA neurons, being reduced by co-infusion of quinpirole [a D (2, 3) receptor agonist] (Rodd et al., 2008). Below, we will discuss the possible cellular mechanisms underlying these psychoactive effects of SAL, in the light of our recent findings in brain slices *in vitro*.

SAL DEPOLARIZES pVTA DA NEURONS *In vitro* AND ACCELERATES THEIR DISCHARGE

Under current-clamp, SAL was found to depolarize the membrane potential of VTA DA neurons in rats (Xie et al., 2012). In keeping with this depolarization, SAL increased the firing rate of DA neurons in a reversible and dose-dependent manner, with a peak effect at 0.1 μM. This dose dependence, however, was biphasic: at concentrations of 0.01–0.1 μM, the firing rate increased with SAL concentration; but at higher concentrations, the increase diminished sharply. Such concentrations are within a pharmacologically relevant range (Matsubara et al., 1987; Haber et al., 1999). This inverted, U-shaped concentration response curve seen *in vitro* is remarkably similar to the concentration dependence of SAL intra-pVTA microinjections effect on locomotor activity: this dose-response curve had an inverted U-shaped profile, with a peak at 30 pmol (Hipolito et al., 2010). Rodd et al. found that 0.03–0.3 μM SAL was readily self-administered when injected directly into the posterior, not anterior VTA of Wistar rats (Rodd et al., 2008). Notably, our *in vitro* and those *in vivo* experiments revealed that SAL is far more potent than ethanol as stimulator of DA neurons in the pVTA. In contrast to the strong activation induced by 0.1 μM SAL, ethanol had a similar effect only at concentrations of 100–200 mM (Brodie et al., 1990; Xiao et al., 2007). Thus, SAL is 1–2 million times more effective than ethanol as stimulator of DA neurons in the pVTA.

GABAergic AND GLUTAMATERGIC TRANSMISSIONS IN THE VTA PLAY A CRITICAL ROLE IN SAL's EFFECT

DISINHIBITION THROUGH THE ACTIVATION OF μ OPIOID RECEPTORS (MORs) ON GABAergic AFFERENTS

The projection neurons in the VTA, mostly DA neurons, are under inhibitory GABAergic control. Several GABAergic inputs are known, including those from GABA neurons in the rostromedial tegmental nucleus (RMTg) (Barrot et al., 2012), local GABA neurons (i.e., interneurons) (Johnson and North, 1992a; Lüscher and Malenka, 2011; Omelchenko et al., 2009; Tan et al., 2012), medium spiny neurons of the NAc and the ventral pallidum (Kalivas, 1993; Kalivas et al., 1993; Hjelmstad et al., 2013). Both $GABA_A$ and $GABA_B$ receptors mediate the inhibitory action of GABA on DA neurons (Johnson and North, 1992a; Brazhnik et al., 2008; Theile et al., 2011). Both *in vivo* and *in vitro*, blockade of $GABA_A$Rs strongly increases DA cell firing (Johnson and North, 1992a; Xiao et al., 2007; Matsui and Williams, 2011; Theile et al., 2011; Guan et al., 2012); GABAergic IPSCs therefore normally dampen the excitability of DA neurons. $GABA_A$R blockade in the VTA increases dopamine levels in the NAc (Ikemoto et al., 1997) and is strongly rewarding (Laviolette and van der Kooy, 2001). Several lines of evidence have linked ethanol-induced reinforcement to the GABAergic system in the VTA. For example, VTA GABA neurons become hyperexcitable during ethanol withdrawal (Gallegos et al., 1999). Both systemic and intra-VTA administration of $GABA_A$R agonists facilitate, whereas antagonists decrease, voluntary ethanol drinking in rats (Smith et al., 1992). In line with these *in vivo* studies, we have recently reported several relevant findings obtained *in vitro*, during recordings of neuronal activity in brain slices from rats (Xiao et al., 2007; Xiao and Ye, 2008). Thus, ethanol inhibited GABA neurons (through activation of MORs); it enhanced DA neuron firing. Moreover, $GABA_A$ antagonists such as bicuculline and gabazine, attenuate the ethanol-induced increase in firing of VTA-DA neurons. Further tests revealed an involvement of μ opioid receptors (MORs) as the MOR agonist DAMGO and MOR antagonist naltrexone significantly attenuated the increase in firing induced by ethanol and even altered the basal firing rate of the DA neurons, indicating ongoing opioid modulation (Xiao et al., 2007;

Xiao and Ye, 2008). By contrast, Theile (Theile et al., 2011) reported that while ethanol-acceleration of the firing rate of VTA DA neurons was increased by picrotoxin, an antagonist of $GABA_A$ and glycine receptors, it was unaffected by naltrexone, and DAMGO did not change the ongoing firing. This apparent difference suggests some links between MORs, $GABA_A$Rs and the effect of ethanol on DA neurons.

Compared to the role of GABA in ethanol abuse, we know much less about how SAL affects GABAergic transmission. Previous studies have found that SAL is a morphine-like alkaloid. It binds to opioid receptors and has opioid-like effects (Fertel et al., 1980; Lucchi et al., 1982). We found (Xie et al., 2012) that both gabazine and naltrexone reduce the acceleration of DA neuronal firing produced by SAL, suggesting that SAL's action may be mediated via MOR on GABAergic neurons. In support of this idea, SAL reduced the frequency of spontaneous IPSCs recorded in DA neurons, without changing their amplitude; but SAL decreased the size of evoked IPSCs and increased the paired-pulse ratio. These observations indicate that SAL depresses GABAergic transmission to DA neurons by an opioid sensitive presynaptic mechanism. Indeed, SAL's effect on sIPSCs was suppressed by naltrexone or DAMGO. MORs are enriched in the VTA and are primarily located on non-DA neurons (Mansour et al., 1995), the RMTg and its efferents to the DA neurons (Jhou et al., 2009; Jalabert et al., 2011; Matsui and Williams, 2011; Hjelmstad et al., 2013). It is generally believed that MORs–mediated inhibition of GABAergic neurons leads to excitation of DA neurons by a disinhibitory mechanism (Johnson and North, 1992a; Jalabert et al., 2011; Matsui and Williams, 2011). In keeping with this idea, both systemic and intra-VTA administrations of MOR agonists increase VTA DA neuron firing and NAc dopamine release (Matthews and German, 1984; Latimer et al., 1987; Di Chiara and Imperato, 1988; Leone et al., 1991; Spanagel et al., 1992). Previous experiments *in vivo* have shown that SAL-associated place preference was blocked by intraperitoneal or local administration of β-Funaltrexamine, an antagonist of MORs (Matsuzawa et al., 2000). Local pretreatment with β-Funaltrexamine hydrochloride also prevented the SAL-evoked increase in NAc dopamine levels (Hipolito et al., 2011). Our observation of pronounced effects of naltrexone on GABAergic IPSCs is strong evidence of ongoing opioid release in the VTA (Xiao et al., 2007; Xiao and Ye, 2008; Xie et al., 2012). Moreover, naltrexone largely eliminated the effects of SAL on DA neurons. The simplest explanation of our results is that SAL activates MORs on GABAergic neurons or their efferents, thus exciting VTA DA neurons by disinhibition. Overall, our *in vitro* findings are consistent with the notion that SAL's excitatory effects in rat pVTA are mediated, at least partly, by activation of MORs (Hipolito et al., 2011) and the resulting suppression of GABAergic inhibition. How SAL activates MORs remains to be clarified. Though known to bind to opioid receptors, it has only a low affinity, significant binding requiring relatively high micromolar concentrations of SAL (Fertel et al., 1980; Lucchi et al., 1982). The binding of SAL lowers the binding of endogenous opioids. Therefore, other mechanisms of SAL action at very low concentrations, such as enhanced release or slower removal of endogenous opioid, should also be considered.

GLUTAMATERGIC TRANSMISSION TO VTA IS ENHANCED BY SAL-INDUCED ACTIVATION OF D1Rs

VTA DA neurons receive numerous glutamatergic afferents from many parts of the brain, including the PFC (Sesack et al., 2003; Geisler et al., 2007) and subcortical structures, such as the pedunculopontine (PPTg) and laterodorsal tegmental (LDTg) nuclei (Charara et al., 1996; Clements et al., 1991; Lavoie and Parent, 1994), the bed nucleus of the stria terminals (BNST) (Georges and Aston-Jones, 2002), the superior colliculus (SC) (Comoli et al., 2003; Dommett et al., 2005), the lateral hypothalamic and preoptic areas, periaqueductal gray, the dorsal and median raphe (Geisler et al., 2007), as well as the lateral habenula (Gonçalves et al., 2012). Some glutamatergic neurons are also present within the VTA (Dobi et al., 2010). Excitatory synaptic inputs which activate α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and NMDA-type ionotropic glutamate receptors in DA neurons are a key component in the regulation of DA cell excitability (Overton and Clark, 1997). Accordingly, iontophoretic applications of both AMPA and NMDA receptor agonists can stimulate DA neuron firing (Christoffersen and Meltzer, 1995; Zhang et al., 1997). Consistently, applications of APV + DNQX (AMPA and NMDA receptor antagonists, respectively) slightly but significantly lower the firing rate of DA neurons (Xie and Ye, 2012).

Glutamatergic transmission plays an important role in the effects of ethanol (Eckardt et al., 1998; Krystal et al., 2003). In several brain regions, ethanol inhibits NMDA and non-NMDA glutamate receptors, as well as glutamate release (Siggins et al., 2005). However, ethanol can increase glutamate release under some circumstances. Systemic administration of ethanol increases glutamate release in the NAc of low-alcohol sensitive rats (Dahchour et al., 2000) and addiction-prone Lewis rats (Selim and Bradberry, 1996). Acute ethanol administration increases glutamate release in the central nucleus of the amygdala from rats receiving chronic ethanol treatment (Roberto et al., 2004; Zhu et al., 2007). Both acute and repeated exposure to low doses of ethanol raised glutamate levels in the pVTA (Ding et al., 2012). We have previously reported that ethanol enhances glutamatergic transmission to VTA DA neurons (Deng et al., 2009; Xiao et al., 2009). Most recently, we found that SAL also enhances glutamatergic transmission to DA neurons in the VTA, increasing the frequency of both firing and spontaneous EPSCs (Xie and Ye, 2012). The application of APV + DNQX substantially attenuated SAL's action on firing, indicating a very substantial glutamatergic component in SAL-induced excitation of DA neurons. The increase in frequency of spontaneous EPSCs was abolished by the sodium channel blocker tetrodotoxin, indicating that SAL's effect involved voltage-dependent Na^+ channels. Since SAL did not alter the amplitude of either sEPSCs or mEPSCs, but increased the $EPSC_2/EPSC_1$ ratio during paired-pulse stimulation, its site of action was probably presynaptic. These findings clearly point to the involvement of glutamatergic transmission in SAL's effects in VTA. Dopamine receptors consist of D1-like (D1 and D5 receptors) and D2-like (D2, D3, and D4 receptors) families. Both D1R and the D2R family (D2R in particular) have been implicated in the mechanisms of drug dependence and abuse (Blum et al., 1990).

Disruption of D1R gene expression (El-Ghundi et al., 1998) or administration of a D1R antagonist (Liu and Weiss, 2002) attenuates or prevents alcohol-seeking behavior. In the VTA, D1Rs are expressed on glutamatergic axons (Lu et al., 1997) but not on the soma of VTA DA neurons (Mansour et al., 1992; Lu et al., 1997). The activation of D1Rs increases glutamate levels in the VTA (Kalivas and Duffy, 1995). In our experiments, SKF83566 (a selective D1R antagonist), suppressed SAL's action on evoked EPSCs in VTA, confirming that the enhancement of glutamatergic transmission was mediated by D1Rs. We also found that SKF83566 attenuated the SAL-induced acceleration of DA neuron firing. SAL may thus increase the somatodendritic release of dopamine; and by raising the extracellular level of dopamine may retrogradely activate the D1Rs on the glutamate-releasing terminals, which in turn increases glutamate release and the excitability of DA neurons. Somatodendritic release of dopamine in the midbrain DA neurons is Na^+ channel-dependent (Threlfell and Cragg, 2007). In keeping with this, we found that the increase in the frequency of spontaneous EPSCs by SAL was abolished by tetrodotoxin. SAL's D1R-dependent effect on glutamatergic transmission is similar to that we observed previously with ethanol (Deng et al., 2009; Xiao et al., 2009); but SAL is much more potent than ethanol (the effective concentrations being 0.1 μM for SAL vs. 40 mM for ethanol). This is consistent with both *in vivo* and *in vitro* findings that the DA system is particularly sensitive to SAL (Hipolito et al., 2011; Rodd et al., 2008; Xie and Ye, 2012).

We showed that the ethanol-induced increase in the release of glutamate (EPSCs) in the VTA was eliminated when dopamine was depleted by pretreatment with reserpine (Deng et al., 2009; Xiao et al., 2009). Since SAL is the condensation product of dopamine and acetaldehyde, depletion of dopamine could prevent the formation of SAL. This could explain why ethanol failed to increase glutamatergic transmission when dopamine was depleted.

SUMMARY

Our *in vitro* findings in combination with *in vivo* experiments by other groups—reviewed here—identify cellular mechanisms underlying SAL's psychoactive effects on the mesolimbic dopamine system. SAL's stimulating action in the pVTA involves modulation of synaptic inputs and intrinsic properties of DA neurons (schematically depicted in **Figure 1**): (1) depolarizing DA neurons and increasing their firing rate; (2) activating MORs

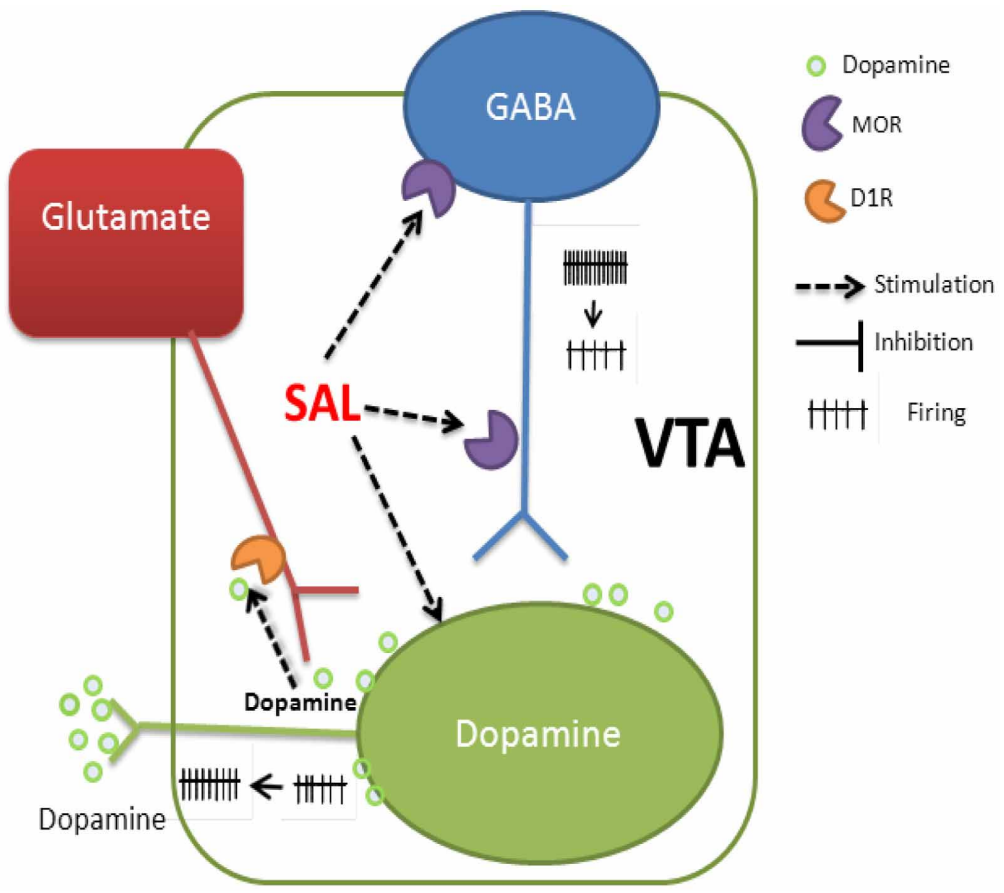


FIGURE 1 | Schematic representation of the mechanisms underlying SAL's excitation of putative DA neurons in rat Pvta. (1) Depolarizing the membrane and increasing the firing rate. (2) Activation of MORs on the GABAergic neurons and/or their afferents reduces GABA release onto DA neurons. (3) Activation of D1Rs at the glutamatergic afferents increases glutamate release onto DA neurons. VTA, ventral tegmental area.

on the GABAergic neurons, which inhibit GABAergic transmission to VTA DA neurons (resulting in disinhibition); (3) enhancing glutamatergic transmission to DA neurons by activating D1Rs situated on glutamatergic afferents. Thus, by acting on both presynaptic and postsynaptic targets on the DA neurons, SAL enhances the discharge of VTA DA neurons and so increases dopamine release in the downstream brain regions. Whatever its precise role—whether as detector of rewarding stimuli (Mirenowicz and Schultz, 1996) or modulating network activity in PFC (Durstewitz et al., 2000; González-Burgos et al., 2002; Lapish et al., 2007)—it is clear that the mesocortical DA system is important in processes leading to addiction. Hence, the novel mechanisms proposed here may contribute to the rewarding properties of SAL observed *in vivo*. Understanding how SAL affects the activity of VTA dopamine neurons could have profound implications for the prevention and treatment of alcoholism.

ACKNOWLEDGMENTS

This work is made possible by the support of NIH AA016964.

REFERENCES

Barrot, M., Sesack, S. R., Georges, F., Pistis, M., Hong, S., and Jhou, T. C. (2012). Braking dopamine systems: a new GABA master structure for mesolimbic and nigrostriatal functions. *J. Neurosci.* 32, 14094–14101.

Blum, K., Noble, E. P., Sheridan, P. J., Montgomery, A., Ritchie, T., Jagadeeswaran, P., et al. (1990). Allelic association of human dopamine D2 receptor gene in alcoholism. *JAMA* 263, 2055–2060.

Brazhnik, E., Shah, F., and Tepper, J. M. (2008). GABAergic afferents activate both GABAA and GABAB receptors in mouse substantia nigra dopaminergic neurons *in vivo*. *J. Neurosci.* 28, 10386–10398.

Brodie, M. S., Shefner, S. A., and Dunwiddie, T. V. (1990). Ethanol increases the firing rate of dopamine neurons of the rat ventral tegmental area *in vitro*. *Brain Res.* 508, 65–69.

Charara, A., Smith, Y., and Parent, A. (1996). Glutamatergic inputs from the pedunculopontine nucleus to midbrain dopaminergic neurons in primates: phaseolus vulgaris-leucoagglutinin anterograde labeling combined with postembedding glutamate and GABA immunohistochemistry. *J. Comp. Neurol.* 364, 254–266.

Chieng, B., Azriel, Y., Mohammadi, S., and Christie, M. J. (2011). Distinct cellular properties of identified dopaminergic and GABAergic neurons in the mouse ventral tegmental area. *J. Physiol.* 589, 3775–3787.

Christoffersen, C. L., and Meltzer, L. T. (1995). Evidence for N-methyl-D-aspartate and AMPA subtypes of the glutamate receptor on substantia nigra dopamine neurons: possible preferential role for N-methyl-D-aspartate receptors. *Neuroscience* 67, 373–381.

Clements, J. R., Toth, D. D., Highfield, D. A., and Grant, S. J. (1991). Glutamate-like immunoreactivity is present within cholinergic neurons of the laterodorsal tegmental and pedunculopontine nuclei. *Adv. Exp. Med. Biol.* 295, 127–142.

Comoli, E., Coizet, V., Boyes, J., Bolam, J. P., Canteras, N. S., Quirk, R. H., et al. (2003). A direct projection from superior colliculus to substantia nigra for detecting salient visual events. *Nat. Neurosci.* 6, 974–980.

Dahchour, A., Hoffman, A., Deitrich, R., and de Witte, P. (2000). Effects of ethanol on extracellular amino acid levels in high-and low-alcohol sensitive rats: a microdialysis study. *Alcohol Alcohol.* 35, 548–553.

Deehan, G. A. Jr., Brodie, M. S., and Rodd, Z. A. (2013a). What is in that drink: the biological actions of ethanol, acetaldehyde, and salsolinol. *Curr. Top. Behav. Neurosci.* 13, 163–184.

Deehan, G. A. Jr., Engleman, E. A., Ding, Z. M., McBride, W. J., and Rodd, Z. A. (2013b). Microinjections of acetaldehyde or salsolinol into the posterior ventral tegmental area increase dopamine release in the nucleus accumbens shell. *Alcohol. Clin. Exp. Res.* 37, 722–729.

Deng, C., Li, K. Y., Zhou, C., and Ye, J. H. (2009). Ethanol enhances glutamate transmission by retrograde dopamine signaling in a postsynaptic neuron/synaptic bouton preparation from the ventral tegmental area. *Neuropsychopharmacology* 34, 1233–1244.

Deng, X. S., and Deitrich, R. A. (2008). Putative role of brain acetaldehyde in ethanol addiction. *Curr. Drug Abuse Rev.* 1, 3–8.

Di Chiara, G., and Imperato, A. (1988). Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc. Natl. Acad. Sci. U.S.A.* 85, 5274–5278.

Ding, Z. M., Engleman, E. A., Rodd, Z. A., and McBride, W. J. (2012). Ethanol increases glutamate neurotransmission in the posterior ventral tegmental area of female wistar rats. *Alcohol. Clin. Exp. Res.* 36, 633–640.

Dobi, A., Margolis, E. B., Wang, H. L., Harvey, B. K., and Morales, M. (2010). Glutamatergic and nonglutamatergic neurons of the ventral tegmental area establish local synaptic contacts with dopaminergic and nondopaminergic neurons. *J. Neurosci.* 30, 218–229.

Dommett, E., Coizet, V., Blaha, C. D., Martindale, J., Lefebvre, V., Walton, N., et al. (2005). How visual stimuli activate dopaminergic neurons at short latency. *Science* 307, 1476–1479.

Duncan, C., and Deitrich, R. A. (1980). A critical evaluation of tetrahydroisoquinoline induced ethanol preference in rats. *Pharmacol. Biochem. Behav.* 13, 265–281.

Durstewitz, D., Seamans, J. K., and Sejnowski, T. J. (2000). Neurocomputational models of working memory. *Nat. Neurosci.* 3(Suppl.), 1184–1191.

Eckardt, M. J., File, S. E., Gessa, G. L., Grant, K. A., Guerri, C., Hoffman, P. L., et al. (1998). Effects of moderate alcohol consumption on the central nervous system. *Alcohol. Clin. Exp. Res.* 22, 998–1040.

El-Ghundi, M., George, S. R., Drago, J., Fletcher, P. J., Fan, T., Nguyen, T., et al. (1998). Disruption of dopamine D1 receptor gene expression attenuates alcohol-seeking behavior. *Eur. J. Pharmacol.* 353, 149–158.

Faraj, B. A., Camp, V. M., Davis, D. C., Lenton, J. D., and Kutner, M. (1989). Elevation of plasma salsolinol sulfate in chronic alcoholics as compared to nonalcoholics. *Alcohol. Clin. Exp. Res.* 13, 155–163.

Fertel, R. H., Greenwald, J. E., Schwarz, R., Wong, L., and Bianchine, J. (1980). Opiate receptor binding and analgesic effects of the tetrahydroisoquinolines salsolinol and tetrahydropapaveroline. *Res. Commun. Chem. Pathol. Pharmacol.* 27, 3–16.

Gallegos, R. A., Lee, R. S., Criado, J. R., Henriksen, S. J., and Steffensen, S. C. (1999). Adaptive responses of gamma-aminobutyric acid neurons in the ventral tegmental area to chronic ethanol. *J. Pharmacol. Exp. Ther.* 291, 1045–1053.

Geisler, S., Derst, C., Veh, R. W., and Zahm, D. S. (2007). Glutamatergic afferents of the ventral tegmental area in the rat. *J. Neurosci.* 27, 5730–5743.

Georges, F., and Aston-Jones, G. (2002). Activation of ventral tegmental area cells by the bed nucleus of the stria terminalis: a novel excitatory amino acid input to midbrain dopamine neurons. *J. Neurosci.* 22, 5173–5187.

Gonçalves, L., Sego, C., and Metzger, M. (2012). Differential projections from the lateral habenula to the rostromedial tegmental nucleus and ventral tegmental area in the rat. *J. Comp. Neurol.* 520, 1278–1300.

González-Burgos, G., Kröner, S., Krimer, L. S., Seamans, J. K., Urban, N. N., Henze, D. A., et al. (2002). Dopamine modulation of neuronal function in the monkey prefrontal cortex. *Physiol. Behav.* 77, 537–543.

Guan, Y., Xiao, C., Krnjevic, K., Xie, G., Zuo, W., and Ye, J. H. (2012). GABAergic actions mediate opposite ethanol effects on dopaminergic neurons in the anterior and posterior ventral tegmental area. *J. Pharmacol. Exp. Ther.* 341, 33–42.

Haber, H., Dumaual, N., Bare, D. J., Melzig, M. F., McBride, W. F., Lumeng, L., et al. (1999). The quantitative determination of R- and S-salsolinol in the striatum and adrenal gland of rats selectively bred for disparate alcohol drinking. *Addict. Biol.* 4, 181–189.

Haber, H., Winkler, A., Putscher, I., Henklein, P., Baeger, I., Georgi, M., et al. (1996). Plasma and urine salsolinol in humans: effect of acute ethanol intake on the enantiomeric composition of salsolinol. *Alcohol. Clin. Exp. Res.* 20, 87–92.

Hipolito, L., Marti-Prats, L., Sanchez-Catalan, M. J., Polache, A., and Granero, L. (2011). Induction of conditioned place preference and dopamine release by salsolinol in posterior VTA of rats: involvement

of mu-opioid receptors. *Neurochem. Int.* 59, 559–562.

Hipolito, L., Sanchez-Catalan, M. J., Granero, L., and Polache, A. (2009). Local salsolinol modulates dopamine extracellular levels from rat nucleus accumbens: shell/core differences. *Neurochem. Int.* 55, 187–192.

Hipolito, L., Sanchez-Catalan, M. J., Marti-Prats, L., Granero, L., and Polache, A. (2012). Revisiting the controversial role of salsolinol in the neurobiological effects of ethanol: old and new vistas. *Neurosci. Biobehav. Rev.* 36, 362–378.

Hipolito, L., Sanchez-Catalan, M. J., Zornoza, T., Polache, A., and Granero, L. (2010). Locomotor stimulant effects of acute and repeated intrategmental injections of salsolinol in rats: role of mu-opioid receptors. *Psychopharmacology (Berl.)* 209, 1–11.

Hjelmstad, G. O., Xia, Y., Margolis, E. B., and Fields, H. L. (2013). Opioid modulation of ventral pallidal afferents to ventral tegmental area neurons. *J. Neurosci.* 33, 6454–6459.

Ikemoto, S., Kohl, R. R., and McBride, W. J. (1997). GABA(A) receptor blockade in the anterior ventral tegmental area increases extracellular levels of dopamine in the nucleus accumbens of rats. *J. Neurochem.* 69, 137–143.

Jalabert, M., Bourdy, R., Courtin, J., Veinante, P., Manzoni, O. J., Barrot, M., et al. (2011). Neuronal circuits underlying acute morphine action on dopamine neurons. *Proc. Natl. Acad. Sci. U.S.A.* 108, 16446–16450.

Jhou, T. C., Fields, H. L., Baxter, M. G., Saper, C. B., and Holland, P. C. (2009). The rostromedial tegmental nucleus (RMTg), a GABAergic afferent to midbrain dopamine neurons, encodes aversive stimuli and inhibits motor responses. *Neuron* 61, 786–800.

Johnson, S. W., and North, R. A. (1992a). Opioids excite dopamine neurons by hyperpolarization of local interneurons. *J. Neurosci.* 12, 483–488.

Johnson, S. W., and North, R. A. (1992b). Two types of neurone in the rat ventral tegmental area and their synaptic inputs. *J. Physiol.* 450, 455–468.

Kalivas, P. W. (1993). Neurotransmitter regulation of dopamine neurons in the ventral tegmental area. *Brain Res. Brain Res. Rev.* 18, 75–113.

Kalivas, P. W., Churchill, L., and Klitenick, M. A. (1993). GABA and enkephalin projection from the nucleus accumbens and ventral pallidum to the ventral tegmental area. *Neuroscience* 57, 1047–1060.

Kalivas, P. W., and Duffy, P. (1995). D1 receptors modulate glutamate transmission in the ventral tegmental area. *J. Neurosci.* 15, 5379–5388.

Karahanian, E., Quintanilla, M. E., Tampier, L., Rivera-Meza, M., Bustamante, D., Gonzalez-Lira, V., et al. (2011). Ethanol as a prodrug: brain metabolism of ethanol mediates its reinforcing effects. *Alcohol. Clin. Exp. Res.* 35, 606–612.

Krystal, J. H., D'Souza, D. C., Mathalon, D., Perry, E., Belger, A., and Hoffman, R. (2003). NMDA receptor antagonist effects, cortical glutamatergic function, and schizophrenia: toward a paradigm shift in medication development. *Psychopharmacology (Berl.)* 169, 215–233.

Lacey, M. G., Mercuri, N. B., and North, R. A. (1989). Two cell types in rat substantia nigra zona compacta distinguished by membrane properties and the actions of dopamine and opioids. *J. Neurosci.* 9, 1233–1241.

Lapish, C. C., Kroener, S., Durstewitz, D., Lavin, A., and Seamans, J. K. (2007). The ability of the mesocortical dopamine system to operate in distinct temporal modes. *Psychopharmacology (Berl.)* 191, 609–625.

Latimer, L. G., Duffy, P., and Kalivas, P. W. (1987). Mu opioid receptor involvement in enkephalin activation of dopamine neurons in the ventral tegmental area. *J. Pharmacol. Exp. Ther.* 241, 328–337.

Laviolette, S. R., and van der Kooy, D. (2001). GABA(A) receptors in the ventral tegmental area control bidirectional reward signalling between dopaminergic and non-dopaminergic neural motivational systems. *Eur. J. Neurosci.* 13, 1009–1015.

Lavoie, B., and Parent, A. (1994). Pedunculopontine nucleus in the squirrel monkey: distribution of cholinergic and monoaminergic neurons in the mesopontine tegmentum with evidence for the presence of glutamate in cholinergic neurons. *J. Comp. Neurol.* 344, 190–209.

Leone, P., Pocock, D., and Wise, R. A. (1991). Morphine-dopamine interaction: ventral tegmental morphine increases nucleus accumbens dopamine release. *Pharmacol. Biochem. Behav.* 39, 469–472.

Liu, X., and Weiss, F. (2002). Additive effect of stress and drug cues on reinstatement of ethanol seeking: exacerbation by history of dependence and role of concurrent activation of corticotropin-releasing factor and opioid mechanisms. *J. Neurosci.* 22, 7856–7861.

Lu, X. Y., Churchill, L., and Kalivas, P. W. (1997). Expression of D1 receptor mRNA in projections from the forebrain to the ventral tegmental area. *Synapse* 25, 205–214.

Lucchi, L., Bosio, A., Spano, P. F., and Trabucchi, M. (1982). Action of ethanol and salsolinol on opiate receptor function. *Brain Res.* 232, 506–510.

Lüscher, C., and Malenka, R. C. (2011). Drug-evoked synaptic plasticity in addiction: from molecular changes to circuit remodeling. *Neuron* 69, 650–663.

Mansour, A., Fox, C. A., Akil, H., and Watson, S. J. (1995). Opioid-receptor mRNA expression in the rat CNS: anatomical and functional implications. *Trends Neurosci.* 18, 22–29.

Mansour, A., Meador-Woodruff, J. H., Zhou, Q., Civelli, O., Akil, H., and Watson, S. J. (1992). A comparison of D1 receptor binding and mRNA in rat brain using receptor autoradiographic and *in situ* hybridization techniques. *Neuroscience* 46, 959–971.

Matsubara, K., Fukushima, S., and Fukui, Y. (1987). A systematic regional study of brain salsolinol levels during and immediately following chronic ethanol ingestion in rats. *Brain Res.* 413, 336–343.

Matsui, A., and Williams, J. T. (2011). Opioid-sensitive GABA inputs from rostromedial tegmental nucleus synapse onto midbrain dopamine neurons. *J. Neurosci.* 31, 17729–17735.

Matsuzawa, S., Suzuki, T., and Misawa, M. (2000). Involvement of mu-opioid receptor in the salsolinol-associated place preference in rats exposed to conditioned fear stress. *Alcohol. Clin. Exp. Res.* 24, 366–372.

Matthews, R. T., and German, D. C. (1984). Electrophysiological evidence for excitation of rat ventral tegmental area dopamine neurons by morphine. *Neuroscience* 11, 617–625.

Melis, M., Diana, M., Enrico, P., Marinelli, M., and Brodie, M. S. (2009). Ethanol and acetaldehyde action on central dopamine systems: mechanisms, modulation, and relationship to stress. *Alcohol* 43, 531–539.

Mirenowicz, J., and Schultz, W. (1996). Preferential activation of midbrain dopamine neurons by appetitive rather than aversive stimuli. *Nature* 379, 449–451.

Morikawa, H., and Morrisett, R. A. (2010). Ethanol action on dopaminergic neurons in the ventral tegmental area: interaction with intrinsic ion channels and neurotransmitter inputs. *Int. Rev. Neurobiol.* 91, 235–288.

Myers, R. D., McCaleb, M. L., and Ruwe, W. D. (1982). Alcohol drinking induced in the monkey by tetrahydropapaveroline (THP) infused into the cerebral ventricle. *Pharmacol. Biochem. Behav.* 16, 995–1000.

Myers, W. D., Mackenzie, L., Ng, K. T., Singer, G., Smythe, G. A., and Duncan, M. W. (1985). Salsolinol and dopamine in rat medial basal hypothalamus after chronic ethanol exposure. *Life Sci.* 36, 309–314.

Nair-Roberts, R. G., Chatelain-Badie, S. D., Benson, E., White-Cooper, H., Bolam, J. P., and Ungless, M. A. (2008). Stereological estimates of dopaminergic, GABAergic and glutamatergic neurons in the ventral tegmental area, substantia nigra and retrorubral field in the rat. *Neuroscience* 152, 1024–1031.

Naoi, M., Maruyama, W., Nakao, N., Ibi, T., Sahashi, K., and Benedetti, M. S. (1998). (R)salsolinol N-methyltransferase activity increases in parkinsonian lymphocytes. *Ann. Neurol.* 43, 212–216.

Oades, R. D., and Halliday, G. M. (1987). Ventral tegmental (A10) system: neurobiology. 1. Anatomy and connectivity. *Brain Res.* 434, 117–165.

Omelchenko, N., Bell, R., and Sesack, S. R. (2009). Lateral habenula projections to dopamine and GABA neurons in the rat ventral tegmental area. *Eur. J. Neurosci.* 30, 1239–1250.

Overton, P. G., and Clark, D. (1997). Burst firing in midbrain dopaminergic neurons. *Brain Res. Brain Res. Rev.* 25, 312–334.

Rehm, J., Mathers, C., Popova, S., Thavorncharoensap, M., Teerawattananon, Y., and Patra, J. (2009). Global burden of disease and injury and economic cost attributable to alcohol use and alcohol-use disorders. *Lancet.* 373, 2223–2233.

Roberto, M., Schweitzer, P., Madamba, S. G., Stouffer, D. G., Parsons, L. H., and Siggins, G. R. (2004). Acute and chronic ethanol alter glutamatergic transmission in rat central amygdala: an *in vitro* and *in vivo* analysis. *J. Neurosci.* 24, 1594–1603.

Rodd, Z. A., Bell, R. L., Zhang, Y., Goldstein, A., Zaffaroni, A., McBride, W. J., et al. (2003).

Salsolinol produces reinforcing effects in the nucleus accumbens shell of alcohol-preferring (P) rats. *Alcohol. Clin. Exp. Res.* 27, 440–449.

Rodd, Z. A., Oster, S. M., Ding, Z. M., Toalston, J. E., Deehan, G., Bell, R. L., et al. (2008). The reinforcing properties of salsolinol in the ventral tegmental area: evidence for regional heterogeneity and the involvement of serotonin and dopamine. *Alcohol. Clin. Exp. Res.* 32, 230–239.

Rojkovicova, T., Mechref, Y., Starkey, J. A., Wu, G., Bell, R. L., McBride, W. J., et al. (2008). Quantitative chiral analysis of salsolinol in different brain regions of rats genetically predisposed to alcoholism. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 863, 206–214.

Rommelspacher, H., Sllstrom Baum, S., Dufeu, P., and Schmidt, L. G. (1995). Determination of (R)- and (S)-salsolinol sulfate and dopamine sulfate levels in plasma of nonalcoholics and alcoholics. *Alcohol* 12, 309–315.

Selim, M., and Bradberry, C. W. (1996). Effect of ethanol on extracellular 5-HT and glutamate in the nucleus accumbens and prefrontal cortex: comparison between the Lewis and Fischer 344 rat strains. *Brain Res.* 716, 157–164.

Sesack, S. R., Carr, D. B., Omelchenko, N., and Pinto, A. (2003). Anatomical substrates for glutamate-dopamine interactions: evidence for specificity of connections and extrasynaptic actions. *Ann. N.Y. Acad. Sci.* 1003, 36–52.

Siggins, G. R., Roberto, M., and Nie, Z. (2005). The tipsy terminal: presynaptic effects of ethanol. *Pharmacol. Ther.* 107, 80–98.

Sjöquist, B., Eriksson, A., and Winblad, B. (1982a). Brain salsolinol levels in alcoholism. *Lancet* 1, 675–676.

Sjöquist, B., Liljequist, S., and Engel, J. (1982b). Increased salsolinol levels in rat striatum and limbic forebrain following chronic ethanol treatment. *J. Neurochem.* 39, 259–262.

Smith, B. R., Robidoux, J., and Amit, Z. (1992). GABAergic involvement in the acquisition of voluntary ethanol intake in laboratory rats. *Alcohol Alcohol.* 27, 227–231.

Spanagel, R., Bartsch, D., Brors, B., Dahmen, N., Deussing, J., Eils, R., et al. (2010). An integrated genome research network for studying the genetics of alcohol addiction. *Addict. Biol.* 15, 369–379.

Spanagel, R., Herz, A., and Shippenberg, T. S. (1992). Opposing tonically active endogenous opioid systems modulate the mesolimbic dopaminergic pathway. *Proc. Natl. Acad. Sci. U.S.A.* 89, 2046–2050.

Starkey, J. A., Mechref, Y., Muzikar, J., McBride, W. J., and Novotny, M. V. (2006). Determination of salsolinol and related catecholamines through on-line preconcentration and liquid chromatography/atmospheric pressure photoionization mass spectrometry. *Anal. Chem.* 78, 3342–3347.

Tan, K. R., Yvon, C., Turiault, M., Mirzabekov, J. J., Doehner, J., Labouèbe, G., et al. (2012). GABA neurons of the VTA drive conditioned place aversion. *Neuron* 73, 1173–1183.

Theile, J. W., Morikawa, H., Gonzales, R. A., and Morrisett, R. A. (2011). GABAergic transmission modulates ethanol excitation of ventral tegmental area dopamine neurons. *Neuroscience* 172, 94–103.

Threlfell, S., and Cragg, S. J. (2007). "Chapter 8, using fast-scan cyclic voltammetry to investigate somatodendritic dopamine release," in *Electrochemical Methods for Neuroscience*, eds A. C. Michael and L. M. Borland (Boca Raton, FL: CRC Press).

Volkow, N. D., Fowler, J. S., Wang, G. J., Swanson, J. M., and Telang, F. (2007). Dopamine in drug abuse and addiction: results of imaging studies and treatment implications. *Arch. Neurol.* 64, 1575–1579.

Xiao, C., Shao, X. M., Olive, M. F., Griffin, W. C. 3rd, Li, K. Y., et al. (2009). Ethanol facilitates glutamatergic transmission to dopamine neurons in the ventral tegmental area. *Neuropsychopharmacology* 34, 307–318.

Xiao, C., and Ye, J. H. (2008). Ethanol dually modulates GABAergic synaptic transmission onto dopaminergic neurons in ventral tegmental area: role of mu-opioid receptors. *Neuroscience* 153, 240–248.

Xiao, C., Zhang, J., Krnjevic, K., and Ye, J. H. (2007). Effects of ethanol on midbrain neurons: role of opioid receptors. *Alcohol. Clin. Exp. Res.* 31, 1106–1113.

Xie, G., and Ye, J. H. (2012). Salsolinol facilitates glutamatergic transmission to dopamine neurons in the posterior ventral tegmental area of rats. *PLoS ONE* 7:e36716. doi: 10.1371/journal.pone.0036716

Xie, G., Hipólito, L., Zuo, W., Polache, A., Granero, L., Krnjevic, K., et al. (2012). Salsolinol stimulates dopamine neurons in slices of posterior ventral tegmental area indirectly by activating μ-opioid receptors. *J. Pharmacol. Exp. Ther.* 341, 43–50.

Yung, W. H., Hausser, M. A., and Jack, J. J. (1991). Electrophysiology of dopaminergic and non-dopaminergic neurones of the guinea-pig substantia nigra pars compacta *in vitro*. *J. Physiol.* 436, 643–667.

Zhang, X. F., Hu, X. T., White, F. J., and Wolf, M. E. (1997). Increased responsiveness of ventral tegmental area dopamine neurons to glutamate after repeated administration of cocaine or amphetamine is transient and selectively involves AMPA receptors. *J. Pharmacol. Exp. Ther.* 281, 699–706.

Zhu, W., Bie, B., and Pan, Z. Z. (2007). Involvement of non-NMDA glutamate receptors in central amygdala in synaptic actions of ethanol and ethanol-induced reward behavior. *J. Neurosci.* 27, 289–298.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 28 February 2013; accepted: 07 May 2013; published online: 24 May 2013.

Citation: Xie G, Krnjević K and Ye J-H (2013) Salsolinol modulation of dopamine neurons. Front. Behav. Neurosci. 7:52. doi: 10.3389/fnbeh.2013.00052

frontiers in
BEHAVIORAL NEUROSCIENCE

GENERAL COMMENTARY
published: 25 June 2013
doi: 10.3389/fnbeh.2013.00074

Salsolinol and ethanol-derived excitation of dopamine mesolimbic neurons: new insights

Ana Polache* and Luis Granero*

Departament de Farmàcia i Tecnología Farmacéutica, Universitat de València, Burjassot, Spain
**Correspondence: ana.polache@uv.es; lfgran@uv.es*

Edited by:
Merce Correa, Universitat Jaume, Spain

A commentary on

Salsolinol modulation of dopamine neurons
by Xie, G., Krnjević, K., and Ye, J.-H. (2013). Front. Behav. Neurosci. 7:52. doi: 10.3389/fnbeh.2013.00052

Evidence supporting the essential role of brain-derived ethanol metabolites in the excitation of dopamine (DA) midbrain neurons has multiplied in the last 10–15 years. The pioneer and influential behavioral studies by CM Aragon and colleagues (see Correa et al., 2012 for a complete review) and more recent data (Sánchez-Catalán et al., 2009; Martí-Prats et al., 2010, 2013) have repeatedly demonstrated the crucial role displayed by acetaldehyde (ACD) in the locomotor and other behavioral responses elicited by ethanol. Although these experiments mainly used an indirect measure (exploratory locomotion) as an index of the excitation of DA neurons in the ventral tegmental area (VTA), results strongly suggested that the manipulations of ethanol brain metabolism determine the response (activation or not) of the DA neurons in the mesolimbic system after ethanol administration.

At the beginning of the past decade, a remarkable leap in the study of the ACD involvement in the ethanol-derived activation of VTA DA neurons was done. Several electrophysiological studies (Foddai et al., 2004; Melis et al., 2007) directly demonstrated that VTA DA neurons are not excited after ethanol administration if ACD production is inhibited. The strength of the provided evidence was very high because, unlike behavioral studies, a direct measure of the neuronal activity after administration of the drug was registered.

These direct and indirect findings confirmed the involvement of ACD in the excitation of the mesolimbic system. But, in spite of their relevance, an important question relative to ACD-derived excitation of the VTA DA neurons remains unresolved. If ACD is the responsible for the excitation of the DA mesolimbic system after ethanol administration, how ACD excites VTA DA neurons? In other words, what mechanism does ACD use to activate DA neurons? As occurs with ethanol, ACD has not any subset of specific receptors on nerve, glial, or other cells in the brain. Moreover, ACD, as other highly toxic aldehydes, is evanescent and reacts instantaneously with other compounds to form new products. Notably, biogenic amines are among the compounds that can react with ACD producing the so-called tetrahydroisoquinolines. The mesolimbic system is particularly enriched with DA, so in this brain region ACD locally formed after ethanol administration can react with DA forming salsolinol (Sal; 1-methyl-6,7-dihidroxy-1,2,3,4-tetrahydroisoquinoline) (Collins and Bigdeli, 1975; Nagatsu, 1997). In fact, in most cases, the results reported show that chronic ethanol treatment produces an increase of Sal levels in different brain areas, such as the striatum, hypothalamus and limbic regions. Moreover, it is also evidenced that the type of treatment i.e., the pattern of ethanol intake, determines the magnitude of enhancement of Sal in brain (see Hipólito et al., 2012 for review). So the question now is: Could Sal be the responsible for the VTA DA neuronal excitation after ethanol administration? In this issue of *Frontiers of Behavioral Neuroscience*, Xie and Ye review direct electrophysiological evidences supporting the role of Sal in the VTA DA neuronal activation after ethanol administration.

As occurred with the evidence supporting the role of ACD in ethanol effects, data initially provided by the scientific community on the involvement of Sal on the ethanol-derived VTA DA excitation were indirect. So, behavioral studies published in the last few years, showed that Sal directly administered into the posterior VTA is able to induce motor activation through a mechanism dependent on mu-opioid receptors (MORs) (Hipólito et al., 2010). Moreover, Sal is also able to induce motor sensitization and CPP (Hipólito et al., 2011), two behavioral responses closely related with activation of the DA mesolimbic system. Importantly, Sal is also self-administered into the posterior VTA (Rodd et al., 2008). A more direct proof supporting the ability of Sal to activate VTA DA neurons was the demonstration that microinjections of Sal into the posterior VTA increase DA levels in NAc shell (Hipólito et al., 2011; Deehan et al., 2013). Nonetheless, the most direct demonstration derives from recent studies using electrophysiological recordings. To adequately appreciate the relevance of these new findings reviewed by Xie and Ye, it is important to remember that previous electrophysiological studies reported by this group in the past decade demonstrated that ethanol indirectly excites (disinhibits) VTA DA neurons (Xiao et al., 2007). Concretely, according to their data, ethanol inhibits, through a mechanism dependent on MORs, the neuronal activity of local GABA neurons which tonically inhibit the activity of VTA DA neurons (Johnson and North, 1992). Could Sal be the responsible for these exciting findings? Using whole-cell patch-clamp recordings to examine the effects of Sal on VTA DA neurons in acute brain slices, Ye and collaborators demonstrate that Sal stimulates DA neurons partly by reducing inhibitory GABAergic transmission through a mechanism dependent on MORs. Xie and Ye also review additional aspects on the mechanism of action of Sal, such as the

ability of Sal to enhance presynaptic glutamatergic transmission onto VTA DA neurons via activation of DA type 1 receptors in the glutamatergic terminal. These and other novel aspects of the mechanism of action of Sal could be crucial to finally understand how ethanol interacts with DA mesolimbic system.

Collectively, the data reviewed suggest that Sal could be the responsible for the activation of the VTA DA neurons after ethanol administration. However, to definitively link Sal to ethanol-derived excitation of DA mesolimbic system after ethanol consumption, it is compulsory resolving, at least, two important interrogations. First, it is crucial to demonstrate the existence of an increase of VTA Sal levels after acute local or systemic ethanol administration. Second, it would be also decisive to establish a clear correlation between changes in VTA Sal levels and excitation of VTA DA neurons after ethanol administration. Future experiments on these crucial issues may be pivotal to unambiguously probe the involvement of Sal in the excitation of DA mesolimbic neurons after ethanol administration.

REFERENCES

Collins, M. A., and Bigdeli, M. G. (1975). Tetrahydroisoquinolines *in vivo*. I. rat brain formation of salsolinol, a condensation product of dopamine and acetaldehyde, under certain conditions during ethanol intoxication. *Life Sci.* 16, 585–601.

Correa, M., Salamone, J. D., Segovia, K. N., Pardo, M., Longoni, R., Spina, L., et al. (2012). Piecing together the puzzle of acetaldehyde as a neuroactive agent. *Neurosci. Biobehav. Rev*. 36, 404–430. doi: 10.1016/j.neubiorev.2011.07.009

Deehan, G. A. Jr., Engleman, E. A., Ding, Z. M., McBride, W. J., and Rodd, Z. A. (2013). Microinjections of acetaldehyde or salsolinol into the posterior ventral tegmental area increase dopamine release in the nucleus accumbens shell. *Alcohol. Clin. Exp. Res.* 37, 722–729. doi: 10.1111/acer.12034

Foddai, M., Dosia, G., Spiga, S., and Diana, M. (2004). Acetaldehyde increases dopaminergic neuronal activity in the VTA. *Neuropsychopharmacology* 29, 530–536. doi: 10.1038/sj.npp.1300326

Hipólito, L., Martí-Prats, L., Sánchez-Catalán, M. J., Polache, A., and Granero, L. (2011). Induction of conditioned place preference and dopamine release by salsolinol in posterior VTA of rats: involvement of μ-opioid receptors. *Neurochem. Int*. 59, 559–562. doi: 10.1016/j.neuint.2011.04.014

Hipólito, L., Sánchez-Catalán, M. J., Martí-Prats, L., Granero, L., and Polache, A. (2012). Revisiting the controversial role of salsolinol in the neurobiological effects of ethanol: old and new vistas. *Neurosci. Biobehav. Rev.* 36, 362–378. doi: 10.1016/j.neubiorev.2011.07.007

Hipólito, L., Sánchez-Catalán, M. J., Zornoza, T., Polache, A., and Granero, L. (2010). Locomotor stimulant effects of acute and repeated intrategmental injections of salsolinol in rats: role of mu-opioid receptors. *Psychopharmacology (Berl.)* 209, 1–11. doi: 10.1007/s00213-009-1751-9

Johnson, S. W., and North, R. A. (1992). Opioids excite dopamine neurons by hyperpolarization of local interneurons. *J. Neurosci*. 12, 483–488.

Martí-Prats, L., Sánchez-Catalán, M. J., Hipólito, L., Orrico, A., Zornoza, T., Polache, A., et al. (2010). Systemic administration of D-penicillamine prevents the locomotor activation after intra-VTA ethanol administration in rats. *Neurosci. Lett.* 483, 143–147. doi: 10.1016/j.neulet.2010.07.081

Martí-Prats, L., Sánchez-Catalán, M. J., Orrico, A., Zornoza, T., Polache, A., and Granero, L. (2013). Opposite motor responses elicited by ethanol in the posterior VTA: the role of acetaldehyde and the non-metabolized fraction of ethanol. *Neuropharmacology* 72C, 204–214. pii: S0028-3908(13)00193-7. doi: 10.1016/j.neuropharm.2013.04.047

Melis, M., Enrico, P., Peana, A. T., and Diana, M. (2007). Acetaldehyde mediates alcohol activation of the mesolimbic dopamine system. *Eur. J. Neurosci*. 26, 2824–2833. doi: 10.1111/j.1460-9568.2007.05887.x

Nagatsu, T. (1997). Isoquinoline neurotoxins in the brain and parkinson's disease. *Neurosci. Res.* 29, 99–111. doi: 10.1016/S0168-0102(97)00083-7

Rodd, Z. A., Oster, S. M., Ding, Z. M., Toalston, J. E., Deehan, G., Bell, R. L., et al. (2008). The reinforcing properties of salsolinol in the ventral tegmental area: evidence for regional heterogeneity and the involvement of serotonin and dopamine. *Alcohol. Clin. Exp. Res.* 32, 230–239. doi: 10.1111/j.1530-0277.2007.00572.x

Sánchez-Catalán, M. J., Hipólito, L., Zornoza, T., Polache, A., and Granero, L. (2009). Motor stimulant effects of ethanol and acetaldehyde injected into the posterior ventral tegmental area of rats: role of opioid receptors. *Psychopharmacology (Berl.)* 204, 641–653. doi: 10.1007/s00213-009-1495-6

Xiao, C., Zhang, J., Krnjević, K., and Ye, J. H. (2007). Effects of ethanol on midbrain neurons: role of opioid receptors. *Alcohol. Clin. Exp. Res.* 31, 1106–1113. doi: 10.1111/j.1530-0277.2007.00405.x

Received: 05 June 2013; accepted: 05 June 2013; published online: 25 June 2013.

Citation: Polache A and Granero L (2013) Salsolinol and ethanol-derived excitation of dopamine mesolimbic neurons: new insights. Front. Behav. Neurosci. 7:74. doi: 10.3389/fnbeh.2013.00074

frontiers in
BEHAVIORAL NEUROSCIENCE

REVIEW ARTICLE
published: 23 August 2013
doi: 10.3389/fnbeh.2013.00104

Elucidating the biological basis for the reinforcing actions of alcohol in the mesolimbic dopamine system: the role of active metabolites of alcohol

Gerald A. Deehan Jr*, Sheketha R. Hauser, Jessica A. Wilden, William A. Truitt and Zachary A. Rodd

Department of Psychiatry, Institute of Psychiatric Research, Indiana University, School of Medicine, Indianapolis, IN, USA

Edited by:
Elio Acquas, University of Cagliari, Italy

Reviewed by:
Miriam Melis, University of Cagliari, Italy
Roberto Ciccocioppo, University of Camerino, Italy
Yedy Israel, University of Chile, Chile

****Correspondence:***
Gerald A. Deehan Jr, Department of Psychiatry, Institute of Psychiatric Research, Indiana University, School of Medicine, 791 Union Drive, Indianapolis, IN 46202-4887, USA
e-mail: gdeehan@iupui.edu

The development of successful pharmacotherapeutics for the treatment of alcoholism is predicated upon understanding the biological action of alcohol. A limitation of the alcohol research field has been examining the effects of alcohol only and ignoring the multiple biological active metabolites of alcohol. The concept that alcohol is a "pro-drug" is not new. Alcohol is readily metabolized to acetaldehyde within the brain. Acetaldehyde is a highly reactive compound that forms a number of condensation products, including salsolinol and iso-salsolinol (acetaldehyde and dopamine). Recent experiments have established that numerous metabolites of alcohol have direct CNS action, and could, in part or whole, mediate the reinforcing actions of alcohol within the mesolimbic dopamine system. The mesolimbic dopamine system originates in the ventral tegmental area (VTA) and projects to forebrain regions that include the nucleus accumbens (Acb) and the medial prefrontal cortex (mPFC) and is thought to be the neurocircuitry governing the rewarding properties of drugs of abuse. Within this neurocircuitry there is convincing evidence that; (1) biologically active metabolites of alcohol can directly or indirectly increase the activity of VTA dopamine neurons, (2) alcohol and alcohol metabolites are reinforcing within the mesolimbic dopamine system, (3) inhibiting the alcohol metabolic pathway inhibits the biological consequences of alcohol exposure, (4) alcohol consumption can be reduced by inhibiting/attenuating the alcohol metabolic pathway in the mesolimbic dopamine system, (5) alcohol metabolites can alter neurochemical levels within the mesolimbic dopamine system, and (6) alcohol interacts with alcohol metabolites to enhance the actions of both compounds. The data indicate that there is a positive relationship between alcohol and alcohol metabolites in regulating the biological consequences of consuming alcohol and the potential of alcohol use escalating to alcoholism.

Keywords: acetaldehyde, salsolinol, ethanol, reinforcement (psychology), reward, dopamine

INTRODUCTION

Alcoholism and alcohol (EtOH) abuse is a global burden. Alcoholism is estimated to be responsible for 3.8% of all global deaths, and cost associated with treatment equivalent to 1% of gross national product of high- and medium-income countries (Rehm et al., 2009). As such, a great deal of research has focused on therapeutic interventions to aid individuals that are currently suffering from alcoholism and a great deal of effort has been put forth to identify neurobiological traits that are common in individuals that are at a high-risk to develop an alcohol-use disorder. However, while several lines of research have emerged focusing on the many different facets of EtOH addiction the biological basis of the reinforcing properties of EtOH has not been completely established. Opposing theories have emerged with some suggesting that it is the action of the EtOH molecule itself that underlies the rewarding properties of EtOH. Others believe that EtOH is simply a "pro-drug" and the rewarding properties of EtOH are dependent on the action of the metabolites/byproducts of EtOH within the brain. The main principles underlying the "pro-drug" theory assert that (1) following EtOH consumption, EtOH concentrations within the body are unable to reach levels that adequately affect the central nervous system (CNS), (2) various behavioral and physiological effects of EtOH endure well past the bioavailability of EtOH in the system, and (3) manipulation of the metabolism of EtOH, and the subsequent formation of the metabolites and/or byproducts, within the system affects most, if not all, of the CNS effects of EtOH. The contrary theory suggests that EtOH affects several neurotransmitter systems thereby exerting its effects within the CNS. Proponents of this theory suggest there is no conclusive evidence that the metabolites of EtOH possess the ability to

Abbreviations: Triazole, 3-amino-1,2,4-triazole; ACD, Acetaldehyde; ADH, Alcohol Dehydrogenase; ALDH, Aldehyde Dehydrogenase; CNS, Central Nervous System; CPP, Conditioned Place Preference; D_2, Dopamine 2; DA, Dopamine; EtOH, Ethanol; GABA, Gamma-aminobutyric Acid; GLU, Glutamate; ICV, Intra-Cerebral Ventricular; IV, Intra-venous; μM, Micro-molar; mM, Milli-molar; MOR, Mu Opioid Receptor; AcbC, Nucleus Accumbens Core; AcbSh, Nucleus Accumbens Shell; Acb, Nucleus Accumbens; $5HT_3$, Serotonin 3; 5-HT, Serotonin; TBCs, Tetrahydrobetacarbolines; SAL, Salsolinol; THIQs, Tetrahydroisoquinoline alkaloids; THP, Tetrahydropapaveraoline; FDA, United States Food and Drug Administration; VTA, Ventral Tegmental Area.

cross the blood brain barrier and the metabolites exists for too short a period to mediate the more persistent effects of EtOH intoxication. Regardless of such polarized stances, EtOH reward within the CNS likely depends on the action of EtOH in conjunction with its metabolites/byproducts. This review will present an overview of the behavioral and neurochemical actions of the neuroactive metabolite acetaldehyde (ACD), and subsequent metabolites/byproducts (i.e., salsolinol) formed through the reaction/condensation of ACD with endogenous compounds, within the central and peripheral nervous systems following EtOH intake.

THE FIRST METABOLITE OF ALCOHOL: ACETALDEHYDE

It has been well established that high levels of ACD within the periphery are associated with aversive symptoms (i.e., flushing, headaches, etc.). The drug disulfuram (tetraethylthiuramdisulphide), which has been approved for the treatment of alcoholism, exacerbates the aversive symptoms of ACD by inhibiting the metabolism of ACD thereby encouraging individuals to abstain from EtOH consumption. The mechanisms of action behind disulfuram treatment were discovered serendipitously. In the early 20th century, a report emerged describing individuals who worked in a metal manufacturing plant experiencing transitory aversive symptoms (i.e., fatigue, shortness of breath, flushing of the face, increased heart rate, headaches) following the consumption of alcoholic beverages (Koelsch, 1914). Such symptoms were subsequently linked to the compound calcium cyanamide, an organic compound used in the production of metals, which the workers were in regular contact with. Similar symptoms were reported shortly thereafter in patients that had consumed ink cap mushrooms prior to drinking EtOH; a reaction that was linked to the amino acid coprine present in the mushroom (Chifflot, 1916; Reynolds and Lowe, 1965). Two decades later, Williams (1937) suggested that the cure of alcoholism may have been discovered as workers at a rubber plant that were exposed to the compound tetramethylthiuram experienced similar aversive symptoms to those outlined above when they consumed EtOH.

Soon thereafter, two researchers, Erik Jacobsen and Jens Hald, began examining tetraethylthiuramdisulphide (disulfuram) as a possible treatment for intestinal worms. Utilizing themselves as test subjects, both men reported experiencing several aversive symptoms following EtOH consumption (i.e., sleepiness, increased heart rate, etc.; Jacobsen, 1958). Follow-up studies indicated that disulfuram, since marketed as antabuse, acted to block aldehyde dehydrogenase, an enzyme that metabolizes ACD, causing increased blood ACD levels thereby increasing the aversive side effects of EtOH consumption (Hald and Jacobsen, 1948). Early studies had already indicated a positive correlation between EtOH intake and increased blood ACD levels such that binge drinkers exhibited ACD levels 35 times greater than controls (Stotz, 1943). However, additional studies indicated that social drinkers treated with antabuse exhibited blood ACD levels 5–10 times greater than individuals that did not receive the treatment (Hald and Jacobsen, 1948; Larsen, 1948). Treating individuals with antabuse, prior to EtOH consumption, allowed for the detection of ACD in the breath (Hald and Jacobsen, 1948). Preclinical research indicated that antabuse rendered ACD detectable in the breath of rabbits following EtOH exposure and research aimed at the identification of the metabolic pathway of EtOH began (Hald et al., 1949a,b).

Over the next 30 years, several theories emerged as to the function of ACD in EtOH-use disorders (Carpenter and Macleod, 1952; Myers and Veale, 1969; Davis and Walsh, 1970; Truitt and Walsh, 1971; Griffiths et al., 1974). A number of theories identified EtOH as a "pro-drug" suggesting alcoholism would be better termed "acetaldhydeism" as ACD was responsible for all of the effects associated with the imbuement of EtOH (Truitt and Walsh, 1971; Raskin, 1975). Contradictory theories asserted that ACD in no way mediated the effects of EtOH. Such assertions were supported by research showing that the consumption of EtOH produced only trace levels of ACD in the cerebrospinal fluid and brain (Kiianmaa and Virtanen, 1978; Pikkarainen et al., 1979; Eriksson et al., 1980) and that ACD was unable to cross the blood brain barrier except when in exceedingly high concentrations (Sippel, 1974; Tabakoff et al., 1976; Eriksson, 1977; Petersen and Tabakoff, 1979). However, Cohen et al. (1980) reported that the local formation of ACD within the brain was possible thereby reestablishing the ACD/EtOH debate.

ACETALDEHYDE AND ALCOHOLISM: A GENETIC PERSPECTIVE

Following consumption, EtOH undergoes a number of reactions as it is metabolized. The primary pathway through which EtOH is eliminated from the body involves the action of the alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) enzymes (for review see: Deehan et al., 2013). The action of ADH oxidizes EtOH which results in the formation of ACD which is subsequently eliminated/metabolized by ALDH into acetate and eliminated from the body (for schematic depiction of EtOH metabolism see **Figure 1**). Alterations in either class of enzyme have been shown to produce alterations in ACD levels. An increase in the formation of ACD has been found to lead to an increase in the aversive symptoms (i.e., flushing, nausea, etc.) associated with EtOH consumption thereby decreasing further motivation to consume EtOH (Peng and Yin, 2009). Genetic studies have identified genetic polymorphisms in both ADH and ALDH which have been linked to a decreased susceptibility to develop an EtOH-use disorder (Edenberg, 2011). For instance, a recent study reported that Mexican Americans expressing the ADH1B*2 genotype were protected against EtOH-dependence (Ehlers et al., 2012). Such protection, against EtOH-dependence, likely occurs through a more rapid oxidation of EtOH resulting in significantly higher levels of peripheral ACD (Hurley and Edenberg, 2012). Research has indicated that an alteration in the expression of the ALDH2 gene results in a slower oxidation of ACD to acetate thereby resulting in a "Disulfiram-like" experience due to greater ACD levels (Ball, 2008). Recent endeavors have identified a polygenic contribution of the ADH gene cluster suggesting a potential role for several of the ADH genes in the development of alcoholism (Frank et al., 2012).

ACETALDEHYDE FORMATION WITHIN THE CNS

Following the intake of EtOH, ACD is formed in the periphery, primarily by the activity of ADH in the liver. However, given high activity of aldehyde dehydrogenase (ALDH; the primary enzyme responsible for metabolizing ACD) within the blood brain barrier

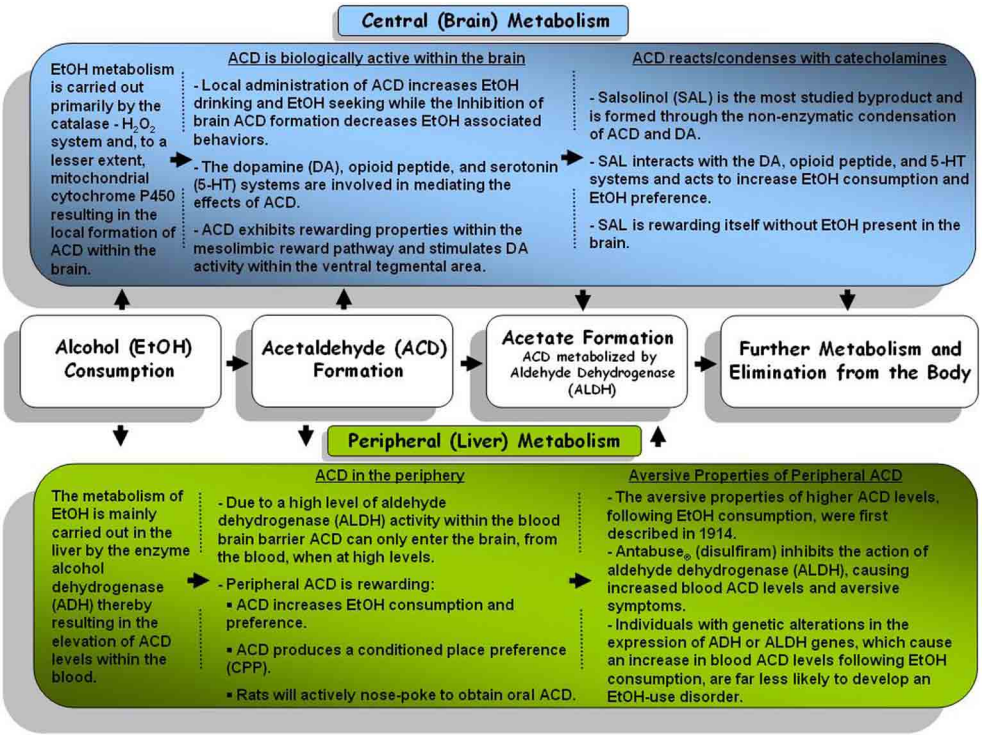


FIGURE 1 | A general schematic representation of the central (brain) and peripheral (body) metabolism pathways for alcohol and alcohol metabolites.

it was widely accepted that very little ACD could commute into the brain from the periphery (Hunt, 1996). Additional studies indicated that higher levels of ACD within the periphery may be capable of overwhelming peripheral ALDH, entering the brain within minutes (Ward et al., 1997; Quertemont et al., 2005). Metabolic activity resulting in the local formation of ACD within brain was not immediately clear and has been the topic of debate for several years. Research has indicated that ADH is not active within the brain and has established that EtOH is primarily metabolized through the activity of the catalase enzyme (Sippel, 1974; Zimatkin, 1991; Smith et al., 1997) and this enzyme remains relatively constant across different rat strains (Rhoads et al., 2012). *In vivo* studies support the activity of catalase as a key component in the formation of brain ACD following EtOH exposure as inhibition of catalase activity subsequently decreased brain ACD levels (Jamal et al., 2007). However, inhibition of catalase does not completely abolish ACD formation. Other metabolic pathways such as mitochondrial cytochrome P450 have also been found to produce ACD locally within the brain following the consumption of EtOH (Zimatkin et al., 1998; Zakhari, 2006). In mice, manipulation of cytochrome P450 has been found to alter overall sensitivity to EtOH (Vasiliou et al., 2006), EtOH consumption, and EtOH stimulated locomotor activity (Correa et al., 2009).

IMPLICATION OF ACD IN THE CENTRAL ACTIONS OF EtOH

Several studies that have made use of compounds that act to inhibit the formation of ACD or sequester ACD into a stable non-reactive adduct. Such experiments have implicated the local formation of ACD as an important aspect of the neurobiological and behavioral aspects of EtOH use/abuse. The compounds sodium azide and/or 3-amino-1,2,4-triazole (triazole) inhibit catalase activity, thereby decreasing ACD formation within the brain, and have been shown to alter EtOH related behaviors. For instance, both sodium azide and triazole significantly altered EtOH-induced locomotor activity when infused into the arcuate nucleus of the hypothalamus (Sanchis-Segura et al., 2005; Pastor and Aragon, 2008). Triazole has also been found to decrease the consumption of EtOH in both rats and mice (Aragon and Amit, 1992; Koechling and Amit, 1994), reduce EtOH induced motor depression in rats (Aragon et al., 1985) and EtOH induced locomotor activity in mice (Escarabajal et al., 2000). However, triazole has also been shown to cause a non-specific reduction in the consumption of saccharin-quinine solution (Rotzinger et al., 1994) and food intake (Tampier et al., 1995). Such data bring into question whether a reduction in EtOH consumption is a function of reduced ACD production or a general reduction in consummatory behavior caused by triazole. Recent studies have utilized a somewhat different approach to limiting the activity of the catalase system. The hydrogen peroxide (H_2O_2) scavenging compounds ebselen and alpha lipoic acid inhibit the formation of ACD through their reduction in the catalase-H_2O_2 reaction and subsequent formation of Compound I (Cohen et al., 1980). Ledesma and colleagues have demonstrated that exposure to both ebselen or alpha lipoic acid inhibit EtOH-stimulated locomotor activity in mice (Ledesma et al., 2012; Ledesma and Aragon, 2013).

Unlike compounds that directly affect brain catalase activity, thiol amino acid compounds, such as D-penicillamine or L-cysteine act to sequester ACD into a non-reactive stable adduct without altering EtOH metabolism (Cederbaum and Rubin, 1976; Nagasawa et al., 1978). Several studies have been conducted using these compounds which have added support for the role of ACD in the behavioral and pharmacological actions of EtOH. For instance, administration of either D-penicillamine or L-cysteine effectively reduced EtOH consumption and decreased EtOH conditioned place preference (CPP) in rats (Font et al., 2006b; Diana et al., 2008; Peana et al., 2008). Intra-cisterna magna injections of D-pennicillamine acted to block EtOH- and/or ACD appetitive conditioning to a surrogate nipple in newborn rats (March et al., 2013) and induced locomotor activity and tactile stimulus preference in preweanling rats (Pautassi et al., 2011). Mice exhibit a decrease in EtOH CPP and a reduction in EtOH-induced motor depression when treated with D-penicillamine (Font et al., 2005, 2006a). L-cysteine has been found to reduce nose-poke responding for ACD and EtOH during acquisition, maintenance, and reinstatement phases of testing (Peana et al., 2010, 2012) as well as inhibit EtOH and ACD induced CPP (Peana et al., 2009). Peripheral and central (intra-VTA) exposure to D-penicillamine significantly reduced expression of the alcohol-deprivation effect (ADE) as observed by a lack on an increase in EtOH consumption during the initial 3 post-abstinence measurements (Orrico et al., 2013). This finding offers support for the role of ACD in the expression of relapse-like behaviors as the ADE has been established as an animal model for EtOH relapse-drinking (for review see: McBride and Li, 1998).

Perhaps the most compelling evidence for the involvement of ACD in the central actions of EtOH has emerged from studies utilizing adenoviral and lentiviral vectors that alter catalase, ADH, or ALDH activity. Approximating the significantly higher activity of the ADH enzyme for individuals expressing the ADH1B*2 gene, mutated cDNA which encoded rADH-47His (the rat analogue for the ADH1B*2 gene) was peripherally administered to the University of Chile Bibulous (UChB) alcohol preferring rat line and resulted in significantly higher ACD blood levels while also significantly reducing EtOH consumption (Rivera-Meza et al., 2010, 2012). Similarly, an adenoviral vector coded for ALDH2 antisense RNA, to approximate clinical condition of reduced ALDH2 activity, produced comparable increases in blood ACD levels and decreases in EtOH consumption (Ocaranza et al., 2008; Rivera-Meza et al., 2012). Studies looking at the central administration of anticatalase (shRNA)- or ADH (rADH1)-encoding lentiviral vectors, which inihibit catalase synthesis or increases the activity of ADH respectively, have been found to alter EtOH-related behaviors. Administration of the anticalatase lentiviral vector into the ventral tegemental area (VTA) significantly reduced EtOH consumption and EtOH stimulated DA release in the AcbSh whereas the rADH1-encoding vector facilitated an increase in EtOH intake (Karahanian et al., 2011). Quintanilla et al. (2012) reported that insertion of an anticatalase viral vector into the VTA resulted in the reduction of EtOH consumption when administered prior to EtOH testing. However, when the viral vector was administered during an ongoing EtOH drinking period, animals only exhibited a reduction in EtOH intake following a period of imposed abstinence during relapse-like drinking (Quintanilla et al., 2012). With regard to ADE expression, an additional study examining the effects of intra-VTA injection of anticatalase viral vector immediately following 67 consecutive days of EtOH exposure and immediately prior to a 15 day EtOH deprivation period, significantly reduced relapse drinking during both a first and second reinstatement of EtOH access (Tampier et al., 2013). Taken as a whole, research utilizing such cutting-edge techniques suggest that ACD possess a substantial role in the neurobiological actions of EtOH.

ACD EXHIBITS REWARDING PROPERTIES

While it is difficult to suggest that the behavioral and neurobiological effects of EtOH are completely dependent on the presence of ACD, there is a substantial amount of literature suggesting that ACD is involved to a significant extent. Studies examining the behavioral effects of ACD, with regard to EtOH reward, have reported that intra-cranial ventricular (ICV) administration of ACD acted to increase the consumption of and preference for EtOH in rodents (Brown et al., 1979, 1980; Amit and Smith, 1985) while peripheral administration of higher doses of ACD produced a conditioned taste aversion in several rat lines (Brown et al., 1978; Aragon et al., 1986; Kunin et al., 2000; Quintanilla et al., 2002; Escarabajal et al., 2003). Utilizing the UChB rat line (an alcohol preferring rat line), researchers have revealed that peripheral ACD exposure, at lower doses (50–100 mg/kg ACD) than those shown to produce a conditioned taste aversion (>200 mg/kg ACD), acted to significantly increase the consumption of EtOH over the two weeks following ACD administration (Tampier and Quintanilla, 2002). Taken together, such findings may suggest that ACD facilitates the development of tolerance to the aversive effects of EtOH thereby increasing EtOH consumption.

It has also been reported that ACD possesses rewarding/reinforcing properties itself as animals readily self-administered both ICV ACD (Amit et al., 1977; Brown et al., 1979, 1980) and intra-venous ACD (Myers et al., 1984a,b; Takayama and Uyeno, 1985). Central ICV administration of ACD produced a CPP (Smith et al., 1984). Extending on such findings, recent endeavors have reported that ACD, whether administered centrally or peripherally, produced a CPP in several rat lines (Quintanilla and Tampier, 2003; Peana et al., 2008; Spina et al., 2010). Adult rats peripherally treated with ACD exhibit a dose-dependent preference to a discrete olfactory stimulus (Quertemont and DeWitte, 2001). Rat pups exhibited a significant preference to an olfactory cue previously paired with ACD exposure (March et al., 2013) while pre-weanling rats exhibited an ACD-dependent stimulation of locomotor activity and tactile stimulus preference following EtOH administration (Nizhnikov et al., 2007; Pautassi et al., 2011). ACD has been shown to dose-dependently alter locomotor activity in adult animals as well. Rodent testing has reported that the central administration of lower doses of ACD resulted in significant increase in locomotor activty (Correa et al., 2003; Sanchez-Catalan et al., 2009) while higher doses, administered either centrally or peripherally, resulted in a significant depression

of locomotor activity (Holtzman and Schneider, 1974; Ortiz et al., 1974; Myers et al., 1987; Durlach et al., 1988; Quertemont et al., 2004; Tambour et al., 2006). An early study also observed comparable biphasic effects utilizing a vapor exposure paradigm to deliver ACD (Ortiz et al., 1974). Recent studies have pursued the evaluation of the reinforcing effects of ACD via the oral route and reported that rats will actively nose-poke (Peana et al., 2010, 2012) or lever press to obtain ACD (Cacace et al., 2012). However, it is unlikely that the effects of oral ACD on the ACD self-administration were mediated via central ACD as Peana et al. (2010, 2012) reported that blood and brain ACD levels did not significantly differ between rats consuming oral ACD and those consuming water (Peana et al., 2010, 2012). Nonetheless, ACD possess rewarding properties itself which are related to (or underlie) the behavioral actions of EtOH.

ACETALDEHYDE REACTIVITY: BYPRODUCTS OF ACETALDEHYDE

Acetaldehyde is a highly reactive compound that interacts with several endogenous neurochemicals in the brain to form a number of additional biologically active products (Cohen and Collins, 1970; Davis and Walsh, 1970; Walsh et al., 1970; Cohen, 1976). With regard to neurobiological and behavioral testing of the byproducts of ACD, the majority of attention has focused on two main classes of compounds which are formed through condensation of ACD with the catecholamines. The first class of compounds, the tetrahydroisoquinoline alkaloids (THIQs), are formed through both the direct and indirect condensation of ACD with the monoamines: dopamine, epinephrine, and norepinephrine (Cohen, 1976). The tetrahydro-beta-carbolines (TBCs) on the other hand, are formed through the reaction of ACD with the indoleamines: tryptophan and tryptamine (Buckholtz, 1980). The THIQs tetrahydropapaveroline (THP) and salsolinol (SAL) have received the most attention as to their role in alcohol use-disorders as both compounds can be detected in the brain following EtOH administration. The TBCs have received considerably less attention and contradictory data exists as to their contribution to the neurobiological effects of EtOH.

TETRAHYDROPAPAVEROLINE

The formation of THP occurs via the condensation of dopaldehyde and dopamine. In this sense, ACD is indirectly associated with the formation of THP as ACD inhibits the breakdown/metabolization of dopaldehyde subsequently increasing THP levels in the brain (Davis and Walsh, 1970). Early studies observed an enhanced preference for EtOH and consumption of EtOH following ICV microinjections of low concentrations of THP in both rodents and primates (Melchior and Myers, 1977; Myers and Melchior, 1977; McCoy et al., 2003) while higher concentrations reduced both EtOH consumption and preference (Duncan and Deitrich, 1980). Manipulation of the mesolimbic DA pathway through microinjections of lower doses of THP into either the ventral tegmental area (VTA) or Nucleus Accumbens (Acb) accentuated EtOH preference in rats (Myers and Privette, 1989; Duncan and Fernando, 1991). Experiments were conducted in an effort to identify the neuroanatomical substrates of both the enhancing and aversive properties of THP with regard to alcohol related behaviors (Privette et al., 1988; Myers and Privette, 1989; Privette and Myers, 1989) however, research on the role of THP in EtOH-use disorders has slowed considerably over the past two decades.

SALSOLINOL

Salsolinol (SAL; 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline) is the most extensively studied byproduct of ACD in relation to EtOH-use disorders and studies aimed at examining the underlying contribution of SAL to the reinforcing properties of EtOH are still in full swing. The *in vivo* formation of SAL occurs primarily through non-enzymatic Pictet-Spengler condensation of DA with ACD (Lee et al., 2010) but has been hypothesized to occur through secondary processes as well (for review see: Hipolito et al., 2012). Several studies have sought to quantify SAL levels within the body and brain following EtOH ingestion with mixed results. Specifically in the rodent brain, studies have shown that EtOH exposure (via oral consumption or experimenter administered EtOH) increased (Rojkovicova et al., 2008) or did not alter (Lee et al., 2010) SAL levels in several brain regions. Nonetheless, there is a substantial amount of evidence suggesting that SAL is intricately involved with the rewarding properties of EtOH.

While the effect of SAL administration on EtOH intake received considerably less attention than that of ACD, early endeavors found that centrally ICV administered SAL caused animals to exhibit an increase in both their consumption of and preference for (Myers and Melchior, 1977; Duncan and Deitrich, 1980; Purvis et al., 1980). Altshuler and Shippenberg (1982) indicated that SAL possess similar discriminative properties compared to EtOH in that animals respond comparably when SAL is substituted for EtOH. More recently, several laboratories have shown that SAL exhibits reinforcing properties in the absence of EtOH. Animals exhibited a CPP for peripheral injections of 10 mg/kg SAL with higher (30 mg/kg) and lower (1 and 3 mg/kg) doses falling in a U-shaped dose response curve (Matsuzawa et al., 2000). Interestingly, when the animals were exposed to a conditioned fear stress (foot shock) the dose response curve shifted to the left (optimal dose: 3 mg/kg) as the animals exhibited a greater sensitivity to the reinforcing properties of SAL (Matsuzawa et al., 2000). Central administration of SAL (intra-VTA) has also been shown to induce a CPP in rats (Hipolito et al., 2011). Much like in response to EtOH, rats will exhibit a biphasic response in SAL-stimulated locomotor activity, specifically when SAL is microinjected into the VTA (Hipolito et al., 2010). Perhaps the most convincing evidence that SAL is reinforcing, even in the absence of EtOH, lies in data showing that animals will readily self-administer SAL into the posterior (p)VTA via intra-cranial self-administration at concentrations far below required to sustain the ICSA or EtOH or ACD (Rodd et al., 2008). Thus, research has outlined a clear role for SAL in the behavioral and neurobiological actions of EtOH and ongoing research is working toward the delineation of the nature of this contribution.

TETRAHYDRO-BETACARBOLINES

The role of TBCs in EtOH-use disorders has received considerably less attention than ACD and/or SAL. Findings have been

somewhat inconsistent as early research indicated that peripheral injections of TBC derivatives reduced EtOH preference (Geller and Purdy, 1975). Central administration (ICV microinjections) of the TBC tryptoline had the opposite effect as it significantly increased both EtOH preference and EtOH consumption (Myers and Melchior, 1977; Tuomisto et al., 1982; Airaksinen et al., 1983; Huttunen and Myers, 1987; Adell and Myers, 1994). Co-administration THP and tryptoline resulted in a synergistic increase in EtOH preference and consumption (Myers and Oblinger, 1977). Hippocampal microinjections of TBCs produced alterations in both 5-HT and norepinephrine levels thereby significantly augmenting EtOH preference and consumption in low alcohol drinking (LAD) rats (Huttunen and Myers, 1987; Adell and Myers, 1995). Additionally, TBCs have been shown to possess an affinity for the delta opioid receptor (Airaksinen et al., 1984). Overall, however, the pharmacological properties of TBCs have yet to be fully examined.

THE NEUROBIOLOGICAL ACTIONS OF EtOH AND EtOH METABOLITES WITHIN THE REWARD PATHWAY

While early studies investigated the effects of the central and/or peripheral administration of ACD and/or SAL on the behavioral actions of EtOH (as discussed above), the dissemination of the underlying mechanisms of ACD and SAL at the neurobiological level has gained significant traction over the past three decades. The advent of a number of novel techniques (i.e., intracranial self-administration; ICSA) has allowed researchers to more thoroughly evaluate the neurobiological actions of drugs of abuse including EtOH and its metabolites. Research to date suggests that the neurobiological actions of EtOH and EtOH metabolites overlap to ultimately affect the development/expression of EtOH-use disorders. This section will present an overview of preclinical research focused on the neurobiological mechanisms within the brain reward pathway that have been identified to play a key role in the rewarding/reinforcing properties of EtOH, ACD, and the THIQs.

Numerous studies have implicated the mesocorticolimbic dopamine reward pathway (MCL) as a key mediator of the rewarding/reinforcing properties of virtually every major drug of abuse including EtOH (for review see: Di Chiara and Imperato, 1988). The MCL originates in the VTA and projects to several forebrain regions including the Acb (Oades and Halliday, 1987). An early study indicated that peripheral EtOH exposure stimulated DA neuronal activity within the substantia nigra (Mereu et al., 1984). A subsequent experiment found that peripheral injections of EtOH (0.5 mg/kg) significantly elevated DA levels within the AcbSh of freely moving rats (Di Chiara and Imperato, 1985). Subsequent research over the past 3 decades has elucidated a cascade of neurochemical events within the MCL that underlie EtOH reinforcement (for review see: Spanagel, 2009). For instance, it has been well documented that EtOH itself primarily targets N-methyl-D-aspartate (NMDA; Lovinger et al., 1989), 5-hydroxytryptamine 3 (5-HT_3; Lovinger and Zhou, 1998), nicotinic acetylcholine (nAch; Narahashi et al., 1999), γ-aminobutyric acid A ($GABA_A$) and glycine (Mihic et al., 1997; Mihic, 1999) receptors. The EtOH molecule also primarily interacts with non-ligand gated ion channels as EtOH inhibits L-type Ca^{2+} channels and opens G protein-activated inwardly rectifying K^+ (GIRKs) channels (Vengeliene et al., 2008). Overall, such primary effects underlie and/or contribute to several secondary effects within the MCL (i.e., increases in DA efflux) that ultimately result in the rewarding/reinforcing properties of EtOH (Spanagel, 2009). Thus, research has established that the neurobiological actions of the EtOH molecule itself are important to the reinforcing properties of EtOH. However, given the dynamic nature of the neurobiological functioning of the MCL and the concurrent actions of EtOH metabolites, the extent to which the actions of the EtOH molecule itself contribute to overall EtOH reinforcement is somewhat tenuous.

Several studies have focused on the role of the projection from the VTA to the Acb in the neurobiological actions of EtOH as well as the metabolites of EtOH (see **Figure 2**). Specifically within the VTA, EtOH and ACD have been shown to activate DA neurons by significantly increasing their firing rate (Gessa et al., 1985; Brodie et al., 1990; Foddai et al., 2004), albeit through differing mechanisms (for review see: Deehan et al., 2013). Sequestering ACD formation through the direct infusion of D-penicillamine into the VTA inhibits DA neuronal activation by the intra-gastric administration of both EtOH and ACD (Enrico et al., 2009). Local application of an ADH (Foddai et al., 2004) or catalase inhibitor (Melis et al., 2007; Diana et al., 2008) in the VTA prevents EtOH stimulated increases in DA neuronal activity. These findings coupled with data from *in vitro* studies showing that ACD stimulates VTA DA neuronal activity at concentrations 1200–2000 fold lower than that required for EtOH suggest that ACD is critical component required for EtOH stimulated DA activity within the VTA (Brodie and Appel, 1998; Brodie et al., 1999; Diana et al., 2008). A recent paper has reported that SAL is also capable of stimulating VTA DA neuronal activity at concentrations 10-1,000 fold lower than the lowest effective concentration of ACD (Xie et al., 2012a).

Relative differences in effective concentrations between EtOH, ACD, and SAL have also been reported by studies examining the behavioral neuropharmacology of these compounds within the VTA. An early study indicated the alcohol preferring (P) rats (an animal model for alcoholism) would readily self-adminster EtOH directly into the VTA exhibiting a U-shaped dose response curve with 100 mg % being the most effective concentration (Gatto et al., 1994). Follow-up studies identified a regional heterogeneity within the VTA as both P and Wistar rats would self-administer EtOH into the posterior (p) VTA but not anterior (a) VTA at doses of 20-80 mM (Rodd et al., 2003, 2005). Much like EtOH, both ACD and SAL are self-administered into the pVTA in an inverted U-shaped dose response pattern and in congruence with neurophysiological data, the pVTA appears to be significantly more sensitive to the rewarding/motivational properties of each compound in a stepwise fashion (SAL > ACD > EtOH). For example, P rats self-administered ACD between the dose ranges of 6–90 μM (Rodd-Henricks et al., 2002; Rodd et al., 2005) whereas SAL ICSA was supported in a dose range of 0.03–0.3 μM (Rodd et al., 2008). For perspective,

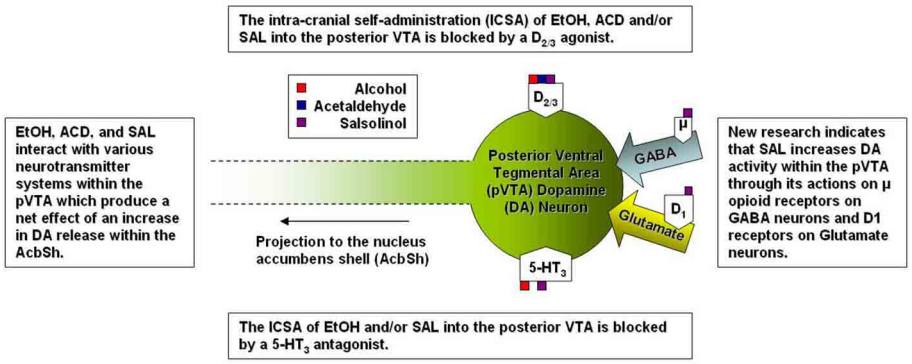


FIGURE 2 | A simplified representation of the sites of action for alcohol and alcohol metabolites on posterior ventral tegmental area dopamine neurons.

the optimal concentration for the ICSA of SAL is approximately 200-fold lower than the most effective concentration of ACD and 300×10^3 lower than the optimal concentration of EtOH. Additionally, the co-infusion of the $D_{2/3}$ agonist quinpirole (100 μM) blocked the ICSA of EtOH, ACD, and SAL into the pVTA (Rodd et al., 2005, 2008) suggesting that of DA neuronal activation within the pVTA is a common mechanism underlying the rewarding/motivational properties of EtOH, ACD, and SAL.

An alternative method to assess the efficacy of a given compound to stimulate DA neurons within the VTA involves microinjecting the compound into the VTA and measuring DA release in downstream projection structures (i.e., the Acb). An early study employed such a paradigm to examine the down-stream effects of microinjections of THP into the VTA on DA efflux within the core (AcbC) and the AcbSh reporting that a 13.6 μM microinfusion of THP increased DA efflux in the AcbC (94%) whereas the same dose decreased DA efflux in the AcbSh (51%; Myers and Robinson, 1999). Given that cannula placement were anterior to the VTA and the THP dose was well above the pharmacological range of the *in vivo* generation of THP (Haber et al., 1997; Baum et al., 1999), it is difficult to resolve whether THP altered DA neuronal activity directly or through a non-specific mechanism. Recent research, however, has utilized similar equipment as that employed for ICSA experiments to examine the effects of intra-pVTA microinjections of EtOH, ACD, and/or SAL on DA levels downstream within the AcbSh (Ding et al., 2009, 2011; Deehan et al., 2013). Ding et al. (2009) reported that pulse microinjections of 200 mg% (∼44 mM) EtOH was the most efficacious dose at stimulating DA efflux in the AcbSh of Wistar rats. Utilizing the same range of doses of ACD and SAL that were reliably self-administered via ICSA (Rodd et al., 2005, 2008), Deehan et al. (2013) reported that Wistar rats exhibited comparable U-shaped dose response curves for DA efflux in the AcbSh following pulse microinjections of ACD and/or SAL into the pVTA. Along the same lines as previous observations utilizing alternative paradigms, DA neurons within the pVTA exhibited a significantly greater sensitivity to ACD and/or SAL compared to EtOH. Pulse microinjections of 23 μM ACD or 0.3 μM SAL were effective at significantly increasing DA efflux within the AcbSh to levels 200 and 300% above baseline respectively (Deehan et al., 2013). Moreover, this was observed for an ACD dose that was over 1800 fold, and a SAL dose that was 147,000 fold lower, than the peak dose of EtOH. These data further suggest that the pVTA is differentially sensitive to EtOH, ACD, and SAL in a manner that is consistent with the production of ACD and SAL through conventional metabolic processes.

The findings from the microinjection/microdialysis study by Deehan et al. (2013) extend on previous research that reported increases in accumbal DA in response to local exposure of higher concentrations of ACD or SAL within the pVTA. The reverse microdialysis of 75 μM ACD in the pVTA stimulated DA release in the AcbSh to 150% of baseline (Melis et al., 2007; Diana et al., 2008) while Hipolito et al. (2011) reported that a microinjection of SAL (150 μM) within the pVTA caused an increase in AcbSh DA to 130% of baseline. However, SAL has been shown to modulate DA levels within the AcbC and AcbSh in an opposing manner. Local perfusion of SAL via reverse microdialysis, over the course of a 20-min sample, significantly increased DA levels in the AcbC but decreased DA levels in the AcbSh (Hipolito et al., 2009) in a manner consistent with the effects of selective μ- and δ-opioid receptor agonists reported by the same lab (Hipolito et al., 2008). Although the lowest concentration of SAL (5 μM) used by Hipolito et al. (2009) was significantly higher than the optimal concentration (0.3 μM) that stimulated activity in DA neurons within the pVTA (Deehan et al., 2013), ICSA studies have shown that the AcbSh is significantly less sensitive to the rewarding properties of SAL with the greatest level of responding exhibited for the 3.0 μM concentration of SAL (Rodd et al., 2003). Additionally, the rewarding properties of SAL within the AcbSh were found to be dependent on post-synaptic activation of DA receptors as the $D_{2/3}$ antagonist (sulpiride) completely abolished ICSA responding for SAL.

Overall, there are several neurobiological mechanisms that underlie the EtOH, ACD, or SAL induced stimulation of DA neuronal activity within the pVTA, not all of which participate

equally across the three compounds. For instance, research has implicated 5-HT_3 receptors in the reinforcing properties of EtOH and SAL but not ACD. The compound ICS 250,390 (a 5-HT_3 receptor antagonist) selectively prevents the ICSA of both EtOH and SAL but does not affect the ICSA of ACD (Rodd et al., 2005, 2008). This stands to reason as EtOH possesses an affinity for 5-HT_3 receptors (Lovinger and White, 1991) and SAL increases the efflux of 5-HT within the rat striatum (Maruyama et al., 1993) but ACD does not exhibit an affinity for $5HT_3$ receptors (Li, 2000). Within the striatum SAL decreases the metabolization of 5-HT through a reduction in metabolizing enzymes resulting in an increase in 5-HT levels to 20 times that of DA (Nakahara et al., 1994). Similar findings have been reported with regard to DA as SAL increases catecholamine levels within the brain through a combination of the inhibition of reuptake (Heikkila et al., 1971; Tuomisto and Tuomisto, 1973; Alpers et al., 1975) and a reduction in the metabolizing enzymes such as catecholmethyltransferase and monoamine oxidase (Collins et al., 1973; Alpers et al., 1975).

From early on, studies had outlined a substantial role for the mu opioid receptor (MOR) in the neurobiological actions of EtOH, ACD, and SAL within the MCL. Naltrexone, a general opioid antagonist with an affinity for all three opioid receptors (mu, delta and kappa), has been approved by the FDA for use in the treatment of alcohol use disorders (Johnson and Ait-Daoud, 2000). Preclinical data indicate that naltrexone decreases both free-choice consumption and the operant self-administration of EtOH (for review see: Gianoulakis, 2009). For instance, EtOH has been found to directly alter the release of opioid peptides (Jarjour et al., 2009) and both naltrexone and β-funaltrexamine (β-FNA; a selective MOR antagonist) reduced the duration of DA release within the AcbSh caused by intra-VTA microinjections of EtOH (Valenta et al., 2013). Microinjections of higher concentrations of EtOH into the VTA stimulate locomotor activity that is prevented by the co-administration of β-FNA (Sanchez-Catalan et al., 2009) or the co-administration of D-penicillamine (Marti-Prats et al., 2010) suggesting that the locomotor activating effects of EtOH within the VTA require MOR activation as well as the presence of ACD.

Both ACD and SAL possess locomotor stimulating properties within the VTA and much like EtOH, the activation of locomotor activity has been reported to be dependent on MOR activation (Sanchez-Catalan et al., 2009; Hipolito et al., 2011). To date, there is a lack of research investigating the effects of MOR manipulation on the self-administration of ACD or SAL. An early study observed a decrease in the IV self-administration of ACD when animals were treated with naloxone (Myers et al., 1984a). Further, the oral self-administration (nose poke responding) of ACD was decreased by naltrexone and naloxonazine (a selective MOR_1 antagonist; Peana et al., 2011). Naltrexone also acted decrease extracellular signal-regulated kinase phosphorylation within the Acb caused by ACD self-administration (Peana et al., 2011). However, the full transgression from MOR activity to increased DA neuronal activity within the pVTA, and subsequent increase in DA release downstream, is indeed complex. Xie and colleagues have reported that SAL stimulates DA neurons within the pVTA indirectly by activating MORs which in turn inhibit of gama-amino butyric acid (GABA) neurons (Xie et al., 2012a) while also increasing glutamatergic signaling into the pVTA (Xie and Ye, 2012b). Overall, it is likely that the rewarding/reinforcing properties of both ACD and SAL in the pVTA are dependent on DA release within the AcbSh and the DA activity is modulated via MORs. After all it has been shown that direct stimulation of MORs increase DA release within the AcbSh (Spanagel et al., 1992) an similar effect to that observed following microinjections of EtOH, ACD, or SAL into the pVTA (Ding et al., 2009; Deehan et al., 2013). Although there are no studies focused on the role of MOR activity in the central self-administration of ACD and/or SAL, the current body of literature has implicated MOR activity within the pVTA as a key mediator of the neurobiological action of ACD and/or SAL on DA neurons. Future research will help to further elucidate other contributory structures and neurochemical systems, within the MCL, with regard to ACD and SAL.

GENERAL SUMMARY

The action of EtOH within the CNS is extremely complex yet the current body of literature has outlined a significant role for ACD and SAL in the modulation of the behavioral and neurological effects of EtOH. It has been shown that EtOH can act directly within the VTA to stimulate DA neurons (Gessa et al., 1985; Lovinger and White, 1991; Brodie et al., 1999; Ye et al., 2001) and accumulating evidence suggests that both ACD and SAL exhibit distinct actions on neurobiological processes within the MCL. The utilization of inhibitory/sequestering agents preventing the conversion from EtOH into ACD clearly affect EtOH consumption and reinforcement further supporting the role for the metabolites of EtOH in EtOH-use disorders. Thus, convergent evidence supports the following assertions: (1) within the CNS, EtOH is capable of altering neurobiological and behavioral processes, (2) evidence exists supporting the notion that the actions of EtOH, are, in part, mediated by the metabolites ACD and SAL formed during metabolic processes, (3) both ACD and SAL possess reinforcing properties within the MCL at levels shown to be pharmacologically relevant, and (4) further research focused on examining the central effects of EtOH and EtOH metabolites will greatly improve our understanding of how these compounds function in regard to the development/expression of EtOH-use disorders. Overall, the manifestation of EtOH-use disorders in the clinical population is undoubtedly a result of a complex and interrelated series of central and peripheral effects of EtOH and the metabolites of EtOH. Research aimed at increasing our understanding of such a complex system will facilitate the development of successful pharmaocterapeutic treatments for individuals suffering from, or are at a high risk to develop, an EtOH-use disorder.

ACKNOWLEDGMENTS

A portion of the research discussed was supported by the National Institutes of Health grants: AA007462, AA007611, and AA014437 from the National Institute on Alcohol Abuse and Alcoholism.

REFERENCES

Adell, A., and Myers, R. D. (1994). Increased alcohol intake in low alcohol drinking rats after chronic infusion of the beta-carboline harman into the hippocampus. *Pharmacol. Biochem. Behav.* 49, 949–953. doi: 10.1016/0091-3057(94)90248-8

Adell, A., and Myers, R. D. (1995). 5-HT, dopamine, norepinephrine, and related metabolites in brain of low alcohol drinking (LAD) rats shift after chronic intrahippocampal infusion of harman. *Neurochem. Res.* 20, 209–215. doi: 10.1007/BF00970546

Airaksinen, M. M., Mahonen, M., Tuomisto, L., Peura, P., and Eriksson, C. J. (1983). Tetrahydro-beta-carbolines: effect on alcohol intake in rats. *Pharmacol. Biochem. Behav.* 18(Suppl. 1), 525–529. doi: 10.1016/0091-3057(83)90230-7

Airaksinen, M. M., Saano, V., Steidel, E., Juvonen, H., Huhtikangas, A., and Gynther, J. (1984). Binding of beta-carbolines and tetrahydroisoquinolines by opiate receptors of the delta-type. *Acta Pharmacol. Toxicol. (Copenh)* 55, 380–385. doi: 10.1111/j.1600-0773.1984.tb01998.x

Alpers, H. S., McLaughlin, B. R., Nix, W. M., and Davis, V. E. (1975). Inhibition of catecholamine uptake and retention in synaptosomal preparations by tetrahydroisoquinoline and tetrahydroprotoberberine alkaloids. *Biochem. Pharmacol.* 24, 1391–1396. doi: 10.1016/0006-2952(75)90361-5

Altshuler, H. L., and Shippenberg, T. S. (1982). Tetrahydroisoquinoline and opioid substrates of alcohol actions. *Prog. Clin. Biol. Res.* 90, 329–344.

Amit, Z., Brown, Z. W., and Rockman, G. E. (1977). Possible involvement of acetaldehyde, norepinephrine and their tetrahydroisoquinoline derivatives in the regulation of ethanol self-administration. *Drug Alcohol Depend.* 2, 495–500. doi: 10.1016/0376-8716(77)90049-7

Amit, Z., and Smith, B. R. (1985). A multi-dimensional examination of the positive reinforcing properties of acetaldehyde. *Alcohol* 2, 367–370. doi: 10.1016/0741-8329(85)90077-1

Aragon, C. M., Abitbol, M., and Amit, Z. (1986). Acetaldehyde may mediate reinforcement and aversion produced by ethanol. An examination using a conditioned taste-aversion paradigm. *Neuropharmacology* 25, 79–83. doi: 10.1016/0028-3908(86)90062-6

Aragon, C. M., and Amit, Z. (1992). The effect of 3-amino-1 2, 4-triazole on voluntary ethanol consumption: evidence for brain catalase involvement in the mechanism of action. *Neuropharmacology* 31, 709–712. doi: 10.1016/0028-3908(92)90150-N

Aragon, C. M., Spivak, K., and Amit, Z. (1985). Blockade of ethanol induced conditioned taste aversion by 3-amino-1 2, 4-triazole: evidence for catalase mediated synthesis of acetaldehyde in rat brain. *Life Sci.* 37, 2077–2084. doi: 10.1016/0024-3205(85)90579-X

Ball, D. (2008). Addiction science and its genetics. *Addiction* 103, 360–367. doi: 10.1111/j.1360-0443.2007.02061.x

Baum, S. S., Hill, R., Kiianmaa, K., and Rommelspacher, H. (1999). Effect of ethanol on (R)- and (S)-Salsolinol, salsoline, and THP in the nucleus accumbens of AA and ANA rats. *Alcohol* 18, 165–169. doi: 10.1016/S0741-8329(98)00080-9

Brodie, M. S., and Appel, S. B. (1998). The effects of ethanol on dopaminergic neurons of the ventral tegmental area studied with intracellular recording in brain slices. *Alcohol. Clin. Exp. Res.* 22, 236–244. doi: 10.1111/j.1530-0277.1998.tb03644.x

Brodie, M. S., Pesold, C., and Appel, S. B. (1999). Ethanol directly excites dopaminergic ventral tegmental area reward neurons. *Alcohol. Clin. Exp. Res.* 23, 1848–1852. doi: 10.1111/j.1530-0277.1999.tb04082.x

Brodie, M. S., Shefner, S. A., and Dunwiddie, T. V. (1990). Ethanol increases the firing rate of dopamine neurons of the rat ventral tegmental area *in vitro*. *Brain Res.* 508, 65–69. doi: 10.1016/0006-8993(90)91118-Z

Brown, Z. W., Amit, Z., and Rockman, G. E. (1979). Intraventricular self-administration of acetaldehyde, but not ethanol, in naive laboratory rats. *Psychopharmacology (Berl)* 64, 271–276. doi: 10.1007/BF00427509

Brown, Z. W., Amit, Z., and Smith, B. (1980). Intraventricular self-administration of acetaldehyde and voluntary consumption of ethanol in rats. *Behav. Neural Biol.* 28, 150–155. doi: 10.1016/S0163-1047(80)91487-9

Brown, Z. W., Amit, Z., Smith, B., and Rockman, G. E. (1978). Differential effects on conditioned taste aversion learning with peripherally and centrally administered acetaldehyde. *Neuropharmacology* 17, 931–935. doi: 10.1016/0028-3908(78)90134-X

Buckholtz, N. S. (1980). Neurobiology of tetrahydro-beta-carbolines. *Life Sci.* 27, 893–903. doi: 10.1016/0024-3205(80)90098-3

Cacace, S., Plescia, F., Barberi, I., and Cannizzaro, C. (2012). Acetaldehyde oral self-administration: evidence from the operant-conflict paradigm. *Alcohol. Clin. Exp. Res.* 36, 1278–1287. doi: 10.1111/j.1530-0277.2011.01725.x

Carpenter, R. K., and Macleod, L. D. (1952). The effects of ethyl alcohol and acetaldehyde on maze behaviour and motor co-ordination in rats. *J. Ment. Sci.* 98, 167–173.

Cederbaum, A. I., and Rubin, E. (1976). Mechanism of the protective action of cysteine and penicillamine against acetaldehyde-induced mitochondrial injury. *Biochem. Pharmacol.* 25, 2179–2185. doi: 10.1016/0006-2952(76)90130-1

Chifflot, M. J. (1916). Sur un cas de rubefaction de la face, tendant a se generalizer a la suite de I'ngestion du Coprinus atramentarius. *Fr. Bull. Soc. Mycol. France* 32, 63.

Cohen, G. (1976). Alkaloid products in the metabolism of alcohol and biogenic amines. *Biochem. Pharmacol.* 25, 1123–1128. doi: 10.1016/0006-2952(76)90357-9

Cohen, G., and Collins, M. (1970). Alkaloids from catecholamines in adrenal tissue: possible role in alcoholism. *Science* 167, 1749–1751. doi: 10.1126/science.167.3926.1749

Cohen, G., Sinet, P. M., and Heikkila, R. (1980). Ethanol oxidation by rat brain *in vivo*. *Alcohol. Clin. Exp. Res.* 4, 366–370. doi: 10.1111/j.1530-0277.1980.tb04833.x

Collins, A. C., Cashaw, J. L., and Davis, V. E. (1973). Dopamine-derived tetrahydroisoquinoline alkaloids–inhibitors of neuroamine metabolism. *Biochem. Pharmacol.* 22, 2337–2348. doi: 10.1016/0006-2952(73)90015-4

Correa, M., Arizzi, M. N., Betz, A., Mingote, S., and Salamone, J. D. (2003). Open field locomotor effects in rats after intraventricular injections of ethanol and the ethanol metabolites acetaldehyde and acetate. *Brain Res. Bull.* 62, 197–202. doi: 10.1016/j.brainresbull.2003.09.013

Correa, M., Viaggi, C., Escrig, M. A., Pascual, M., Guerri, C., Vaglini, F., et al. (2009). Ethanol intake and ethanol-induced locomotion and locomotor sensitization in Cyp2e1 knockout mice. *Pharmacogenet. Genomics* 19, 217–225. doi: 10.1097/FPC.0b013e328324e726

Davis, V. E., and Walsh, M. J. (1970). Alcohol, amines, and alkaloids: a possible biochemical basis for alcohol addiction. *Science* 167, 1005–1007. doi: 10.1126/science.167.3920.1005

Deehan, G. A. Jr., Brodie, M. S., and Rodd, Z. A. (2013). "What is in that drink: the biological actions of ethanol, acetaldehyde, and salsolinol," in *Current Topics in Behavioral Neurosciences: Behavioral Neurobiology of Alcohol Addiction,* eds W. H Sommer and R. Spanagel (Berlin, Heidelberg: Springer-Verlag), 163–184.

Deehan GA Jr., Engleman, E. A., Ding, Z. M., McBride, W. J.,and Rodd, Z. A. (2013). Microinjections of acetaldehyde or salsolinol into the posterior ventral tegmental area increase dopamine release in the nucleus accumbens shell. *Alcohol. Clin. Exp. Res.* 37, 722–729. doi: 10.1111/acer.12034

Di Chiara, G., and Imperato, A. (1985). Ethanol preferentially stimulates dopamine release in the nucleus accumbens of freely moving rats. *Eur. J. Pharm.* 115, 131–132. doi: 10.1016/0014-2999(85)90598-9

Di Chiara, G., and Imperato, A. (1988). Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc. Natl. Acad. Sci. U.S.A.* 85, 5274–5278. doi: 10.1073/pnas.85.14.5274

Diana, M., Peana, A. T., Sirca, D., Lintas, A., Melis, M., and Enrico, P. (2008). Crucial role of acetaldehyde in alcohol activation of the mesolimbic dopamine system. *Ann. N.Y. Acad. Sci.* 1139, 307–317. doi: 10.1196/annals.1432.009

Ding, Z. M., Oster, S. M., Hall, S. R., Engleman, E. A., Hauser, S. R., McBride, W. J., et al. (2011). The stimulating effects of ethanol on ventral tegmental area dopamine neurons projecting to the ventral pallidum and medial prefrontal cortex in female Wistar rats: regional difference and involvement of serotonin-3 receptors. *Psychopharmacology (Berl)* 216, 245–255. doi: 10.1007/s00213-011-2208-5

Ding, Z. M., Rodd, Z. A., Engleman, E. A., and McBride, W. J. (2009). Sensitization of ventral tegmental area dopamine neurons to the stimulating effects of ethanol. *Alcohol. Clin. Exp. Res.* 33, 1571–1581. doi: 10.1111/j.1530-0277.2009.00985.x

Duncan, C., and Deitrich, R. A. (1980). A critical evaluation of tetrahydroisoquinoline induced ethanol preference in rats. *Pharmacol. Biochem. Behav.* 13, 265–281. doi: 10.1016/0091-3057(80)90083-0

Duncan, C. C., and Fernando, P. W. (1991). Effects of tetrahydropapaveroline in the nucleus accumbens and the ventral tegmental area on ethanol preference in the rat. *Alcohol* 8, 87–90. doi: 10.1016/0741-8329(91)91314-R

Durlach, J., Rinjard, P., Sprince, H., and Smith, G. G. (1988). Similar antagonistic effects of Ca N-acetylhomotaurinate on depression of motor activity and lethality induced by acetaldehyde or ethanol. *Methods Find. Exp. Clin. Pharmacol.* 10, 437–447.

Edenberg, H. J. (2011). Common and rare variants in alcohol dependence. *Biol. Psychiatry* 70, 498–499. doi: 10.1016/j.biopsych.2011.07.007

Ehlers, C. L., Liang, T., and Gizer, I. R. (2012). ADH and ALDH polymorphisms and alcohol dependence in Mexican and Native Americans. *Am. J. Drug Alcohol Abuse* 38, 389–394. doi: 10.3109/00952990.2012.694526

Enrico, P., Sirca, D., Mereu, M., Peana, A. T., Lintas, A., and Golosio, A., et al. (2009). Acetaldehyde sequestering prevents ethanol-induced stimulation of mesolimbic dopamine transmission. *Drug Alcohol Depend.* 100, 265–271. doi: 10.1016/j.drugalcdep.2008.10.010

Eriksson, C. J. (1977). Acetaldehyde metabolism *in vivo* during ethanol oxidation. *Adv. Exp. Med. Biol.* 85A, 319–341.

Eriksson, C. J., Hillbom, M. E., and Sovijarvi, A. R. (1980). Difficulties in measuring human blood acetaldehyde concentrations during ethanol intoxication. *Adv. Exp. Med. Biol.* 126, 439–451.

Escarabajal, M. D., De Witte, P., and Quertemont, E. (2003). Role of acetaldehyde in ethanol-induced conditioned taste aversion in rats. *Psychopharmacology (Berl.)* 167, 130–136.

Escarabajal, D., Miquel, M., and Aragon, C. M. (2000). A psychopharmacological study of the relationship between brain catalase activity and ethanol-induced locomotor activity in mice. *J. Stud. Alcohol* 61, 493–498.

Foddai, M., Dosia, G., Spiga, S., and Diana, M. (2004). Acetaldehyde increases dopaminergic neuronal activity in the VTA. *Neuropsychopharmacology* 29, 530–536. doi: 10.1038/sj.npp.1300326

Font, L., Aragon, C. M., and Miquel, M. (2006a). Ethanol-induced conditioned place preference, but not aversion, is blocked by treatment with D-penicillamine, an inactivation agent for acetaldehyde. *Psychopharmacology (Berl)* 184, 56–64. doi: 10.1007/s00213-005-0224-z

Font, L., Aragon, C. M., and Miquel, M. (2006b). Voluntary ethanol consumption decreases after the inactivation of central acetaldehyde by d-penicillamine. *Behav. Brain Res.* 171, 78–86. doi: 10.1016/j.bbr.2006.03.020

Font, L., Miquel, M., and Aragon, C. M. (2005). Prevention of ethanol-induced behavioral stimulation by D-penicillamine: a sequestration agent for acetaldehyde. *Alcohol. Clin. Exp. Res.* 29, 1156–1164. doi: 10.1097/01.ALC.0000171945.30494.AF

Frank, J., Cichon, S., Treutlein, J., Ridinger, M., Mattheisen, M., Hoffmann, P., et al. (2012). Genome-wide significant association between alcohol dependence and a variant in the ADH gene cluster. *Addict. Biol.* 17, 171–180. doi: 10.1111/j.1369-1600.2011.00395.x

Gatto, G. J., McBride, W. J., Murphy, J. M., Lumeng, L., and Li, T. K. (1994). Ethanol self-infusion into the ventral tegmental area by alcohol-preferring rats. *Alcohol* 11, 557–564. doi: 10.1016/0741-8329(94)90083-3

Geller, I., and Purdy, R. (1975). Alteration of ethanol preference in rats; effects of beta-carbolines. *Adv. Exp. Med. Biol.* 59, 295–301.

Gessa, G. L., Muntoni, F., Collu, M., Vargiu, L., and Mereu, G. (1985). Low doses of ethanol activate dopaminergic neurons in the ventral tegmental area. *Bran Res.* 348, 201–203. doi: 10.1016/0006-8993(85)90381-6

Gianoulakis, C. (2009). Endogenous opioids and addiction to alcohol and other drugs of abuse. *Curr. Top. Med. Chem.* 9, 999–1015. doi: 10.2174/156802609789630956

Griffiths, P. J., Littleton, J. M., and Ortiz, A. (1974). Proceedings: chronic administration of acetaldehyde to mice: behavioural and biochemical similarities with chronic ethanol administration. *Br. J. Pharmacol.* 50, 441P.

Haber, H., Roske, I., Rottmann, M., Georgi, M., and Melzig, M. F. (1997). Alcohol induces formation of morphine precursors in the striatum of rats. *Life Sci.* 60, 79–89. doi: 10.1016/S0024-3205(96)00597-8

Hald, J., and Jacobsen, E. (1948). A drug sensitizing the organism to ethyl alcohol. *Lancet* 2, 1001–1004. doi: 10.1016/S0140-6736(48)91514-1

Hald, J., Jacobsen, E., and Larsen, V. (1949a). Formation of acetaldehyde in the organism in relation to dosage of antabuse (tetraethylthiuramdisulphide) and to alcohol-concentration in blood. *Acta Pharmacol. Toxicol. (Copenh)* 5, 179–188. doi: 10.1111/j.1600-0773.1949.tb03384.x

Hald, J., Jacobsen, E., and Larsen, V. (1949b). The rate of acetaldehyde metabolism in isolated livers and hind limbs of rabbits treated with antabuse (tetraethylthiuramdisulphide). *Acta Pharmacol. Toxicol. (Copenh)* 5, 298–308. doi: 10.1111/j.1600-0773.1949.tb03395.x

Heikkila, R., Cohen, G., and Dembiec, D. (1971). Tetrahydroisoquinoline alkaloids: uptake by rat brain homogenates and inhibition of catecholamine uptake. *J. Pharmacol. Exp. Ther.* 179, 250–258.

Hipolito, L., Marti-Prats, L., Sanchez-Catalan, M. J., Polache, A., and Granero, L. (2011). Induction of conditioned place preference and dopamine release by salsolinol in posterior VTA of rats: Involvement of mu-opioid receptors. *Neurochem. Int.* 59, 559–562. doi: 10.1016/j.neuint.2011.04.014

Hipolito, L., Sanchez-Catalan, M. J., Granero, L., and Polache, A. (2009). Local salsolinol modulates dopamine extracellular levels from rat nucleus accumbens: shell/core differences. *Neurochem. Int.* 55, 187–192. doi: 10.1016/j.neuint.2009.02.014

Hipolito, L., Sanchez-Catalan, M. J., Marti-Prats, L., Granero, L., and Polache, A. (2012). Revisiting the controversial role of salsolinol in the neurobiological effects of ethanol: old and new vistas. *Neurosci. Biobehav. Rev.* 36, 362–378. doi: 10.1016/j.neubiorev.2011.07.007

Hipolito, L., Sanchez-Catalan, M. J., Zanolini, I., Polache, A., and Granero, L. (2008). Shell/core differences in mu- and delta-opioid receptor modulation of dopamine efflux in nucleus accumbens. *Neuropharmacology* 55, 183–189. doi: 10.1016/j.neuropharm.2008.05.012

Hipolito, L., Sanchez-Catalan, M. J., Zornoza, T., Polache, A., and Granero, L. (2010). Locomotor stimulant effects of acute and repeated intrategmental injections of salsolinol in rats: role of mu-opioid receptors. *Psychopharmacology (Berl)* 209, 1–11. doi: 10.1007/s00213-009-1751-9

Holtzman, S. G., and Schneider, F. H. (1974). Comparison of acetaldehyde and ethanol: depression of motor activity in mice. *Life Sci.* 14, 1243–1250. doi: 10.1016/0024-3205(74)90432-9

Hunt, W. A. (1996). Role of acetaldehyde in the actions of ethanol on the brain–a review. *Alcohol* 13, 147–151. doi: 10.1016/0741-8329(95)02026-8

Hurley, T. D., and Edenberg, H. J. (2012). Genes encoding enzymes involved in ethanol metabolism. *Alcohol Res.* 34, 339–344.

Huttunen, P., and Myers, R. D. (1987). Anatomical localization in hippocampus of tetrahydro-beta-carboline-induced alcohol drinking in the rat. *Alcohol* 4, 181–187. doi: 10.1016/0741-8329(87)90041-3

Jacobsen, E. (1958). Et Præparat Fødes. *Medicinisk Forum* 58, 58–67.

Jamal, M., Ameno, K., Uekita, I., Kumihashi, M., Wang, W., and Ijiri, I. (2007). Catalase mediates acetaldehyde formation in the striatum of free-moving rats. *Neurotoxicology* 28, 1245–1248. doi: 10.1016/j.neuro.2007.05.002

Jarjour, S., Bai, L., and Gianoulakis, C. (2009). Effect of acute ethanol administration on the release of opioid peptides from the midbrain including the ventral tegmental area. *Alcohol. Clin. Exp. Res.* 33, 1033–1043. doi: 10.1111/j.1530-0277.2009.00924.x

Johnson, B. A., and Ait-Daoud, N. (2000). Neuropharmacological treatments for alcoholism: scientific basis and clinical findings. *Psychopharmacology* 149, 327–344. doi: 10.1007/s002130000371

Karahanian, E., Quintanilla, M. E., Tampier, L., Rivera-Meza, M., Bustamante, D., Gonzalez-Lira, V., et al. (2011). Ethanol as a prodrug: brain metabolism of ethanol mediates its reinforcing effects. *Alcohol. Clin. Exp. Res.* 35, 606–612. doi: 10.1111/j.1530-0277.2011.01439.x

Kiianmaa, K., and Virtanen, P. (1978). Ethanol and acetaldehyde levels in cerebrospinal fluid during ethanol oxidation in the rat. *Neurosci. Lett.* 10, 181–186. doi: 10.1016/0304-3940(78)90032-0

Koechling, U. M., and Amit, Z. (1994). Effects of 3-amino-1 2, 4-triazole on brain catalase in the mediation of ethanol consumption in mice. *Alcohol* 11, 235–239. doi: 10.1016/0741-8329(94)90036-1

Koelsch, F. (1914). Uber neuartige gewerbliche Errankungen in Kalkstickstoffbetrieben. *Munch. Med. Wochschr* 61, 1869–1970.

Kunin, D., Latendresse, M. W., Gaskin, S., Smith, B. R., and Amit,

Z. (2000). Preexposure effects of nicotine and acetaldehyde on conditioned taste aversion induced by both drugs. *Pharmaocol. Biochem. Behav.* 66, 695–699. doi: 10.1016/S0091-3057(00)00284-7

Larsen, V. (1948). The effect of experimental animals of antabuse (tetraethylthiuramdisulphide) in combination with alcohol. *Acta Pharmacol.* 4, 321–332. doi: 10.1111/j.1600-0773.1948.tb03354.x

Ledesma, J. C., and Aragon, C. (2013). Acquisition and reconditioning of ethanol-induced conditioned place preference in mice is blocked by the H_2O_2 scavenger alpha lipoic acid. *Psychopharmacology* 226, 673–685. doi: 10.1007/s00213-012-2831-9

Ledesma, J. C., Font, L., and Aragon, C. (2012). The H_2O_2 scavenger ebselen decreases ethanol-induced locomotor stimulation in mice. *Drug Alcohol Depend.* 124, 42–49. doi: 10.1016/j.drugalcdep.2011.12.003

Lee, J., Ramchandani, V. A., Hamazaki, K., Engleman, E. A., McBride, W. J., Li, T. K., et al. (2010). A critical evaluation of influence of ethanol and diet on salsolinol enantiomers in humans and rats. *Alcohol. Clin. Exp. Res.* 34, 242–250. doi: 10.1111/j.1530-0277.2009.01087.x

Li, T. K. (2000). Pharmacogenetics of responses to alcohol and genes that influence alcohol drinking. *J. Stud. Alcohol* 61, 5–12.

Lovinger, D. M., and White, G. (1991). Ethanol potentiation of 5-hydroxytryptamine3 receptor-mediated ion current in neuroblastoma cells and isolated adult mammalian neurons. *Mol. Pharmacol.* 40, 263–270.

Lovinger, D. M., White, G., and Weight, F. F. (1989). Ethanol inhibits NMDA-activated ion current in hippocampal neurons. *Science* 243, 1721–1724. doi: 10.1126/science.2467382

Lovinger, D. M., and Zhou, Q. (1998). Alcohol effects on the 5-HT3 ligand-gated ion channel. *Toxicol. Lett.* 100-101, 239–246. doi: 10.1016/S0378-4274(98)00191-X

Marti-Prats, L., Sanchez-Catalan, M. J., Hipolito, L., Orrico, A., Zornoza, T., Polache, A., et al. (2010). Systemic administration of D-penicillamine prevents the locomotor activation after intra-VTA ethanol administration in rats. *Neurosci. Lett.* 483, 143–147. doi: 10.1016/j.neulet.2010.07.081

March, S. M., Abate, P., Spear, N. E., and Molina, J. C. (2013). The role of acetaldehyde in ethanol reinforcement assessed by Pavlovian conditioning in newborn rats. *Psychopharmacology* 226, 491–499. doi: 10.1007/s00213-012-2920-9

Maruyama, W., Nakahara, D., Dostert, P., Hashiguchi, H., Ohta, S., Hirobe, M., et al. (1993). Selective release of serotonin by endogenous alkaloids, 1-methyl-6, 7-dihydroxy-1 2, 3, 4-tetrahydroisoquinolines, (R)- and (S)salsolinol, in the rat striatum; *in vivo* microdialysis study. *Neurosci. Lett.* 149, 115–118. doi: 10.1016/0304-3940(93)90749-B

Matsuzawa, S., Suzuki, T., and Misawa, M. (2000). Involvement of mu-opioid receptor in the salsolinol-associated place preference in rats exposed to conditioned fear stress. *Alcohol. Clin. Exp. Res.* 24, 366–372.

McBride, W. J., and Li, T. K. (1998). Animal models of alcoholism: neurobiology of high alcohol-drinking behavior in rodents. *Crit. Rev. Neurobiol.* 12, 339–369. doi: 10.1615/CritRevNeurobiol.v12.i4.40

McCoy, J. G., Strawbridge, C., McMurtrey, K. D., Kane, V. B., and Ward, C. P. (2003). A re-evaluation of the role of tetrahydropapaveroline in ethanol consumption in rats. *Brain Res. Bull.* 60, 59–65. doi: 10.1016/S0361-9230(03)00018-2

Melchior, C. L., and Myers, R. D. (1977). Preference for alcohol evoked by tetrahydropapaveroline (THP) chronically infused in the cerebral ventricle of the rat. *Pharmacol. Biochem. Behav.* 7, 19–35. doi: 10.1016/0091-3057(77)90006-5

Melis, M., Enrico, P., Peana, A. T., and Diana, M. (2007). Acetaldehyde mediates alcohol activation of the mesolimbic dopamine system. *Eur. J. Neurosci.* 26, 2824–2833. doi: 10.1111/j.1460-9568.2007.05887.x

Mereu, G., Fadda, F., and Gessa, G. L. (1984). Ethanol stimulates the firing rate of nigral dopaminergic neurons in unanesthetized rats. *Brain Res.* 292, 63–69. doi: 10.1016/0006-8993(84)90890-4

Mihic, S. J. (1999). Acute effects of ethanol on GABAA and glucine receptor function. *Neurochem. Int.* 35, 115–123. doi: 10.1016/S0197-0186(99)00053-4

Mihic, S. J., Ye, Q., Wick, M. J., Koltchine, V. V., Krasowski, M. D., Fin, S. E., et al. (1997). Sites of alcohol and volatile anaesthetic action on GABA(A) and glucine receptors. *Nature* 389, 385–389. doi: 10.1038/38738

Myers, W. D., Gibson, S., Ng, K. T., and Singer, G. (1987). Sex differences in acetaldehyde on body temperature and open-field performance in the rat. *Drug Alcohol Depend.* 19, 1–6. doi: 10.1016/0376-8716(87)90081-0

Myers, R. D., and Melchior, C. L. (1977). Alcohol drinking: abnormal intake caused by tetrahydropapaveroline in brain. *Science* 196, 554–556. doi: 10.1126/science.557839

Myers, W. D., Ng, K. T., and Singer, G. (1984a). Effects of naloxone and buprenorphine on intravenous acetaldehyde self-injection in rats. *Physiol. Behav.* 33, 449–455. doi: 10.1016/0031-9384(84)90168-9

Myers, W. D., Ng, K. T., Marzuki, S., Myers, R. D., and Singer, G. (1984b). Alteration of alcohol drinking in the rat by peripherally self-administered acetaldehyde. *Alcohol* 1, 229–236. doi: 10.1016/0741-8329(84)90103-4

Myers, R. D., and Oblinger, M. M. (1977). Alcohol drinking in the rat induced by acute intra-cerebral infusion of two tetrahydroisoquinolines and a beta-carboline. *Drug Alcohol Depend.* 2, 469–483. doi: 10.1016/0376-8716(77)90047-3

Myers, R. D., and Privette, T. H. (1989). A neuroanatomical substrate for alcohol drinking: identification of tetrahydropapaveroline (THP)-reactive sites in the rat brain. *Brain Res. Bull.* 22, 899–911. doi: 10.1016/0361-9230(89)90035-X

Myers, R. D., and Robinson, D. E. (1999). Tetrahydropapaveroline injected in the ventral tegmental area shifts dopamine efflux differentially in the shell and core of nucleus accumbens in high-ethanol-preferring (HEP) rats. *Alcohol* 18, 83–90. doi: 10.1016/S0741-8329(99)00008-7

Myers, R. D., and Veale, W. L. (1969). Alterations in volitional alcohol intake produced in rats by chronic intraventricular infusions of acetaldehyde, paraldehyde or methanol. *Arch. Int. Pharmacodyn. Ther.* 180, 100–113.

Nagasawa, H. T., Goon, D. J., and DeMaster, E. G. (1978). 2 5, 5-Trimethylthiazolidine-4-carboxylic acid, a D(-)-penicillamine-directed pseudometabolite of ethanol. Detoxication mechanism for acetaldehyde. *J. Med. Chem.* 21, 1274–1279. doi: 10.1021/jm00210a019

Nakahara, D., Maruyama, W., Hashiguti, H., and Naoi, M. (1994). Characterization of the in vivo action of (R)-salsolinol, an endogenous metabolite of alcohol, on serotonin and dopamine metabolism: a microdialysis study. *Brain Res.* 644, 226–232. doi: 10.1016/0006-8993(94)91684-5

Narahashi, T., Aistrup, G. L., Marszalec, W., and Nagata, K. (1999). Neuronal nicotinic acetylcholine receptors: a new target site of ethanol. *Neurochem. Int.* 35, 131–141. doi: 10.1016/S0197-0186(99)00055-8

Nizhnikov, M. E., Molina, J. C., and Spear, N. E. (2007). Central reinforcing effects of ethanol are blocked by catalase inhibition. *Alcohol* 41, 525–534. doi: 10.1016/j.alcohol.2007.08.006

Oades, R. D., and Halliday, G. M. (1987). Ventral tegemental (A10) system: neurobiology. 1. Anatomy connectivity. *Brain Res.* 434, 117–165.

Ocaranza, P., Quintanilla, M. E., Tampier, L., Karahanian, E., Sapag, A., and Israel, Y. (2008). Gene therapy reduces ethanol intake in an animal model of alcohol dependence. *Alcohol. Clin. Exp. Res.* 32, 52–57. doi: 10.1111/j.1530-0277.2007.00553.x

Orrico, A., Hipolito, L., Sanchez-Catalan, M. J., Marti-Prats, L., Zornoza, T., Granero, L., et al. (2013). Efficacy of D-penicillamine, a sequestering acetaldehyde agent, in the prevention of alcohol relapse drinking in rats. *Psychopharmacology* 228, 563–575. doi: 10.1007/s00213-013-3065-1

Ortiz, A., Griffiths, P. J., and Littleton, J. M. (1974). A comparison of the effects of chronic administration of ethanol and acetaldehyde to mice: evidence for a role of acetaldehyde in ethanol dependence. *J. Pharm. Pharmacol.* 26, 249–260. doi: 10.1111/j.2042-7158.1974.tb09266.x

Pastor, R., and Aragon, C. (2008). Ethanol injected into the hypothalamic arcuate nucleus induced behavioral stimulation in rats: an effect prevented by catalase inhibition and naltrexone. *Behav. Pharmacol.* 19, 698–705. doi: 10.1097/FBP.0b013e328315ecd7

Pautassi, R. M., Nizhnikov, M. E., Fabio, M. C., and Spear, N. E. (2011). An acetaldehyde-sequestering agent inhibits appetitive reinforcement and behavioral stimulation induced by ethanol in preweanling rats. *Pharmacol. Biochem. Behav.* 97, 462–469. doi: 10.1016/j.pbb.2010.10.005

Peana, A. T., Assaretti, A. R., Muggironi, G., Enrico, P., and Diana, M. (2009). Reduction of ethanol-derived acetaldehyde induced motivational properties

by L-cysteine. *Alcohol. Clin. Exp. Res.* 33, 43–48. doi: 10.1111/j.1530-0277.2008.00809.x

Peana, A. T., Enrico, P., Assaretti, A. R., Pulighe, E., Muggironi, G., Nieddu, M., et al. (2008). Key role of ethanol-derived acetaldehyde in the motivational properties induced by intragastric ethanol: a conditioned place preference study in the rat. *Alcohol. Clin. Exp. Res.* 32, 249–258. doi: 10.1111/j.1530-0277.2007.00574.x

Peana, A. T., Muggironi, G., and Diana, M. (2010). Acetaldehyde-reinforcing effects: a study on oral self-administration behavior. *Front. Psychiatry* 1:23. doi: 10.3389/fpsyt.2010.00023.

Peana, A. T., Muggironi, G., Fois, G. R., Zinellu, M., Sirca, D., and Diana, M. (2012). Effect of L-systeine on acetaldehyde self-administration. *Alcohol* 46, 489–497. doi: 10.1016/j.alcohol.2011.10.004

Peana, A. T., Muggironi, G., Fois, G. R., Zinellu, M., Vinci, S., and Acquas, E. (2011). Effect of opioid receptor blockade on acetaldehyde self-administration and ERK phosphorylation in the rat nucleus accumbens. *Alcohol* 45, 773–783. doi: 10.1016/j.alcohol.2011.06.003

Peng, G. S., and Yin, S. J. (2009). Effect of the allelic variants of Aldehyde dehydrogenase ALDH2*2 and alcohol dehydrogenase ADH1B*2 on blood acetaldehyde concentrations. *Hum. Genomics* 3, 121–127. doi: 10.1186/1479-7364-3-2-121

Petersen, D. R., and Tabakoff, B. (1979). Characterization of brain acetaldehyde oxidizing systems in the mouse. *Drug Alcohol Depend.* 4, 137–144. doi: 10.1016/0376-8716(79)90054-1

Pikkarainen, P. H., Salaspuro, M. P., and Lieber, C. S. (1979). A method for the determination of "free" acetaldehyde in plasma. *Alcohol. Clin. Exp. Res.* 3, 259–261. doi: 10.1111/j.1530-0277.1979.tb05311.x

Privette, T. H., Hornsby, R. L., and Myers, R. D. (1988). Buspirone alters alcohol drinking induced in rats by tetrahydropapaveroline injected into brain monoaminergic pathways. *Alcohol* 5, 147–152. doi: 10.1016/0741-8329(88)90012-2

Privette, T. H., and Myers, R. D. (1989). Anatomical mapping of tetrahy -dropapaveroline-reactive sites in brain mediating suppression of alcohol drinking in the rat. *Brain Res. Bull.* 22, 1039–1048. doi: 10.1016/0361-9230(89)90017-8

Purvis, P. L., Hirst, M., and Baskerville, J. C. (1980). Voluntary ethanol consumption in the rat following peripheral administrations of 3-carboxy-salsolinol. *Subst. Alcohol Actions. Misuse* 1, 439–445.

Quertemont, E., and DeWitte, P. (2001). Conditioned stimulus preference after acetaldehyde but not ethanol injections. *Pharmacol. Biochem. Behav.* 68, 449–454. doi: 10.1016/S0091-3057(00)00486-X

Quertemont, E., Grant, K. A., Correa, M., Arizzi, M. N., Salamone, J. D., Tambour, S., et al. (2005). The role of acetaldehyde in the central effects of ethanol. *Alcohol. Clin. Exp. Res.* 29, 221–234. doi: 10.1097/01.ALC.0000156185.39073.D2

Quertemont, E., Tambour, S., Bernaerts, P., Zimatkin, S. M., and Tirelli, E. (2004). Behavioral characterization of acetaldehyde in C57BL/6J mice: locomotor, hypnotic, anxiolytic and amnesic effects. *Psychopharmacology (Berl.)* 177, 84–92. doi: 10.1007/s00213-004-1911-x

Quintanilla, M. E., Callejas, O., and Tampier, L. (2002). Aversion to acetaldehyde: differences in low-alcohol drinking (UChA) and high-alcohol drinking (UChB) rats. *Alcohol* 26, 69–74. doi: 10.1016/S0741-8329(01)00197-5

Quintanilla, M. E., and Tampier, L. (2003). Acetaldehyde-reinforcing effects: differences in low-alcohol-drinking (UChA) and high-alcohol-drinking (UChB) rats. *Alcohol* 31, 63–69. doi: 10.1016/j.alcohol.2003.07.001

Quintanilla, M. E., Tampier, L., Karahanian, E., Rivera-Meza, M., Herrera-Marschitz, M., and Israel, Y. (2012). Reward and Relapse: complete gene-induced dissociation in an animal model of alcohol dependence. *Alcohol. Clin. Exp. Res.* 36, 517–522. doi: 10.1111/j.1530-0277.2011.01606.x

Raskin, N. H. (1975). Editorial: alcoholism or acetaldehydism. *N. Engl. J. Med.* 292, 422–423. doi: 10.1056/NEJM197502202920811

Rehm, J., Mathers, C., Popova, S., Thavorncharoensap, M., Teerawattananon, Y., and Patra, J. (2009). Global burden of disease and injury and economic cost attributable to alcohol use and alcohol-use disorders. *Lancet* 373, 2223–2233. doi: 10.1016/S0140-6736(09)60746-7

Reynolds, W. A., and Lowe, F. H. (1965). Mushrooms and a toxic reaction to alcohol: report of Four Cases. *N. Engl. J. Med.* 272, 630–631. doi: 10.1056/NEJM196503252721209

Rhoads, D. E., Contreras, C., and Fathalla, S. (2012). Brain levels of catalase remain constant through strain, development, and chronic alcohol challenges. *Enzyme Res.* 2012, 1–6. doi: 10.1155/2012/572939

Rivera-Meza, M., Quintanilla, M. E., and Tampier, L. (2012). Reduction of ethanol consumption in alcohol-preferring rats by dual expression gene transfer. *Alcohol Alcohol.* 47, 102–108. doi: 10.1093/alcalc/agr161

Rivera-Meza, M., Quintanilla, M. E., Tampier, L., Mura, C. V., Sapag, A., and Israel, Y. (2010). Mechanism of protection against alcoholism by an alcohol dehydrogenase polymorphism: development of an animal model. *FASEB J.* 24, 266–274. doi: 10.1096/fj.09-132563

Rodd, Z. A., Bell, R. L., Zhang, Y., Goldstein, A., Zaffaroni, A., McBride, W. J., et al. (2003). Salsolinol produces reinforcing effects in the nucleus accumbens shell of alcohol-preferring (P) rats. *Alcohol. Clin. Exp. Res.* 27, 440–449. doi: 10.1097/01.ALC.0000056612.89957.B4

Rodd, Z. A., Bell, R. L., Zhang, Y., Murphy, J. M., Goldstein, A., Zaffaroni, A., et al. (2005). Regional heterogeneity for the intracranial self-administration of ethanol and acetaldehyde within the ventral tegmental area of alcohol-preferring (P) rats: involvement of dopamine and serotonin. *Neuropsychopharmacology* 30, 330–338. doi: 10.1038/sj.npp.1300561

Rodd, Z. A., Oster, S. M., Ding, Z. M., Toalston, J. E., Deehan, G., Bell, R. L., et al. (2008). The reinforcing properties of salsolinol in the ventral tegmental area: evidence for regional heterogeneity and the involvement of serotonin and dopamine. *Alcohol. Clin. Exp. Res.* 32, 230–239. doi: 10.1111/j.1530-0277.2007.00572.x

Rodd-Henricks, Z. A., Melendez, R. I., Zaffaroni, A., Goldstein, A., McBride, W. J., and Li, T. K. (2002). The reinforcing effects of acetaldehyde in the posterior ventral tegmental area of alcohol-preferring rats. *Pharmacol. Biochem. Behav.* 72, 55–64. doi: 10.1016/S0091-3057(01)00733-X

Rojkovicova, T., Mechref, Y., Starkey, J. A., Wu, G., Bell, R. L., McBride, W. J., et al. (2008). Quantitative chiral analysis of salsolinol in different brain regions of rats genetically predisposed to alcoholism. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* 863, 206–214. doi: 10.1016/j.jchromb.2008.01.016

Rotzinger, S., Smith, B. R., and Amit, Z. (1994). Catalase inhibition attenuates the acquisition of ethanol and saccharin-quinine consumption in laboratory rats. *Behav. Pharmacol.* 5, 203–209. doi: 10.1097/00008877-199404000-00012

Sanchez-Catalan, M. J., Hipolito, L., Zornoza, T., Polache, A., and Granero, L. (2009). Motor stimulant effects of ethanol and acetaldehyde injected into the posterior ventral tegmental area of rats: role of opioid receptors. *Psychopharmacology* 204, 641–653. doi: 10.1007/s00213-009-1495-6

Sanchis-Segura, C., Correa, M., Miquel, M., and Aragon, C. (2005). Catalase inhibition in the arcuate nucleus blocks ethanol effects on the locomotor activity of rats. *Neurosci. Lett.* 376, 66–70. doi: 10.1016/j.neulet.2004.11.025

Sippel, H. W. (1974). The acetaldehyde content in rat brain during ethanol metabolism. *J. Neurochem.* 23, 451–452. doi: 10.1111/j.1471-4159.1974.tb04380.x

Smith, B. R., Amit, Z., and Splawinsky, J. (1984). Conditioned place preference induced by intraventricular infusions of acetaldehyde. *Alcohol* 1, 193–195. doi: 10.1016/0741-8329(84)90097-1

Smith, B. R., Aragon, C. M., and Amit, Z. (1997). Catalase and the production of brain acetaldehyde: a possible mediator of the psychopharmacological effects of ethanol. *Addict. Biol.* 2, 277–289. doi: 10.1080/13556219772570

Spanagel, R. (2009). Alcoholism: a systems approach from molecular physiology to addictive behavior. *Physiol. Rev.* 89, 649–705. doi: 10.1152/physrev.00013.2008

Spanagel, R., Herz, A.,and Shippenberg, T. S. (1992). Opposing tonically active endogenous opioid systems modulate the mesolimbic dopaminergic pathway. *Proc. Natl. Acad. Sci. U.S.A.* 89, 2046–2050. doi: 10.1073/pnas.89.6.2046

Spina, L., Longoni, R., Vinci, S., Ibba, F., Peana, A. T., Muggironi, G., et al. (2010). Role of dopamine D1 receptors and extracellular signal regulated kinase in the motivational properties of acetaldehyde as assessed by place preference conditioning. *Alcohol. Clin. Exp. Res.* 34, 607–616. doi: 10.1111/j.1530-0277.2009.01129.x

Stotz, E. (1943). A colorimetric determination of acetaldehyde in blood. *J. Biol. Chem.* 148, 585–591.

Tabakoff, B., Anderson, R. A.,and Ritzmann, R. F. (1976). Brain acetaldehyde after ethanol administration. *Biochem. Pharmacol.* 25, 1305–1309. doi: 10.1016/0006-2952(76)90094-0

Takayama, S.,and Uyeno, E. T. (1985). Intravenous self-administration of ethanol and acetaldehyde by rats. *Yakubutsu. Seishin. Kodo* 5, 329–334.

Tambour, S., Didone, V., Tirelli, E.,and Quertemont, E. (2006). Locomotor effects of ethanol and acetaldehyde after peripheral and intraventricular injections in Swiss and C57BL/6J mice. *Behav. Brain Res.* 172, 145–154. doi: 10.1016/j.bbr.2006.05.010

Tampier, L., and Quintanilla, M. E. (2002). Effect of acetaldehyde on acute tolerance and ethanol consumption in drinker and non-drinker rats. *J. Stud. Alcohol* 63, 257–262.

Tampier, L., Quintanilla, M. E., Karahanian, E., Rivera-Meza, M., Herrera-Marschitz, M.,and Israel, Y. (2013). The alcohol deprivation effect: marked inhibition by anticatalase gene administration into the ventral tegmental area in rats. *Alcohol. Clin. Exp. Res.* 37, 1278–1285. doi: 10.1111/acer.12101

Tampier, L., Quintanilla, M. E., Mardones, J. (1995). Effects of aminotriazole on ethanol, water, and food intake and on brain catalase in UChA and UChB rats. *Alcohol* 12, 341–344. doi: 10.1016/0741-8329(95)00014-I

Truitt, E. B. Jr., and Walsh, M. J. (1971). "The role of acetaldehyde in the actions of ethanol," in *The Biology of Alcoholism,* eds B. Kissin and H. Begleiter (New York, NY: Plenum Press), 161–195. doi: 10.1007/978-1-4615-6525-3_5

Tuomisto, L., Airaksinen, M. M., Peura, P.,and Eriksson, C. J. (1982). Alcohol drinking in the rat: increases following intracerebroventricular treatment with tetrahydro-beta-carbolines. *Pharmacol. Biochem. Behav.* 17, 831–836. doi: 10.1016/0091-3057(82)90367-7

Tuomisto, L., and Tuomisto, J. (1973). Inhibition of monoamine uptake in synaptosomes by tetrahydroharmane and tetrahydroisoquinoline compounds. *Naunyn Schmiedebergs Arch. Pharmacol.* 279, 371–380. doi: 10.1007/BF00500802

Valenta, J. P., Job, M. O., Mangieri, R. A., Schier, C. J., Howard, E. C., and Gonzales, R. A. (2013). μ-Opioid receptors in the stimulation of mesolimbic dopamine activity by ethanol and morphine in Long-Evans rats: a delayed effect of ethanol. *Psychopharmacology* 228, 389–400. doi: 10.1007/s00213-013-3041-9

Vasiliou, V., Ziegler, T. L., Bludeau, P., Petersen, D. R., Gonzalez, F. J.,and Deitrich, R. A. (2006). CYP2E1 and catalase influence ethanol sensitivity in the central nervous system. *Pharmacogenet. Genomics* 16, 51–58. doi: 10.1097/01.fpc.0000182777.95555.56

Vengeliene, V., Bilbao, A., Molander, A., and Spanagel, R. (2008). Neuropharmacology of alcohol addiction. *Br. J. Pharmacol.* 154, 299–315. doi: 10.1038/bjp.2008.30

Walsh, M. J., Davis, V. E., and Yamanaka, Y. (1970). Tetrahydropapaveroline: an alkaloid metabolite of dopamine *in vitro*. *J. Pharmacol. Exp. Ther.* 174, 388–400.

Ward, R. J., Colantuoni, C., Dahchour, A., Quertemont, E.,and De Witte, P. (1997). Acetaldehyde-induced changes in monoamine and amino acid extracellular microdialysate content of the nucleus accumbens. *Neuropharmacology* 36, 225–232. doi: 10.1016/S0028-3908(97)00007-5

Williams, E. E. (1937). Effects of alcohol on workers with carbon disulfide. *J. Am. Med. Assoc.* 109, 1472–1473.

Xie, G., Hipolito, L., Zuo, W., Polache, A., Granero, L., Krnjevic, K., and Ye, J. H. (2012a). Salsolinol stimulates dopamine neurons in slices of posterior ventral tegmental area indirectly by activating μ-opioid receptors. *J. Pharmacol. Exp. Ther.* 341, 43–50. doi: 10.1124/jpet.111.186833

Xie, G., and Ye, J. H. (2012b). Salsolinol facilitates glutamatergic transmission to dopamine neurons in the posterior ventral tegmental area of rats. *PLoS ONE* 7:e36716. doi: 10.1371/journal.pone.0036716

Ye, J. H., Tao, L., Ren, J., Schaefer, R., Krnjevic, K., Liu, P. L. et al. (2001). Ethanol potentiation of glycine-induced responses in dissociated neurons of rat ventral tegmental area. *J. Pharmacol. Exp. Ther.* 296, 77–83.

Zakhari, S. (2006). Overview: how is alcohol metabolized by the body. *Alcohol Res. Health* 29, 245–254.

Zimatkin, S. M. (1991). Histochemical study of aldehyde dehydrogenase in the rat CNS. *J. Neurochem.* 56, 1–11. doi: 10.1111/j.1471-4159.1991.tb02555.x

Zimatkin, S. M., Liopo, A. V.,and Deitrich, R. A. (1998). Distribution and kinetics of ethanol metabolism in rat brain. *Alcohol. Clin. Exp. Res.* 22, 1623–1627. doi: 10.1111/j.1530-0277.1998.tb03958.x

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 06 May 2013; paper pending published: 01 June 2013; accepted: 27 July 2013; published online: 23 August 2013.

Citation: Deehan GA Jr, Hauser SR, Wilden JA, Truitt WA and Rodd ZA (2013) Elucidating the biological basis for the reinforcing actions of alcohol in the mesolimbic dopamine system: the role of active metabolites of alcohol. Front. Behav. Neurosci. 7:104. doi: 10.3389/fnbeh.2013.00104

This article was submitted to the journal Frontiers in Behavioral Neuroscience.

frontiers in
BEHAVIORAL NEUROSCIENCE

REVIEW ARTICLE
published: 31 July 2013
doi: 10.3389/fnbeh.2013.00093

Involvement of the endogenous opioid system in the psychopharmacological actions of ethanol: the role of acetaldehyde

Laura Font*, Miguel Á. Luján and Raúl Pastor

Area de Psicobiología, Universitat Jaume I, Castellón, Spain

Edited by:
Merce Correa, University Jaume I, Spain

Reviewed by:
Elio Acquas, University of Cagliari, Italy
María J. Sánchez-Catalán, Centre National de Recherche Scientifique (CNRS), Institute of Cellular and Integrative Neurosciences (INCI) UPR3212, France

****Correspondence:***
Laura Font, Area de Psicobiología, Universitat Jaume I, Avda. Sos Baynat s/n, 12071 Castellón, Spain
e-mail: laura.font@uji.es

Significant evidence implicates the endogenous opioid system (EOS) (opioid peptides and receptors) in the mechanisms underlying the psychopharmacological effects of ethanol. Ethanol modulates opioidergic signaling and function at different levels, including biosynthesis, release, and degradation of opioid peptides, as well as binding of endogenous ligands to opioid receptors. The role of β-endorphin and μ-opioid receptors (OR) have been suggested to be of particular importance in mediating some of the behavioral effects of ethanol, including psychomotor stimulation and sensitization, consumption and conditioned place preference (CPP). Ethanol increases the release of β-endorphin from the hypothalamic arcuate nucleus (NArc), which can modulate activity of other neurotransmitter systems such as mesolimbic dopamine (DA). The precise mechanism by which ethanol induces a release of β-endorphin, thereby inducing behavioral responses, remains to be elucidated. The present review summarizes accumulative data suggesting that the first metabolite of ethanol, the psychoactive compound acetaldehyde, could participate in such mechanism. Two lines of research involving acetaldehyde are reviewed: (1) implications of the formation of acetaldehyde in brain areas such as the NArc, with high expression of ethanol metabolizing enzymes and presence of cell bodies of endorphinic neurons and (2) the formation of condensation products between DA and acetaldehyde such as salsolinol, which exerts its actions via OR.

Keywords: ethanol, acetaldehyde, endogenous opioid system, salsolinol, behavior, animal

ETHANOL AND THE OPIOID SYSTEM

Evidence indicates that ethanol modulates the activity of different components of the endogenous opioid system (EOS), with a large body of data supporting the implication of opioid ligands and receptors in the mediation of some of the psychopharmacological effects of ethanol.

THE ENDOGENOUS OPIOID SYSTEM AT A GLANCE

The opioid peptide precursors proopiomelanocortin (POMC), proenkephalin (PENK) or prodynorphin (PDYN) (Kieffer and Gavériaux-Ruff, 2002) are the source for the respective peptides β-endorphin, enkephalin, and dynorphin (Nylander and Roman, 2012). These endogenous ligands activate G-protein-coupled μ-, ∂-, and κ-opioid receptors (OR) (μ-OR, ∂-OR and κ-OR), which differ in their affinities and response profiles (Evans et al., 1992; Knapp et al., 1995; Kieffer and Evans, 2009). β-endorphin presents higher affinity for μ- than ∂-, and reduced affinity for κ-OR (Roth-Deri et al., 2008; Trigo et al., 2010). Enkephalin binding to ∂-OR is greater than that for μ-OR (Khachaturian et al., 1985; Raynor et al., 1994; Akil et al., 1998) and dynorphin shows specific affinity for κ-OR (Chavkin et al., 1982; Simon, 1991; Roth-Deri et al., 2008; Trigo et al., 2010). Ethanol can modulate opioidergic transmission at different levels, including synthesis, release, and degradation of opioid peptides, and binding of endogenous ligands to OR (for a review see, Méndez and Morales-Mulia, 2008). Since β-endorphin signaling has been specially implicated in the behavioral effects of ethanol, the present review will focus on the effects of ethanol on this component of the EOS. In this regard, although OR and ligands are widely distributed through the brain, there are important neuroanatomical determinants related to β-endorphin distribution that are worth highlighting. β-endorphin-synthesizing cell bodies are primarily located in the hypothalamic arcuate nucleus (NArc) (Chronwall, 1985). Important brain regions for drug-induced effects such as the nucleus accumbens (NAcb) are under tonic control of β-endorphinic innervations from the NArc (Chronwall, 1985; Khachaturian et al., 1985; Spanagel et al., 1992; Gianoulakis, 2001). These NArc β-endorphin projections exert this control through the direct activation of OR located at the NAcb and by an indirect pathway via OR in the ventral tegmental area (VTA), which in turn modulate NAcb activity via VTA-NAcb dopamine (DA) neurons (Mansour et al., 1988; Di Chiara and North, 1992; Spanagel et al., 1992).

ETHANOL-INDUCED MODULATION OF β-ENDORPHINIC NEUROTRANSMISSION

Acute administration of ethanol induces the release of β-endorphin; an effect found in hypothalamic cell cultures and

tissue preparations (Gianoulakis, 1990; Boyadjieva and Sarkar, 1994; de Waele et al., 1994; Reddy et al., 1995; De et al., 2002). Ethanol also produces *in vivo* increases in β-endorphin content at the level of the hypothalamus (Schulz et al., 1980; Patel and Pohorecky, 1989), NAcb (Anwer and Soliman, 1995; Olive et al., 2001; Marinelli et al., 2003a), midbrain including the VTA (Rasmussen et al., 1998; Jarjour et al., 2009) and the central amygdala (CeA) (Lam et al., 2008). Some studies, however, have found inconsistent results, probably related to procedural and methodological differences (Seizinger et al., 1983; Popp and Erickson, 1998; Rasmussen et al., 1998; Leriche and Méndez, 2010). Increased levels of enkephalin in the hypothalamus (Schulz et al., 1980; Seizinger et al., 1983; Milton et al., 1991) and NAcb (Marinelli et al., 2003b) have also been found after acute ethanol.

Long-term exposure to ethanol primarily induces a decrease in POMC expression (Boyadjieva and Sarkar, 1997; Rasmussen et al., 2002; Oswald and Wand, 2004) and in hypothalamic β-endorphin release and levels (Boyadjieva and Sarkar, 1994; Oswald and Wand, 2004). A limited number of studies reported an increase in biosynthesis of POMC and POMC mRNA expression (Seizinger et al., 1984; Gianoulakis et al., 1988) as well as an initial increase followed by a gradual return to normal levels (Wand, 1990). Also, some authors found an increase or no effect on β-endorphin release (Boyadjieva and Sarkar, 1994; Oswald and Wand, 2004). Discrepancies might be attributable to the method of ethanol administration, ethanol dose, time course of drug exposure, administration route and differences in the development of tolerance. Also, it has been observed that alcohol-induced changes depend on the brain region investigated as well as the species and strain of animals used (Gianoulakis, 2001; Méndez and Morales-Mulia, 2008).

EVIDENCE OF BEHAVIORAL EFFECTS OF ETHANOL MEDIATED BY THE ENDOGENOUS OPIOID SYSTEM

Given that β-endorphin, and also enkephalin, activate μ-OR, extensive research has investigated the role of μ-OR in the behavioral effects of ethanol (Gianoulakis, 1993; Herz, 1997; Sanchis-Segura et al., 2000; Thorsell, 2013). Here we will focus on the involvement of these components of the EOS in several behavioral effects of ethanol, including psychomotor stimulation and sensitization, consumption, and associative learning (with a special focus on conditioned place preference (CPP)).

Psychomotor stimulation and sensitization

Increased psychomotor stimulation induced by ethanol in mice can be blocked with non-selective opioid receptor antagonists such as naloxone or naltrexone (Kiianmaa et al., 1983; Camarini et al., 2000; Sanchis-Segura et al., 2004; Pastor et al., 2005; Pastor and Aragon, 2006). Some pharmacological strategies have suggested the existence of three so-called subtypes of μ-OR; μ_1, μ_2, and, μ_3 (Pasternak, 2001a,b; Cadet et al., 2003) and several studies have shown that μ- and specifically the $\mu_{1/2}$ - and μ_3-OR subtypes, but not δ- or κ-OR, are involved in the motor stimulant effects of ethanol in adult mice (Pastor et al., 2005), and also in rats during early development (Arias et al., 2010; Pautassi et al., 2012). Other studies conducted in mice have suggested that this involvement of μ-OR in ethanol stimulation is debatable (Cunningham et al., 1998; Gevaerd et al., 1999; Holstein et al., 2005). Consistent with the EOS involvement, however, a lesion of the NArc produces a decrease in ethanol-induced stimulation in mice (Sanchis-Segura et al., 2000), and knockout mice deficient in β-endorphin showed attenuated ethanol-induced stimulation (Dempsey and Grisel, 2012). Also, in rats, naltrexone prevents activation produced by ethanol when locally administered in the NArc (Pastor and Aragon, 2008) and intra-VTA blockade of the μ-OR using either naltrexone or the irreversible and selective μ-OR antagonist β-funaltrexamine reduces ethanol-induced locomotor stimulation (Sánchez-Catalán et al., 2009). Additionally, chronic naltrexone, which upregulates μ-OR (Unterwald et al., 1998; Lesscher et al., 2003), enhances the stimulant effects of ethanol in mice (Sanchis-Segura et al., 2004).

A critical role of the EOS in the motor sensitizing effects of ethanol has also been proposed (Camarini et al., 2000; Miquel et al., 2003; Pastor and Aragon, 2006). Unspecific OR antagonism prevents development (Camarini et al., 2000) but not expression (Abrahao et al., 2008) of ethanol-induced locomotor sensitization. μ-OR are particularly involved in ethanol sensitization (Camarini et al., 2000), without a clear role of any of the μ-OR subtypes in mediating this process; $\mu_{1/2}$ -OR antagonism slowed down, but did not block development of sensitization (Pastor and Aragon, 2006). Facilitation of ethanol-induced sensitization found after a period of voluntary alcohol consumption in mice was also seen to be absent in μ-OR deficient CXBK mice (Tarragón et al., 2012). The involvement of μ-OR in ethanol sensitization might be related to ethanol-induced increases in β-endorphin release as a recent study demonstrated that β-endorphin-deficient mice do not show locomotor sensitization to ethanol (Dempsey and Grisel, 2012). Also, animals with selective lesions of the NArc show prevented sensitization to ethanol (Miquel et al., 2003; Pastor et al., 2011). Altogether these data suggest that opioids and specifically β-endorphins, via μ-OR, might be critical mediators of ethanol-induced neuroplasticity underlying psychomotor sensitization.

Ethanol consumption

Numerous studies conducted during the last few decades showed that systemic, as well as local administration of opioid antagonists decrease ethanol consumption under a variety of schedules in different animal species (for reviews see Herz, 1997; Gianoulakis, 2001; Oswald and Wand, 2004; Modesto-Lowe and Fritz, 2005). These conclusions have also been supported by the use of OR knockout mouse models (Roberts et al., 2000; Méndez and Morales-Mulia, 2008). This strong pre-clinical basis has lead to the use of opioid antagonists in alcoholism pharmacotherapy (O'Malley et al., 1992). In rodents, the use of non-selective, as well as selective μ-OR antagonists proved to be effective at reducing ethanol consumption (Méndez and Morales-Mulia, 2008). However, the effects of these manipulations have been seen to be, in some cases, non-specific; fat, saccharin, sucrose and water intake were also reduced by these manipulations (Krishnan-Sarin et al., 1995; Nielsen et al., 2008; Rao et al., 2008; Simms et al., 2008; Corwin and Wojnicki, 2009; Wong et al., 2009). These data are compatible with the interpre-

tation that OR, and especially μ-OR might be a key mediator of the processing of positive reinforcement, both at emotional and motivational levels (Herz, 1997; Peciña and Berridge, 2005).

In general, data obtained with κ-OR or δ-OR manipulations are less conclusive. A recent review of the literature indicates that κ-OR stimulation generally antagonizes the reinforcing effects of alcohol whereas κ-OR blockade has no consistent effect (Wee and Koob, 2010). Dynorphin/κ-OR system appears to be involved in the negative reinforcing effects of ethanol by producing an aversive effect rather than by directly modulating the rewarding mechanism of ethanol (Wee and Koob, 2010; Walker et al., 2012). However, under an alcohol dependent-state, antagonism of κ-OR results effective in decreasing ethanol voluntary consumption (Wee and Koob, 2010; Walker et al., 2012). It has been reported that blockade of δ-OR either attenuates (Lê et al., 1993; Froehlich, 1995; Krishnan-Sarin et al., 1995; June et al., 1999; Hyytiä and Kiianmaa, 2001; Ciccocioppo et al., 2002), increases (Margolis et al., 2008) or has no effect on ethanol intake (Ingman et al., 2003). These discrepancies may be related to dynamic changes in δ-OR efficacy during ethanol exposure (Margolis et al., 2008). All these data support the participation of the POMC and PENK systems in maintaining alcohol consumption (Froehlich et al., 1991; Vengeliene et al., 2008).

Associative learning and conditioned place preference

It has been suggested that the EOS participates in the underlying mechanisms mediating conditioned effects induced by abused drugs, including ethanol. This implication is supported by two groups of experiments. On one hand, evidence indicates that OR antagonists attenuate cue-induced reinstatement of previously extinguished responding for ethanol self-administration (Lê et al., 1999; Ciccocioppo et al., 2002, 2003; Liu and Weiss, 2002; Burattini et al., 2006; Dayas et al., 2007; Marinelli et al., 2009), which suggests a role of EOS in cue-induced incentive motivational effects influencing ethanol-seeking behavior. This interpretation is consistent with clinical data showing that opioid antagonists increase abstinence duration periods in alcohol abusers (O'Malley et al., 1992), probably by reducing cue-induced seeking behavior. On the other hand, pretreatment with opioid receptor antagonism, while not influencing the acquisition of ethanol-induced CPP, reduces the expression and facilitates the extinction of this drug-free conditioned response (Bormann and Cunningham, 1997; Middaugh and Bandy, 2000; Kuzmin et al., 2003; Pastor et al., 2011). Mice lacking μ-OR also showed attenuated ethanol CPP (Hall et al., 2001). Further studies have suggested that expression of ethanol-induced CPP depends on OR located in the VTA, CeA, as well as anterior cingulated cortex (Bechtholt and Cunningham, 2005; Bie et al., 2009; Gremel et al., 2011). Additionally, a neurotoxic lesion of the β-endorphin neurons of the NArc, showed a facilitated extinction of ethanol-induced CPP (Pastor et al., 2011). β-endorphin and μ-OR appear to be therefore critically involved in the mechanisms underlying ethanol CPP. As Cunningham and collaborators have suggested, it is possible that altered opioid signaling might in turn alter conditioned motivation that normally maintains cue-induced seeking behavior during CPP testing (Cunningham et al., 1998). It is interesting to mention that pharmacological blockade of δ-OR with naltrindole in the CeA reduces expression of CPP induced by ethanol in rats (Bie et al., 2009). Activation of κ-OR has been shown to blunt acquisition of ethanol CPP (Logrip et al., 2009). Supporting these results, κ-OR knockout mice also showed enhanced ethanol CPP (Femenía and Manzanares, 2012).

ACETALDEHYDE: A PSYCHOACTIVE METABOLITE

The specific mechanism by which ethanol modulates the activity of the EOS remains to be understood. Evidence indicates that one possible mechanism might involve the role of acetaldehyde, the first metabolite of ethanol (Miquel et al., 2003; Sanchis-Segura et al., 2005b; Pastor and Aragon, 2008). Acetaldehyde is a psychoactive compound that produces behavioral and neurochemical effects suggested to mediate at least some of the effects of ethanol. Acetaldehyde is self-administered orally (Peana et al., 2010, 2012; Cacace et al., 2012) and directly into the brain (Brown et al., 1979; McBride et al., 2002; Rodd-Henricks et al., 2002; Peana et al., 2011). Its administration induces CPP (Smith et al., 1984; Quertemont and De Witte, 2001; Peana et al., 2009; Spina et al., 2010) as well as behavioral stimulation and sensitization when centrally administered (Arizzi et al., 2003; Correa et al., 2003a,b, 2009; Rodd et al., 2005; Arizzi-LaFrance et al., 2006; Sánchez-Catalán et al., 2009). The oxidation of ethanol to acetaldehyde in the brain is essentially mediated by the catalase-H_2O_2 system (Aragon et al., 1992a; Gill et al., 1992). Reduced brain catalase activity, which have been seen to decrease ethanol-derived central acetaldehyde formation in brain tissue preparations (Hamby-Mason et al., 1997) and in the brain of free-moving rats (Jamal et al., 2007), decreases ethanol consumption (Aragon and Amit, 1992; Koechling and Amit, 1994; Correa et al., 2004; Karahanian et al., 2011), ethanol-induced locomotor stimulation (Aragon et al., 1992b; Correa et al., 1999b, 2004; Sanchis-Segura et al., 1999a,b,c; Pastor et al., 2002; Pastor and Aragon, 2008), the anxiolityc effects of alcohol (Correa et al., 2008) and modulates ethanol-induced CPP (Font et al., 2008). Strategies aimed at increasing the production of brain acetaldehyde via an enhancement in activity of the enzymatic catalase system have also been used. These manipulations produced an increase in the motor stimulant properties of ethanol in mice (Correa et al., 1999a, 2000; Pastor et al., 2002). Other ethanol-induced effects such as taste aversion (Aragon et al., 1985) and social memory recognition have also been seen to be modulated by changes in brain catalase (Manrique et al., 2005).

Apart from brain catalase manipulation, the direct inactivation of acetaldehyde has also been shown to reduce ethanol effects, including drinking (Font et al., 2006a) and alcohol-induced relapse drinking (Orrico et al., 2013), CPP (Font et al., 2006b; Peana et al., 2008) and motor stimulation (Font et al., 2005; Martí-Prats et al., 2010; Pautassi et al., 2011).

ACETALDEHYDE-INDUCED CHANGES IN THE OPIOIDERGIC NEUROTRANSMISSION

The NArc, the main site of β-endorphin synthesis in the brain, is one of areas with the highest levels of catalase expression (Moreno et al., 1995; Zimatkin and Lindros, 1996) and lower levels of the acetaldehyde-degrading enzyme aldehyde dehydroge-

nase (Zimatkin et al., 1992). Therefore, it has been thus suggested that catalase-dependent formation of acetaldehyde into the NArc might mediate ethanol-induced increases in the release of β-endorphin from the NArc in turn activating OR at the level of the VTA/NAcb to stimulate behavioral and neurophysiological actions (Sanchis-Segura et al., 2005a; Pastor and Aragon, 2008). Supporting this hypothesis, several authors (Reddy and Sarkar, 1993; Pastorcic et al., 1994; Reddy et al., 1995) have demonstrated that ethanol-induced increases in hypothalamic β-endorphin release are, indeed, mediated by acetaldehyde (Reddy and Sarkar, 1993; Pastorcic et al., 1994; Reddy et al., 1995). Hypothalamic cell cultures exposed to ethanol (12.5–100 μM) led to the formation of acetaldehyde (8–24 μM) and similar concentrations of acetaldehyde (12.5–50 μM) were able to stimulate β-endorphin release when tested in the absence of ethanol (Reddy and Sarkar, 1993; Pastorcic et al., 1994). Moreover, pretreatment of hypothalamic cell cultures with catalase inhibitors caused dose-dependent decreases in ethanol-stimulated β-endorphin secretion (Reddy et al., 1995).

Another line of research linking the EOS and acetaldehyde is the investigation of the actions of salsolinol (for a review see Hipólito et al., 2012), the condensation product of DA and acetaldehyde. Salsolinol has been shown to alter enkephalin-receptor site binding (Lucchi et al., 1982) and other OR an effect that is blocked by naloxone (Fertel et al., 1980). Interestingly, intra-NAcb administration of salsolinol increases DA levels when microinjected in the core and decreases DA levels if the administration is in the NAcb shell (Hipólito et al., 2009) in a similar way to μ- and δ-OR agonists (Hipólito et al., 2008). It has been demonstrated that μ_1-OR receptors exert a tonic modulatory control over activity of the DA system (Di Chiara and North, 1992; Devine et al., 1993). Thus, one possible mechanism by which salsolinol exerts its effects on the OR could be disinhibiting DA neurons in the VTA. Upholding this hypothesis, intra-posterior VTA administration of salsolinol induces a μ-OR dependent increase in DA levels in the NAcb shell (Hipólito et al., 2011). Accordingly, it has been recently shown that salsolinol excites DA neurons of the VTA, by activating μ-OR on local GABA interneurons (Xie et al., 2012).

EVIDENCE OF BEHAVIORAL EFFECTS OF ACETALDEHYDE MEDIATED BY THE ENDOGENOUS OPIOID SYSTEM

Whereas accumulating evidence indicates that the EOS participates in the behavioral effects of ethanol, only few studies have studied the involvement of this system in acetaldehyde effects. Self-administration of acetaldehyde appears to be mediated by the EOS; high doses of naloxone reduced intravenous acetaldehyde self-administration in rats, and naltrexone reduced the maintenance, the deprivation effect, and operant break points of acetaldehyde voluntary consumption (Myers et al., 1984; Peana et al., 2011). Treatment with naloxonazine, a specific μ_1-OR antagonist reduces maintenance of acetaldehyde oral self-administration (Peana et al., 2011). Blockade of μ-OR using either naltrexone or the irreversible and selective μ-OR antagonist β-funaltrexamine suppress the locomotor stimulation effect of acetaldehyde when microinjected into the rat posterior VTA (Sánchez-Catalán et al., 2009). Additionally, Hipólito et al. (2010) have provided data supporting the hypothesis that acetaldehyde may mediate the actions of ethanol through a mechanism dependent on μ-OR activation. These authors showed that intra-posterior VTA injections of salsolinol induced locomotor stimulation and sensitization in rats; stimulation (but not sensitization) was prevented by μ-OR antagonism. Finally, Sanchis-Segura et al. (2005b) demonstrated that administration of a catalase inhibitor directly into the NArc is sufficient to prevent the effects of ethanol on rat locomotion. Conversely, locomotor stimulation induced by ethanol injected directly into the NArc, was prevented by catalase inhibition or naltrexone, indicating a link between the behavioral effects of a reduction in acetaldehyde formation and the antagonism of μ-OR (Pastor and Aragon, 2008). The NArc, therefore, may represent a critical site to link two independent but related hypotheses: (1) the hypothesis proposing that acetaldehyde may mediate some of the psychopharmacological actions attributed to ethanol (Aragon et al., 1992a; Smith et al., 1997; Quertemont et al., 2005; Correa et al., 2012) and (2) the hypothesis that suggests that the β-endorphin/μ-OR system participate in the reinforcing and psychomotor effects of ethanol (Stinus et al., 1980; Herz, 1997; Gianoulakis, 2001; Sanchis-Segura et al., 2005b; Pastor and Aragon, 2008). Early findings also suggested a role of the opioidergic system in mediating CPP induced by salsolinol in rats (Matsuzawa et al., 2000). Antagonism of μ-OR attenuated CPP induced by salsolinol when achieved under fear stress (Matsuzawa et al., 2000). Moreover, intra-posterior VTA administration of salsolinol, that produced CPP in rats, also produced an increase in DA in the NAcb that was suppressed by β-funaltrexamine administration (Hipólito et al., 2011).

SUMMARY AND PERSPECTIVES

In the present review we have summarized consistent results indicating that the EOS, and particularly β-endorphin and μ-OR, are critically involved in the psychopharmacological effects of ethanol. Additionally, we have reviewed a large body of data that indicates that the first metabolite of ethanol, acetaldehyde, might be responsible for the activation of β-endorphin release and μ-OR signaling after ethanol administration. There are two main lines of research suggesting a link between acetaldehyde and the EOS: (1) formation of acetaldehyde in brain areas such as the NArc, with high expression of ethanol metabolizing enzymes and presence of cell bodies of endorphinic neurons and (2) the formation of condensation products between DA and acetaldehyde such as salsolinol, which exerts its actions via μ-OR. To a certain degree both lines of research show important incompatibility. The fact that the lesions of the NArc are sufficient to block ethanol-induced behaviors challenge the putative role of salsolinol formed in other non-hypothalamic areas. Future studies will need to explore how to reconcile those two sets of data, and to clarify what is sufficient and/or necessary for acetaldehyde to induce behavioral responses mediated by the EOS. Finally, it is interesting to mention that most of the data suggesting a role of the EOS in acetaldehyde-induced behavioral effects have been linked to acetaldehyde-induced changes in the opioid system that are suggested to impact behavior via modulation of the DA system (Peana et al., 2011). Ethanol as well

as acetaldehyde activate firing of dopaminergic neurons in the VTA (Foddai et al., 2004; Diana et al., 2008) and stimulate DA transmission in the NAcb (Melis et al., 2007; Enrico et al., 2009; Sirca et al., 2011), effects that are prevented by D-penicillamine, a sequestering agent of acetaldehyde (Enrico et al., 2009). A recent study demonstrates that in rats, ethanol and acetaldehyde induce via DA D_1 receptors, ERK phosphorylation in the NAcb and extended amygdala (Vinci et al., 2010). This effect is blocked by D-penicillamine and by naltrexone, suggesting that the opiodergic modulation of the reinforcing properties of acetaldehyde could be mediated by the dopaminergic system (Vinci et al., 2010; Peana et al., 2011). There are other effects such as ethanol-induced CPP, ethanol drinking in some non-operant conditions and even ethanol-induced sensitization that appear to have a less straightforward involvement of DA signaling (Risinger et al., 1992; Broadbent et al., 1995; Spina et al., 2010; Young et al., 2013). Future research will need to investigate DA-dependent and independent mechanisms by which acetaldehyde might induce behavioral responses via its modulation of the EOS.

ACKNOWLEDGMENTS

This work was supported by grants from Fundación Bancaixa (P1-1A2011-05), Spain.

REFERENCES

Abrahao, K. P., Quadros, I. M., and Souza-Formigoni, M. L. (2008). Morphine attenuates the expression of sensitization to ethanol, but opioid antagonists do not. *Neuroscience* 156, 857–864. doi: 10.1016/j.neuroscience.2008.08.012

Akil, H., Owens, C., Gutstein, H., Taylor, L., Curran, E., and Watson, S. (1998). Endogenous opioids: overview and current issues. *Drug Alcohol Depend.* 51, 127–140. doi: 10.1016/s0376-8716(98)00071-4

Anwer, J., and Soliman, M. R. (1995). Ethanol-induced alterations in beta-endorphin levels in specific rat brain regions: modulation by adenosine agonist and antagonist. *Pharmacology* 51, 364–369. doi: 10.1159/000139348

Aragon, C. M., and Amit, Z. (1992). The effect of 3-amino-1,2,4-triazole on voluntary ethanol consumption: evidence for brain catalase involvement in the mechanism of action. *Neuropharmacology* 31, 709–712. doi: 10.1016/0028-3908(92)90150-n

Aragon, C. M., Pesold, C. N., and Amit, Z. (1992b). Ethanol-induced motor activity in normal and acatalasemic mice. *Alcohol* 9, 207–211. doi: 10.1016/0741-8329(92)90055-f

Aragon, C. M., Rogan, F., and Amit, Z. (1992a). Ethanol metabolism in rat brain homogenates by a catalase-H2O2 system. *Biochem. Pharmacol.* 44, 93–98. doi: 10.1016/0006-2952(92)90042-h

Aragon, C. M., Spivak, K., and Amit, Z. (1985). Blockade of ethanol induced conditioned taste aversion by 3-amino-1,2,4-triazole: evidence for catalase mediated synthesis of acetaldehyde in rat brain. *Life Sci.* 37, 2077–2084. doi: 10.1016/0024-3205(85)90579-x

Arias, C., Molina, J. C., and Spear, N. E. (2010). Differential role of mu, delta and kappa opioid receptors in ethanol-mediated locomotor activation and ethanol intake in preweanling rats. *Physiol. Behav.* 99, 348–454. doi: 10.1016/j.physbeh.2009.11.012

Arizzi, M. N., Correa, M., Betz, A. J., Wisniecki, A., and Salamone, J. D. (2003). Behavioral effects of intraventricular injections of low doses of ethanol, acetaldehyde, and acetate in rats: studies with low and high rate operant schedules. *Behav. Brain Res.* 147, 203–210. doi: 10.1016/s0166-4328(03)00158-x

Arizzi-LaFrance, M. N., Correa, M., Aragon, C. M., and Salamone, J. D. (2006). Motor stimulant effects of ethanol injected into the substantia nigra pars reticulata: importance of catalase-mediated metabolism and the role of acetaldehyde. *Neuropsychopharmacology* 31, 997–1008. doi: 10.1038/sj.npp.1300849

Bechtholt, A. J., and Cunningham, C. L. (2005). Ethanol-induced conditioned place preference is expressed through a ventral tegmental area dependent mechanism. *Behav. Neurosci.* 119, 213–223. doi: 10.1037/0735-7044.119.1.213

Bie, B., Zhu, W., and Pan, Z. Z. (2009). Ethanol-induced delta-opioid receptor modulation of glutamate synaptic transmission and conditioned place preference in central amygdala. *Neuroscience* 160, 348–358. doi: 10.1016/j.neuroscience.2009.02.049

Bormann, N. M., and Cunningham, C. L. (1997). The effects of naloxone on expression and acquisition of ethanol place conditioning in rats. *Pharmacol. Biochem. Behav.* 58, 975–982.

Boyadjieva, N. I., and Sarkar, D. K. (1994). Effects of chronic alcohol on immunoreactive beta-endorphin secretion from hypothalamic neurons in primary cultures: evidence for alcohol tolerance, withdrawal, and sensitization responses. *Alcohol. Clin. Exp. Res.* 18, 1497–1501. doi: 10.1006/mcne.1994.1071

Boyadjieva, N. I., and Sarkar, D. K. (1997). Effects of ethanol on basal and prostaglandin E1-induced increases in beta-endorphin release and intracellular cAMP levels in hypothalamic cells. *Alcohol. Clin. Exp. Res.* 21, 1005–1009. doi: 10.1111/j.1530-0277.1997.tb04245.x

Broadbent, J., Grahame, N. J., and Cunningham, C. L. (1995). Haloperidol prevents ethanol-stimulated locomotor activity but fails to block sensitization. *Psychopharmacology* 120, 475–482. doi: 10.1007/bf02245821

Brown, Z. W., Amit, Z., and Rockman, G. E. (1979). Intraventricular self-administration of acetaldehyde, but not ethanol, in naive laboratory rats. *Psychopharmacology* 64, 271–276. doi: 10.1007/bf00427509

Burattini, C., Gill, T. M., Aicardi, G., and Janak, P. H. (2006). The ethanol self-administration context as a reinstatement cue: acute effects of naltrexone. *Neuroscience* 139, 877–887. doi: 10.1016/j.neuroscience.2006.01.009

Cacace, S., Plescia, F., Barberi, I., and Cannizzaro, C. (2012). Acetaldehyde oral self-administration: evidence from the operant-conflict paradigm. *Alcohol. Clin. Exp. Res.* 36, 1278–1287. doi: 10.1111/j.1530-0277.2011.01725.x

Cadet, P., Mantione, K. J., and Stefano, G. B. (2003). Molecular identification and functional expression of mu 3, a novel alternatively spliced variant of the human mu opiate receptor gene. *J. Immunol.* 170, 5118–5123.

Camarini, R., Nogueira Pires, M. L., and Calil, H. M. (2000). Involvement of the opioid system in the development and expression of sensitization to the locomotor-activating effect of ethanol. *Int. J. Neuropsychopharmacol.* 3, 303–309. doi: 10.1017/s146114570000211x

Chavkin, C., James, I. F., and Goldstein, A. (1982). Dynorphin is a specific endogenous ligand of the kappa opioid receptor. *Science* 215, 413–415. doi: 10.1126/science.6120570

Chronwall, B. M. (1985). Anatomy and physiology of the neuroendocrine arcuate nucleus. *Peptides* 6 (Suppl. 2), 1–11. doi: 10.1016/0196-9781(85)90128-7

Ciccocioppo, R., Martin-Fardon, R., and Weiss, F. (2002). Effect of selective blockade of mu(1) or delta opioid receptors on reinstatement of alcohol-seeking behavior by drug-associated stimuli in rats. *Neuropsychopharmacology* 27, 391–399. doi: 10.1016/s0893-133x(02)00302-0

Ciccocioppo, R., Lin, D., Martin-Fardon, R., and Weiss, F. (2003). Reinstatement of ethanol-seeking behavior by drug cues following single versus multiple ethanol intoxication in the rat: effects of naltrexone. *Psychopharmacology* 168, 208–215. doi: 10.1007/s00213-002-1380-z

Correa, M., Arizzi, M. N., Betz, A., Mingote, S., and Salamone, J. D. (2003a). Open field locomotor effects in rats after intraventricular injections of ethanol and the ethanol metabolites acetaldehyde and acetate. *Brain Res. Bull.* 62, 197–202. doi: 10.1016/j.brainresbull.2003.09.013

Correa, M., Arizzi, M. N., Betz, A., Mingote, S., and Salamone, J. D. (2003b). Locomotor stimulant effects of intraventricular injections of low doses of ethanol in rats: acute and repeated administration. *Psychopharmacology* 170, 368–375. doi: 10.1007/s00213-003-1557-0

Correa, M., Arizzi-LaFrance, M. N., and Salamone, J. D. (2009). Infusions of acetaldehyde into the arcuate nucleus of the hypothalamus induce motor activity in rats. *Life Sci.* 84, 321–327. doi: 10.1016/j.lfs.2008.12.013

Correa, M., Manrique, H. M., Font, L., Escrig, M. A., and Aragon, C. M. (2008). Reduction in the anxiolytic effects of ethanol by centrally formed acetaldehyde: the role of catalase inhibitors and acetaldehyde-sequestering agents. *Psychopharmacology* 200, 455–464. doi: 10.1007/s00213-008-1219-3

Correa, M., Miquel, M., and Aragon, C. M. (2000). Lead acetate potentiates brain catalase activity and enhances ethanol-induced locomotion in mice. *Pharmacol. Biochem. Behav.* 66, 137–142. doi: 10.1016/s0091-3057(00)00204-5

Correa, M., Miquel, M., Sanchis-Segura, C., and Aragon, C. M. (1999a). Acute lead acetate administration potentiates ethanol-induced locomotor activity in mice: the role of brain catalase. *Alcohol. Clin. Exp. Res.* 23, 799–805.

Correa, M., Miquel, M., Sanchis-Segura, C., and Aragon, C. M. (1999b). Effects of chronic lead administration on ethanol-induced locomotor and brain catalase activity. *Alcohol* 19, 43–49. doi: 10.1016/s0741-8329(99)00023-3

Correa, M., Salamone, J. D., Segovia, K. N., Pardo, M., Longoni, R., Spina, L., et al. (2012). Piecing together the puzzle of acetaldehyde as a neuroactive agent. *Neurosci. Biobehav. Rev.* 36, 404–430. doi: 10.1016/j.neubiorev.2011.07.009

Correa, M., Sanchis-Segura, C., Pastor, R., and Aragon, C. M. (2004). Ethanol intake and motor sensitization: the role of brain catalase activity in mice with different genotypes. *Physiol. Behav.* 82, 231–40. doi: 10.1016/j.physbeh.2004.03.033

Corwin, R. L., and Wojnicki, F. H. (2009). Baclofen, raclopride, and naltrexone differentially affect intake of fat and sucrose under limited access conditions. *Behav. Pharmacol.* 20, 537–548. doi: 10.1097/fbp.0b013e3283313168

Cunningham, C. L., Henderson, C. M., and Bormann, N. M. (1998). Extinction of ethanol-induced conditioned place preference and conditioned place aversion: effects of naloxone. *Psychopharmacology* 139, 62–70. doi: 10.1007/s002130050690

Dayas, C. V., Liu, X., Simms, J. A., and Weiss, F. (2007). Distinct patterns of neural activation associated with ethanol seeking: effects of naltrexone. *Biol. Psychiatry* 61, 979–989. doi: 10.1016/j.biopsych.2006.07.034

De, A., Boyadjieva, N., and Sarkar, D. K. (2002). Role of protein kinase C in control of ethanol-modulated beta-endorphin release from hypothalamic neurons in primary cultures. *J. Pharmacol. Exp. Ther.* 301, 119–128. doi: 10.1124/jpet.301.1.119

de Waele, J. P., Kiianmaa, K., and Gianoulakis, C. (1994). Spontaneous and ethanol-stimulated in vitro release of beta-endorphin by the hypothalamus of AA and ANA rats. *Alcohol. Clin. Exp. Res.* 18, 1468–1473. doi: 10.1111/j.1530-0277.1994.tb01452.x

Dempsey, S., and Grisel, J. E. (2012). Locomotor sensitization to EtOH: contribution of β-Endorphin. *Front. Mol. Neurosci.* 5:87. doi: 10.3389/fnmol.2012.00087

Devine, D. P., Leone, P., and Wise, R. A. (1993). Mesolimbic dopamine neurotransmission is increased by administration of mu-opioid receptor antagonists. *Eur. J. Pharmacol.* 243, 55–64. doi: 10.1016/0014-2999(93)90167-g

Di Chiara, G., and North, R. A. (1992). Neurobiology of opiate abuse. *Trends Pharmacol. Sci.* 13, 185–193. doi: 10.1016/0165-6147(92)90062-b

Diana, M., Peana, A. T., Sirca, D., Lintas, A., Melis, M., and Enrico, P. (2008). Crucial role of acetaldehyde in alcohol activation of the mesolimbic dopamine system. *Ann. NY Acad. Sci.* 1139, 307–317. doi: 10.1196/annals.1432.009

Enrico, P., Sirca, D., Mereu, M., Peana, A. T., Lintas, A., Golosio, A., et al. (2009). Acetaldehyde sequestering prevents ethanol-induced stimulation of mesolimbic dopamine transmission. *Drug Alcohol Depend.* 100, 265–271. doi: 10.1016/j.drugalcdep.2008.10.010

Evans, C. J., Keith, D. E. Jr., Morrison, H., Magendzo, K., and Edwards, R. H. (1992). Cloning of a delta opioid receptor by functional expression. *Science* 258, 1952–1955. doi: 10.1126/science.1335167

Femenía, T., and Manzanares, J. (2012). Increased ethanol intake in prodynorphin knockout mice is associated to changes in opioid receptor function and dopamine transmission. *Addict. Biol.* 17, 322–337. doi: 10.1111/j.1369-1600.2011.00378.x

Fertel, R. H., Greenwald, J. E., Schwarz, R., Wong, L., and Bianchine, J. (1980). Opiate receptor binding and analgesic effects of the tetrahydroisoquinolines salsolinol and tetrahydropapaveroline. *Res. Commun. Chem. Pathol. Pharmacol.* 27, 3–16.

Foddai, M., Dosia, G., Spiga, S., and Diana, M. (2004). Acetaldehyde increases dopaminergic neuronal activity in the VTA. *Neuropsychopharmacology* 29, 530–536. doi: 10.1038/sj.npp.1300326

Font, L., Aragon, C. M., and Miquel, M. (2006a). Voluntary ethanol consumption decreases after the inactivation of central acetaldehyde by D-penicillamine. *Behav. Brain Res.* 171, 78–86. doi: 10.1016/j.bbr.2006.03.020

Font, L., Miquel, M., and Aragon, C. (2005). Prevention of ethanol induced behavioral stimulation by D-penicillamine: a sequestration agent for acetaldehyde. *Alcohol. Clin. Exp. Res.* 29, 1156–1164. doi: 10.1097/01.alc.0000171945.30494.af

Font, L., Miquel, M., and Aragon, C. (2006b). Ethanol-induced conditioned place preference, but not aversion, is blocked by treatment with D-penicillamine, an inactivation agent for acetaldehyde. *Psychopharmacology* 184, 56–64. doi: 10.1007/s00213-005-0224-z

Font, L., Miquel, M., and Aragon, C. M. (2008). Involvement of brain catalase activity in the acquisition of ethanol-induced conditioned place preference. *Physiol. Behav.* 93, 733–741. doi: 10.1016/j.physbeh.2007.11.026

Froehlich, J. C. (1995). Genetic factors in alcohol self-administration. *J. Clin. Psychiatry* 56(Suppl. 7), 15–23.

Froehlich, J. C., Zweifel, M., Harts, J., Lumeng, L., and Li, T. K. (1991). Importance of delta opioid receptors in maintaining high alcohol drinking. *Psychopharmacology* 103, 467–472. doi: 10.1007/bf02244246

Gevaerd, M. S., Sultowski, E. T., and Takahashi, R. N. (1999). Combined effects of diethylpropion and alcohol on locomotor activity of mice: participation of the dopaminergic and opioid systems. *Braz. J. Med. Biol. Res.* 32, 1545–1550. doi: 10.1590/S0100-879X1999001200015

Gianoulakis, C. (1990). Characterization of the effects of acute ethanol administration on the release of beta-endorphin peptides by the rat hypothalamus. *Eur. J. Pharmacol.* 180, 21–29. doi: 10.1016/0014-2999(90)90588-w

Gianoulakis, C. (1993). Endogenous opioids and excessive alcohol consumption. *J. Psychiatry Neurosci.* 18, 148–156.

Gianoulakis, C. (2001). Influence of the endogenous opioid system on high alcohol consumption and genetic predisposition to alcoholism. *J. Psychiatry Neurosci.* 26, 304–318.

Gianoulakis, C., Hutchison, W. D., and Kalant, H. (1988). Effects of ethanol treatment and withdrawal on biosynthesis and processing of proopiomelanocortin by the rat neurointermediate lobe. *Endocrinology* 122, 817–825. doi: 10.1210/endo-122-3-817

Gill, K., Menez, J. F., Lucas, D., and Deitrich, R. A. (1992). Enzymatic production of acetaldehyde from ethanol in rat brain tissue. *Alcohol. Clin. Exp. Res.* 16, 910–915. doi: 10.1111/j.1530-0277.1992.tb01892.x

Gremel, C. M., Young, E. A., and Cunningham, C. L. (2011). Blockade of opioid receptors in anterior cingulate cortex disrupts ethanol-seeking behavior in mice. *Behav. Brain Res.* 219, 358–362. doi: 10.1016/j.bbr.2010.12.033

Hall, F. S., Sora, I., and Uhl, G. R. (2001). Ethanol consumption and reward are decreased in μ-opiate receptor knockout mice. *Psychopharmacology* 154, 43–49. doi: 10.1007/s002130000622

Hamby-Mason, R., Chen, J. J., Schenker, S., Perez, A., and Henderson, G. I. (1997). Catalase mediates acetaldehyde formation from ethanol in fetal and neonatal rat brain. *Alcohol. Clin. Exp. Res.* 21, 1063–72. doi: 10.1111/j.1530-0277.1997.tb04255.x

Herz, A. (1997). Endogenous opioid systems and alcohol addiction. *Psychopharmacology* 129, 99–111. doi: 10.1007/s002130050169

Hipólito, L., Martí-Prats, L., Sánchez-Catalán, M. J., Polache, A., and Granero, L. (2011). Induction of conditioned place preference and dopamine release by salsolinol in posterior VTA of rats: involvement of μ-opioid receptors. *Neurochem. Int.* 59, 559–562. doi: 10.1016/j.neuint.2011.04.014

Hipólito, L., Sánchez-Catalán, M. J., Granero, L., and Polache, A. (2009). Local salsolinol modulates dopamine extracellular levels from rat nucleus accumbens: shell/core differences. *Neurochem. Int.* 55, 187–192. doi: 10.1016/j.neuint.2009.02.014

Hipólito, L., Sánchez-Catalán, M. J., Martí-Prats, L., Granero, L., and Polache, A. (2012). Revisiting the controversial role of salsolinol in the neurobiological effects of ethanol: old and new vistas. *Neurosci. Biobehav. Rev.* 36, 362–378. doi: 10.1016/j.neubiorev.2011.07.007

Hipólito, L., Sánchez-Catalán, M. J., Zanolini, I., Polache, A., and Granero, L. (2008). Shell/core differences in mu- and delta-opioid receptor modulation of dopamine efflux in nucleus accumbens. *Neuropharmacology* 55, 183–189. doi: 10.1016/j.neuropharm.2008.05.012

Hipólito, L., Sánchez-Catalán, M. J., Zornoza, T., Polache, A., and Granero, L. (2010). Locomotor stimulant effects of acute and repeated intrategmental injections of salsolinol in rats : role of μ-opioid receptors. *Psychopharmacology* 209, 1–11. doi: 10.1007/s00213-009-1751-9

Holstein, S. E., Pastor, R., Meyer, P. J., and Phillips, T. J. (2005). Naloxone does not attenuate the locomotor effects of ethanol in FAST, SLOW, or two heterogeneous stocks of mice. *Psychopharmacology* 182, 277–289. doi: 10.1007/s00213-005-0066-8

Hyytiä, P., and Kiianmaa, K. (2001). Suppression of ethanol responding by centrally administered CTOP and naltrindole in AA and Wistar rats. *Alcohol. Clin. Exp. Res.* 25, 25–33. doi: 10.1111/j.1530-0277.2001.tb02123.x

Ingman, K., Salvadori, S., Lazarus, L., Korpi, E. R., and Honkanen, A. (2003). Selective delta-opioid receptor antagonist N,N(CH3)2-Dmt-Tic-OH does not reduce ethanol intake in alcohol-preferring AA rats. *Addict. Biol.* 8, 173–179. doi: 10.1080/1355621031000117400

Jamal, M., Ameno, K., Uekita, I., Kumihashi, M., Wang, W., and Ijiri, I. (2007). Catalase mediates acetaldehyde formation in the striatum of free-moving rats. *Neurotoxicology* 28, 1245–1248. doi: 10.1016/j.neuro.2007.05.002

Jarjour, S., Bai, L., and Gianoulakis, C. (2009). Effect of acute ethanol administration on the release of opioid peptides from the midbrain including the ventral tegmental area. *Alcohol. Clin. Exp. Res.* 33, 1033–1043. doi: 10.1111/j.1530-0277.2009.00924.x

June, H. L., Grey, C., Warren-Reese, C., Durr, L. F., Ricks-Cord, A., Johnson, A., et al. (1999). The opioid receptor antagonist nalmefene reduces responding maintained by ethanol presentation: preclinical studies in ethanol-preferring and outbred wistar rats. *Alcohol. Clin. Exp. Res.* 22, 2174–2185. doi: 10.1111/j.1530-0277.1998.tb05931.x

Karahanian, E., Quintanilla, M. E., Tampier, L., Rivera-Meza, M., Bustamante, D., Gonzalez-Lira, V., et al. (2011). Ethanol as a prodrug: brain metabolism of ethanol mediates its reinforcing effects. *Alcohol. Clin. Exp. Res.* 35, 606–612. doi: 10.1111/j.1530-0277.2011.01439.x

Khachaturian, H., Lewis, M. E., Alessi, N. E., and Watson, S. J. (1985). Time of origin of opioid peptide-containing neurons in the rat hypothalamus. *J. Comp. Neurol.* 236, 538–546. doi: 10.1002/cne.902360409

Kieffer, B. L., and Evans, C. J. (2009). Opioid receptors: from binding sites to visible molecules in vivo. *Neuropharmacology* 56(Suppl. 1), 205–212. doi: 10.1016/j.neuropharm.2008.07.033

Kieffer, B. L., and Gavériaux-Ruff, C. (2002). Exploring the opioid system by gene knockout. *Prog. Neurobiol.* 66, 285–306. doi: 10.1016/s0301-0082(02)00008-4

Kiianmaa, K., Hoffman, P., and Tabakoff, B. (1983). Antagonism of the behavioral effects of ethanol by naltrexone in BALB/c, C57BL/6, and DBA/2 mice. *Psychopharmacology* 79, 291–294. doi: 10.1007/bf00433403

Knapp, R. J., Malatynska, E., Collins, N., Fang, L., Wang, J. Y., Hruby, V. J., et al. (1995). Molecular biology and pharmacology of cloned opioid receptors. *FASEB J.* 9, 516–525.

Koechling, U. M., and Amit, Z. (1994). Effects of 3-amino-1,2,4-triazole on brain catalase in the mediation of ethanol consumption in mice. *Alcohol* 11, 235–239. doi: 10.1016/0741-8329(94)90036-1

Krishnan-Sarin, S., Portoghese, P. S., Li, T. K., and Froehlich, J. C. (1995). The delta 2-opioid receptor antagonist naltriben selectively attenuates alcohol intake in rats bred for alcohol preference. *Pharmacol. Biochem. Behav.* 52, 153–159. doi: 10.1016/0091-3057(95)00080-g

Kuzmin, A., Sandin, J., Terenius, L., and Ogren, S. O. (2003). Acquisition, expression, and reinstatement of ethanol-induced conditioned place preference in mice: effects of opioid receptor-like 1 receptor agonists and naloxone. *J. Pharmacol. Exp. Ther.* 304, 310–318. doi: 10.1124/jpet.102.041350

Lam, M. P., Marinelli, P. W., Bai, L., and Gianoulakis, C. (2008). Effects of acute ethanol on opioid peptide release in the central amygdala: an in vivo microdialysis study. *Psychopharmacology* 201, 261–271. doi: 10.1007/s00213-008-1267-8

Lê, A. D., Poulos, C. X., Harding, S., Watchus, J., Juzytsch, W., and Shaham, Y. (1999). Effects of naltrexone and fluoxetine on alcohol self-administration and reinstatement of alcohol seeking induced by priming injections of alcohol and exposure to stress. *Neuropsychopharmacology* 21, 435–444. doi: 10.1016/s0893-133x(99)00024-x

Lê, A. D., Poulos, C. X., Quan, B., and Chow, S. (1993). The effects of selective blockade of delta and mu opiate receptors on ethanol consumption by C57BL/6 mice in a restricted access paradigm. *Brain Res.* 630, 330–332. doi: 10.1016/0006-8993(93)90672-a

Leriche, M., and Méndez, M. (2010). Ethanol exposure selectively alters beta-endorphin content but not [3H]-DAMGO binding in discrete regions of the rat brain. *Neuropeptides* 44, 9–16. doi: 10.1016/j.npep.2009.11.009

Lesscher, H. M., Bailey, A., Burbach, J. P., Van Ree, J. M., Kitchen, I., and Gerrits, M. A. (2003). Receptor-selective changes in mu-, delta- and kappa-opioid receptors after chronic naltrexone treatment in mice. *Eur. J. Neurosci.* 17, 1006–1012.

Liu, X., and Weiss, F. (2002). Additive effect of stress and drug cues on reinstatement of ethanol seeking: exacerbation by history of dependence and role of concurrent activation of corticotropin-releasing factor and opioid mechanisms. *J. Neurosci.* 22, 7856–7861.

Logrip, M. L., Janak, P. H., and Ron, D. (2009). Blockade of ethanol reward by the kappa opioid receptor. *Alcohol* 43, 359–365. doi: 10.1016/j.alcohol.2009.05.001

Lucchi, L., Bosio, A., Spano, P. F., and Trabucchi, M. (1982). Action of ethanol and salsolinol on opiate receptor function. *Brain Res.* 232, 506–510. doi: 10.1016/0006-8993(82)90297-9

Manrique, H. M., Miquel, M., and Aragon, C. M. (2005). Brain catalase mediates potentiation of social recognition memory produced by ethanol in mice. *Drug Alcohol Depend.* 79, 343–350. doi: 10.1016/j.drugalcdep.2005.02.007

Mansour, A., Khachaturian, H., Lewis, M. E., Akil, H., and Watson, S. J. (1988). Anatomy of CNS opioid receptors. *Trends Neurosci.* 11, 308–314. doi: 10.1016/0166-2236(88)90093-8

Margolis, E. B., Fields, H. L., Hjelmstad, G. O., and Mitchell, J. M. (2008). Delta-opioid receptor expression in the ventral tegmental area protects against elevated alcohol consumption. *J. Neurosci.* 28, 12672–12681. doi: 10.1523/jneurosci.4569-08.2008

Marinelli, P. W., Funk, D., Harding, S., Li, Z., Juzytsch, W., and Lê, A. D. (2009). Roles of opioid receptor subtypes in mediating alcohol seeking induced by discrete cues and context. *Eur. J. Neurosci.* 30, 671–678. doi: 10.1111/j.1460-9568.2009.06851.x

Marinelli, P. W., Quirion, R., and Gianoulakis, C. (2003a). Estradiol valerate and alcohol intake: a comparison between Wistar and Lewis rats and the putative role of endorphins. *Behav. Brain Res.* 139, 59–67. doi: 10.1016/s0166-4328(02)00057-8

Marinelli, P. W., Quirion, R., and Gianoulakis, C. (2003b). A microdialysis profile of beta-endorphin and catecholamines in the rat nucleus accumbens following alcohol administration. *Psychopharmacology* 169, 60–67. doi: 10.1007/s00213-003-1490-2

Martí-Prats, L., Sánchez-Catalán, M. J., Hipólito, L., Orrico, A., Zornoza, T., Polache, A., et al. (2010). Systemic administration of D-penicillamine prevents the locomotor activation after intra-VTA ethanol administration in rats. *Neurosci. Lett.* 483, 143–147. doi: 10.1016/j.neulet.2010.07.081

Matsuzawa, S., Suzuki, T., and Misawa, M. (2000). Involvement of mu-opioid receptor in the salsolinol-associated place preference in rats exposed to conditioned fear stress. *Alcohol. Clin. Exp. Res.* 24, 366–372. doi: 10.1111/j.1530-0277.2000.tb04624.x

McBride, W. J., Li, T. K., Deitrich, R. A., Zimatkin, S., Smith, B. R., and Rodd-Henricks, Z. A. (2002). Involvement of acetaldehyde in alcohol addiction. *Alcohol. Clin. Exp. Res.* 26, 114–119.

Melis, M., Enrico, P., Peana, A. T., and Diana, M. (2007). Acetaldehyde mediates alcohol activation of the mesolimbic dopamine system. *Eur. J. Neurosci.* 26, 2824–33. doi: 10.1111/j.1460-9568.2007.05887.x

Méndez, M., and Morales-Mulia, M. (2008). Role of mu and delta opioid receptors in alcohol drinking behaviour. *Curr. Drug Abuse Rev.* 1, 239–252. doi: 10.2174/1874473710801020239

Middaugh, L. D., and Bandy, A. L. (2000). Naltrexone effects on ethanol consumption and response to ethanol conditioned cues in C57BL/6 mice. *Psychopharmacology* 151, 321–327. doi: 10.1007/s002130000479

Milton, G. W., Verhaert, P. D., and Downer, R. G. (1991). Immunofluorescent localization of dopamine-like and leucine-enkephalin-like neurons in the supraoesophageal ganglia of the american cockroach, periplaneta americana. *Tissue Cell* 23, 331–340. doi: 10.1016/0040-8166(91)90051-t

Miquel, M., Font, L., Sanchis-Segura, C., and Aragon, C. M. (2003). Neonatal administration of monosodium glutamate prevents the development of ethanol- but not psychostimulant-induced sensitization: a putative role of the arcuate nucleus. *Eur. J. Neurosci.* 17, 2163–2170. doi: 10.1046/j.1460-9568.2003.02646.x

Modesto-Lowe, V., and Fritz, E. M. (2005). The opioidergic-alcohol link: implications for treatment. *CNS Drugs* 19, 693–707. doi: 10.2165/00023210-200519080-00005

Moreno, S., Mugnaini, E., and Cerù, M. P. (1995). Immunocytochemical localization of catalase in the central nervous system of the rat. *J. Histochem. Cytochem.* 43, 1253–1267. doi: 10.1177/43.12.8537642

Myers, W., Ng, K., and Singer, G. (1984). Effects of naloxone and buprenorphine on intravenous acetaldehyde self-injection in rats. *Physiol. Behav.* 33, 449–455. doi: 10.1016/0031-9384(84)90168-9

Nielsen, C. K., Simms, J. A., Pierson, H. B., Li, R., Saini, S. K., Ananthan, S., et al. (2008). A novel delta opioid receptor antagonist, SoRI-9409, produces a selective and long-lasting decrease in ethanol consumption in heavy-drinking rats. *Biol. Psychiatry* 64, 974–981. doi: 10.1016/j.biopsych.2008.07.018

Nylander, I., and Roman, E. (2012). Neuropeptides as mediators of the early-life impact on the brain; implications for alcohol use disorders. *Front. Mol. Neurosci.* 5:77. doi: 10.3389/fnmol.2012.00077

Olive, M. F., Koenig, H. N., Nannini, M. A., and Hodge, C. W. (2001). Stimulation of endorphin neurotransmission in the nucleus accumbens by ethanol, cocaine, and amphetamine. *J. Neurosci.* 21, RC184.

O'Malley, S. S., Jaffe, A. J., Chang, G., Schottenfeld, R. S., Meyer, R. E., and Rounsaville, B. (1992). Naltrexone and coping skills therapy for alcohol dependence. A controlled study. *Arch. Gen. Psychiatry* 49, 881–887. doi: 10.1001/archpsyc.1992.01820110045007

Orrico, A., Hipólito, L., Sánchez-Catalán, M. J., Martí-Prats, L., Zornoza, T., Granero, L., et al. (2013). Efficacy of D-penicillamine, a sequestering acetaldehyde agent, in the prevention of alcohol relapse-like drinking in rats. *Psychopharmacology* doi: 10.1007/s00213-013-3065-1. [Epub ahead of print]

Oswald, L. M., and Wand, G. S. (2004). Opioids and alcoholism. *Physiol. Behav.* 81, 339–358. doi: 10.1016/j.physbeh.2004.02.008

Pasternak, G. W. (2001a). The pharmacology of mu analgesics: from patients to genes. *Neuroscientist* 7, 220–231. doi: 10.1177/107385840100700307

Pasternak, G. W. (2001b). Insights into mu opioid pharmacology the role of mu opioid receptor subtypes. *Life Sci.* 68, 2213–229. doi: 10.1016/S0024-3205(01)01008-6

Pastor, R., and Aragon, C. M. (2006). The role of opioid receptor subtypes in the development of behavioral sensitization to ethanol. *Neuropsychopharmacology* 31, 1489–1499. doi: 10.1038/sj.npp.1300928

Pastor, R., and Aragon, C. M. (2008). Ethanol injected into the hypothalamic arcuate nucleus induces behavioral stimulation in rats: an effect prevented by catalase inhibition and naltrexone. *Behav. Pharmacol.* 19, 698–705. doi: 10.1097/fbp.0b013e328315ecd7

Pastor, R., Font, L., Miquel, M., Phillips, T. J., and Aragon, C. M. (2011). Involvement of the beta-endorphin neurons of the hypothalamic arcuate nucleus in ethanol-induced place preference conditioning in mice. *Alcohol. Clin. Exp. Res.* 35, 2019–2029. doi: 10.1111/j.1530-0277.2011.01553.x

Pastor, R., Sanchis-Segura, C., and Aragon, C. M. (2002). Ethanol-stimulated behaviour in mice is modulated by brain catalase activity and H2O2 rate of production. *Psychopharmacology* 165, 51–59. doi: 10.1007/s00213-002-1241-9

Pastor, R., Sanchis-Segura, C., and Aragon, C. (2005). Effect of selective antagonism of mu(1)-, mu(1/2)-, mu(3)-, and delta-opioid receptors on the locomotor-stimulating actions of ethanol. *Drug Alcohol Depend.* 78, 289–295. doi: 10.1016/j.drugalcdep.2004.11.007

Pastorcic, M., Boyadjieva, N., and Sarkar, D. K. (1994). Comparison of the effects of alcohol and acetaldehyde on proopiomelanocortin mRNA levels and beta-endorphin secretion from hypothalamic neurons in primary cultures. *Mol. Cell. Neurosci.* 5, 580–586. doi: 10.1006/mcne.1994.1071

Patel, V. A., and Pohorecky, L. A. (1989). Acute and chronic ethanol treatment on beta-endorphin and catecholamine levels. *Alcohol* 6, 59–63. doi: 10.1016/0741-8329(89)90074-8

Pautassi, R. M., Nizhnikov, M. E., Acevedo, M. B., and Spear, N. E. (2012). Early role of the κ opioid receptor in ethanol-induced reinforcement. *Physiol. Behav.* 105, 1231–1241. doi: 10.1016/j.physbeh.2012.01.003

Pautassi, R. M., Nizhnikov, M. E., Fabio, M. C., and Spear, N. E. (2011). An acetaldehyde-sequestering agent inhibits appetitive reinforcement and behavioral stimulation induced by ethanol in preweanling rats. *Pharmacol. Biochem. Behav.* 97, 462–469. doi: 10.1016/j.pbb.2010.10.005

Peana, A. T., Assaretti, A. R., Muggironi, G., Enrico, P., and Diana, M. (2009). Reduction of ethanol-derived acetaldehyde induced motivational properties by L-cysteine. *Alcohol. Clin. Exp. Res.* 33, 43–48. doi: 10.1111/j.1530-0277.2008.00809.x

Peana, A. T., Enrico, P., Assaretti, A. R., Pulighe, E., Muggironi, G., Nieddu, M., et al. (2008). Key role of ethanol-derived acetaldehyde in the motivational properties induced by intragastric ethanol: a conditioned place preference study in the rat. *Alcohol. Clin. Exp. Res.* 32, 249–258. doi: 10.1111/j.1530-0277.2007.00574.x

Peana, A. T., Muggironi, G., and Diana, M. (2010). Acetaldehyde-reinforcing effects; a study on oral self-administration behavior. *Front. Psychiatry* 1:23. doi: 10.3389/fpsyt.2010.00023

Peana, A. T., Muriggironi, G., Fois, G. R., Zinellu, M., Sirca, D., and Diana, M. (2012). Effect of (L)-cysteine on acetaldehyde self-administration. *Alcohol* 46, 489–497. doi: 10.1016/j.alcohol.2011.10.004

Peana, A. T., Muggironi, G., Fois, G. R., Zinellu, M., Vinci, S., and Acquas, E. (2011). Effect of opioid receptor blockade on acetaldehyde self-administration and ERK phosphorylation in the rat nucleus accumbens. *Alcohol* 45, 773–783. doi: 10.1016/j.alcohol.2011.06.003

Peciña, S., and Berridge, K. C. (2005). Hedonic hot spot in nucleus accumbens shell: where do mu-opioids cause increased hedonic impact of sweetness?. *J. Neurosci.* 25, 11777–11786. doi: 10.1523/jneurosci.2329-05.2005

Popp, R. L., and Erickson, C. K. (1998). The effect of an acute ethanol exposure on the rat brain POMC opiopeptide system. *Alcohol* 16, 139–148. doi: 10.1016/s0741-8329(98)00003-2

Quertemont, E., and De Witte, P. (2001). Conditioned stimulus preference after acetaldehyde but not ethanol injections. *Pharmacol. Biochem. Behav.* 68, 449–454. doi: 10.1016/s0091-3057(00)00486-x

Quertemont, E., Tambour, S., and Tirelli, E. (2005). The role of acetaldehyde in the neurobehavioral effects of ethanol: a comprehensive review of animal studies. *Prog. Neurobiol.* 75, 247–274. doi: 10.1016/j.pneurobio.2005.03.003

Rao, R. E., Wojnicki, F. H., Coupland, J., Ghosh, S., and Corwin, R. L. (2008). Baclofen, raclopride, and naltrexone differentially reduce solid fat emulsion intake under limited access conditions. *Pharmacol. Biochem. Behav.* 89, 581–590. doi: 10.1016/j.pbb.2008.02.013

Rasmussen, D. D., Boldt, B. M., Wilkinson, C. W., and Mitton, D. R. (2002). Chronic daily ethanol and withdrawal: 3. Forebrain pro-opiomelanocortin gene expression and implications for dependence, relapse, and deprivation effect. *Alcohol. Clin. Exp. Res.* 26, 535–546. doi: 10.1111/j.1530-0277.2002.tb02572.x

Rasmussen, D. D., Bryant, C. A., Boldt, B. M., Colasurdo, E. A., Levin, N., and Wilkinson, C. W. (1998). Acute alcohol effects on opiomelanocortinergic regulation. *Alcohol. Clin. Exp. Res.* 22, 789–801. doi: 10.1111/j.1530-0277.1998.tb03870.x

Raynor, K., Kong, H., Chen, Y., Yasuda, K., Yu, L., Bell, G. I., et al. (1994). Pharmacological characterization of the cloned kappa-, delta-, and mu-opioid receptors. *Mol. Pharmacol.* 45, 330–334.

Reddy, B. V., and Sarkar, D. K. (1993). Effect of alcohol, acetaldehyde, and salsolinol on beta-endorphin secretion from the hypothalamic neurons in primary cultures. *Alcohol. Clin. Exp. Res.* 17, 1261–1267. doi: 10.1111/j.1530-0277.1993.tb05239.x

Reddy, B. V., Boyadjieva, N., and Sarkar, D. K. (1995). Effect of ethanol, propanol, butanol, and catalase enzyme blockers on beta-endorphin secretion from primary cultures of hypothalamic neurons: evidence for a mediatory role of acetaldehyde in ethanol stimulation of beta-endorphin release. *Alcohol. Clin. Exp. Res.* 19, 339–344. doi: 10.1111/j.1530-0277.1995.tb01512.x

Risinger, F. O., Dickinson, S. D., and Cunningham, C. L. (1992) Haloperidol reduces ethanol-induced motor activity stimulation but not conditioned place preference. *Psychopharmacology* 107, 453–456. doi: 10.1007/bf02245175

Roberts, A. J., McDonald, J. S., Heyser, C. J., Kieffer, B. L., Matthes,

H. W., Koob, G. F., et al. (2000). Mu-Opioid receptor knockout mice do not self-administer alcohol. *J. Pharmacol. Exp. Ther.* 293, 1002–1008.

Rodd, Z., Bell, R. L., Zhang, Y., Murphy, J. M., Goldstein, A., Zaffaroni, A., et al. (2005). Regional heterogeneity for the intracranial self-administration of ethanol and acetaldehyde within the ventral tegmental area of alcohol-preferring (P) rats: involvement of dopamine and serotonin. *Neuropsychopharmacology* 30, 330–338. doi: 10.1038/sj.npp.1300561

Rodd-Henricks, Z. A., Melendez, R. I, Zaffaroni, A., Goldstein, A., McBride, W. J., and Lu, T. K. (2002). The reinforcing effects of acetaldehyde in the posterior ventral tegmental area of alcohol-preferring rats. *Pharmacol. Biochem. Behav.* 72, 55–64. doi: 10.1016/s0091-3057(01)00733-x

Roth-Deri, I., Green-Sadan, T., and Yadid, G. (2008). Beta-endorphin and drug-induced reward and reinforcement. *Prog. Neurobiol.* 86, 1–21. doi: 10.1016/j.pneurobio.2008.06.003

Sánchez-Catalán, M. J., Hipólito, L., Zornoza, T., Polache, A., and Granero, L. (2009). Motor stimulant effects of ethanol and acetaldehyde injected into the posterior ventral tegmental area of rats: role of opioid receptors. *Psychopharmacology* 204, 641–653. doi: 10.1007/s00213-009-1495-6

Sanchis-Segura, C., Correa, M., and Aragon, C. M. (2000). Lession on the hypothalamic arcuate nucleus by estradiol valerate results in a blockade of ethanol-induced locomotion. *Behav. Brain Res.* 114, 57–63. doi: 10.1016/s0166-4328(00)00183-2

Sanchis-Segura, C., Correa, M., Miquel, M., and Aragon, C. M. (2005b). Catalase inhibition in the arcuate nucleus blocks ethanol effects on the locomotor activity of rats. *Neurosci. Lett.* 376, 66–70. doi: 10.1016/j.neulet.2004.11.025

Sanchis-Segura, C., Grisel, J. E., Olive, M. F., Ghozland, S., Koob, G. F., Roberts, A. J., et al. (2005a). Role of the endogenous opioid system on the neuropsychopharmacological effects of ethanol: new insights about an old question. *Alcohol. Clin. Exp. Res.* 29, 1522–1527. doi: 10.1097/01.alc.0000174913.60384.e8

Sanchis-Segura, C., Miquel, M., Correa, M., and Aragon, C. M. (1999a). Daily injections of cyanamide enhance both ethanol-induced locomotion and brain catalase activity. *Behav. Pharmacol.* 10, 459–465. doi: 10.1097/00008877-199909000-00004

Sanchis-Segura, C., Miquel, M., Correa, M., and Aragon, C. M. (1999b). The catalase inhibitor sodium azide reduces ethanol-induced locomotor activity. *Alcohol* 19, 37–42. doi: 10.1016/s0741-8329(99)00016-6

Sanchis-Segura, C., Miquel, M., Correa, M., and Aragon, C. M. (1999c). Cyanamide reduces brain catalase and ethanol-induced locomotor activity: is there a functional link? *Psychopharmacology* 144, 83–89. doi: 10.1007/s002130050980

Sanchis-Segura, C., Pastor, R., and Aragon, C. M. (2004). Opposite effects of acute versus chronic naltrexone administration on ethanol-induced locomotion. *Behav. Brain Res.* 153, 61–67. doi: 10.1016/j.bbr.2003.11.003

Schulz, R., Wüster, M., Duka, T., and Herz, A. (1980). Acute and chronic ethanol treatment changes endorphin levels in brain and pituitary. *Psychopharmacology* 68, 221–227. doi: 10.1007/bf00428107

Seizinger, B. R., Bovermann, K., Maysinger, D., Höllt, V., and Herz, A. (1983). Differential effects of acute and chronic ethanol treatment on particular opioid peptide systems in discrete regions of rat brain and pituitary. *Pharmacol. Biochem. Behav.* 18(Suppl. 1), 361–369. doi: 10.1016/0091-3057(83)90200-9

Seizinger, B. R., Höllt, V., and Herz, A. (1984). Effects of chronic ethanol treatment on the in vitro biosynthesis of pro-opiomelanocortin and its posttranslational processing to beta-endorphin in the intermediate lobe of the rat pituitary. *J. Neurochem.* 43, 607–613. doi: 10.1111/j.1471-4159.1984.tb12778.x

Simms, J. A., Steensland, P., Medina, B., Abernathy, K. E., Chandler, L. J., Wise, R., et al. (2008). Intermittent access to 20% ethanol induces high ethanol consumption in Long-Evans and Wistar rats. *Alcohol. Clin. Exp. Res.* 32, 1816–1823. doi: 10.1111/j.1530-0277.2008.00753.x

Simon, E. J. (1991). Opioid receptors and endogenous opioid peptides. *Med. Res. Rev.* 11, 357–374. doi: 10.1002/med.2610110402

Sirca, D., Enrico, P., Mereu, M., Peana, A. T., and Diana, M. (2011). L-cysteine prevents ethanol-induced stimulation of mesolimbic dopamine transmission. *Alcohol. Clin. Exp. Res.* 35, 862–869. doi: 10.1111/j.1530-0277.2010.01416.x

Smith, B. R., Amit, Z., and Splawinsky, J. (1984). Conditioned place preference induced by intraventricular infusions of acetaldehyde. *Alcohol* 1, 193–195. doi: 10.1016/0741-8329(84)90097-1

Smith, B. R., Aragon, C. M. G., and Amit, Z. (1997). Catalase and the production of central acetaldehyde: a possible mediator of the psychopharmacological effects of ethanol. *Addict. Biol.* 2, 277–289. doi: 10.1080/13556219772570

Spanagel, R., Herz, A., and Shippenberg, T. S. (1992). Opposing tonically active endogenous opioid systems modulate the mesolimbic dopaminergic pathway. *Proc. Natl. Acad. Sci. U S A* 89, 2046–2050. doi: 10.1073/pnas.89.6.2046

Spina, L., Longoni, R., Vinci, S., Ibba, F., Peana, A. T., Muggironi, G., et al. (2010). Role of dopamine D1 receptors and extracellular signal regulated kinase in the motivational properties of acetaldehyde as assessed by place preference conditioning. *Alcohol. Clin. Exp. Res.* 34, 607–616. doi: 10.1111/j.1530-0277.2009.01129.x

Stinus, L., Koob, G. F., Ling, N., Bloom, F. E., and Le Moal, M. (1980). Locomotor activation induced by infusion of endorphins into the ventral tegmental area: evidence for opiate-dopamine interactions. *Proc. Natl. Acad. Sci. U S A* 77, 2323–2327. doi: 10.1073/pnas.77.4.2323

Tarragón, E., Baliño, P., Aragon, C. M., and Pastor, R. (2012). Ethanol drinking-in-the-dark facilitates behavioral sensitization to ethanol in C57BL/6J, BALB/cByJ, but not in mu-opioid receptor deficient CXBK mice. *Pharmacol. Biochem. Behav.* 101, 14–23. doi: 10.1016/j.pbb.2011.11.014

Thorsell, A. (2013). The μ-opioid receptor and treatment response to naltrexone. *Alcohol Alcohol.* 48, 402–408. doi: 10.1093/alcalc/agt030

Trigo, J. M., Martin-García, E., Berrendero, F., Robledo, P., and Maldonado, R. (2010). The endogenous opioid system: a common substrate in drug addiction. *Drug Alcohol Depend.* 108, 183–194. doi: 10.1016/j.drugalcdep.2009.10.011

Unterwald, E. M., Anton, B., To, T., Lam, H., and Evans, C. J. (1998). Quantitative immunolocalization of mu opioid receptors: regulation by naltrexone. *Neuroscience* 85, 897–905. doi: 10.1016/s0306-4522(97)00659-3

Vengeliene, V., Bilbao, A., Molander, A., and Spanagel, R. (2008). Neuropharmacology of alcohol addiction. *Br. J. Pharmacol.* 154, 299–315. doi: 10.1038/bjp.2008.30

Vinci, S., Ibba, F., Longoni, R., Spina, L., Spiga, S., and Acquas, E. (2010). Acetaldehyde elicits ERK phosphorylation in the rat nucleus accumbens and extended amygdala. *Synapse* 64, 916–927. doi: 10.1002/syn.20811

Walker, B. M., Valdez, G. R., McLaughlin, J. P., and Bakalkin, G. (2012). Targeting dynorphin/kappa opioid receptor systems to treat alcohol abuse and dependence. *Alcohol* 46, 359–370. doi: 10.1016/j.alcohol.2011.10.006

Wand, G. S. (1990). Differential regulation of anterior pituitary corticotrope function is observed in vivo but not in vitro in two lines of ethanol-sensitive mice. *Alcohol. Clin. Exp. Res.* 14, 100–106. doi: 10.1111/j.1530-0277.1990.tb00454.x

Wee, S., and Koob, G. F. (2010). The role of the dynorphin-kappa opioid system in the reinforcing effects of drugs of abuse. *Psychopharmacology* 210, 121–135. doi: 10.1007/s00213-010-1825-8

Wong, K. J., Wojnicki, F. H., and Corwin, R. L. (2009). Baclofen, raclopride, and naltrexone differentially affect intake of fat/sucrose mixtures under limited access conditions. *Pharmacol. Biochem. Behav.* 92, 528–536. doi: 10.1016/j.pbb.2009.02.002

Xie, G., Hipólito, L., Zuo, W., Polache, A., Granero, L., Krnjevic, K., et al. (2012). Salsolinol stimulates dopamine neurons in slices of posterior ventral tegmental area indirectly by activating-opioid receptors. *J. Pharmacol. Exp. Ther.* 341, 43–50. doi: 10.1124/jpet.111.186833

Young, E. A., Dreumont, S. E., and Cunningham, C. L. (2013). Role of nucleus accumbens dopamine receptor subtypes in the learning and expression of alcohol-seeking behavior. *Neurobiol. Learn. Mem.* doi: 10.1016/j.nlm.2013.05.004. [Epub ahead of print]

Zimatkin, S. M., and Lindros, K. O. (1996). Distribution of catalase in rat brain: aminergic neurons as possible targets for ethanol effects. *Alcohol Alcohol.* 31, 167–174. doi: 10.1093/oxfordjournals.alcalc.a008128

Zimatkin, S. M., Rout, U. K., Koivusalo, M., Bühler, R., and Lindros, K. O. (1992). Regional distribution of low-Km mitochondrial aldehyde dehydrogenase in the rat central nervous system. *Alcohol. Clin. Exp. Res.* 16, 1162–1167. doi: 10.1111/j.1530-0277.1992.tb00713.x

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 03 May 2013; accepted: 10 July 2013; published online: 31 July 2013.
Citation: Font L, Luján MÁ and Pastor R (2013) Involvement of the endogenous opioid system in the psychopharmacological actions of ethanol: the role of acetaldehyde. Front. Behav. Neurosci. 7:93. doi:10.3389/fnbeh.2013.00093

frontiers in
BEHAVIORAL NEUROSCIENCE

ORIGINAL RESEARCH ARTICLE
published: 11 June 2013
doi: 10.3389/fnbeh.2013.00064

Acetaldehyde as a drug of abuse: insight into AM281 administration on operant-conflict paradigm in rats

Fulvio Plescia, Anna Brancato, Rosa A. M. Marino and Carla Cannizzaro*

Laboratory of Neuropsychopharmacology, Section of Pharmacology, Department of Sciences for Health Promotion and Mother and Child Care "Giuseppe D'Alessandro", University of Palermo, Palermo, Italy

Edited by:
Merce Correa, Universidad Jaume, Spain

Reviewed by:
John D. Salamone, University of Connecticut, USA
Marco Diana, University of Sassari, Italy

****Correspondence:***
Carla Cannizzaro, Laboratory of Neuropsychopharmacology, Department of Sciences for Health Promotion and Mother and Child Care "Giuseppe D'Alessandro", University of Palermo, Via del Vespro 129, 90127 Palermo, Italy
e-mail: carla.cannizzaro@unipa.it; carla.cannizzaro@katamail.com

Increasing evidence focuses on acetaldehyde (ACD) as the mediator of the rewarding and motivational properties of ethanol. Indeed, ACD stimulates dopamine release in the nucleus accumbens and it is self-administered under different conditions. Besides the dopaminergic transmission, the endocannabinoid system has been reported to play an important role in ethanol central effects, modulating primary alcohol rewarding effect, drug-seeking, and relapse behavior. Drug motivational properties are highlighted in operant paradigms which include response-contingent punishment, a behavioral equivalent of compulsive drug use despite adverse consequences. The aim of this study was thus to characterize ACD motivational and rewarding properties employing an operant-conflict paradigm in which rats, trained to lever press in order to get ACD solution (0.9%), undergo extinction, reinstatement and conflict sessions, according to a modified Geller–Seifter procedure. Furthermore, the role played by CB1 receptor system in modulating ACD-induced effects were investigated through the administration of CB1 receptor antagonist, AM281 (1 mg/kg, i.p.) during the extinction-, relapse-, and conflict-experiments. Our results indicate that ACD is able to induce and maintain an operant behavior, a high number of responses during extinction, an increase in the lever presses during the reinstatement phase, and a higher emission of punished responses during the conflict experiments, when compared to controls. The administration of AM281 is able to decrease ACD-seeking behavior during extinction, the number of lever presses during reinstatement and to strongly decrease the punished responses for ACD. Our data strengthen the idea that ACD may be responsible for the central effects of ethanol, and pinpoint at the CB1 system as one of the neural substrates underlying its addictive properties.

Keywords: acetaldehyde, lever pressing, relapse, Geiller–Seifter procedure, CB1 receptor blockade/antagonism

INTRODUCTION

Evidence obtained in preclinical studies suggests that Acetaldehyde (ACD), the first metabolite of ethanol, is biologically active and may play a pivotal role in the rewarding, motivational and addictive properties of alcohol, as recently reviewed by Correa et al. (2012) and Deehan et al. (2013a).

ACD is obtained from ethanol oxidative metabolism, which occurs by peripheral alcohol dehydrogenase, and by central catalase and CYP2E1 (Zimatkin et al., 1998; Arizzi-LaFrance et al., 2006; Zakhari, 2006; Jamal et al., 2007). High blood levels of ACD enter the brain, likely overwhelming the aldehyde dehydrogenase present in the blood-brain barrier (Quertemont et al., 2005).

ACD is able to affect dopamine neurotransmission, increasing neuronal firing in the ventral tegmental area (VTA), thus stimulating DA release in the nucleus accumbens (NAcc) shell (Melis et al., 2007; Deehan et al., 2013b). Recent reports show that the intra-VTA administration of a lentiviral vector, able to inhibit catalase synthesis, and hence central ACD production, nearly abolishes voluntary ethanol intake, as well as decreases ethanol-induced DA release in the NAcc shell (Karahanian et al., 2011), further supporting the compelling theory that ethanol may be acting as a prodrug. Behavioral studies confirm that ACD administration is able to induce conditioned place preference (Smith et al., 1984; Peana et al., 2008); furthermore rats readily self-administer ACD solution in operant conditions through several routes: centrally (Amit et al., 1977; Brown et al., 1979; Rodd et al., 2005), and peripherally (Myers et al., 1982; Peana et al., 2010; Cacace et al., 2012).

Besides the dopaminergic transmission, the brain endocannabinoid (EC) system plays an important role in value-attribution processing and in the modulation of reward-seeking behavior for different drugs of abuse (Serrano and Parsons, 2011), in view of its role as fine modulator of incoming inputs within the VTA (Melis et al., 2012).

In particular, CB1 receptor manipulation is reported to affect ethanol-related behavior, and in fact CB1 antagonism decreases both voluntary ethanol intake and relapse to ethanol in several experimental models (Arnone et al., 1997; Colombo et al., 1998; Gallate and McGregor, 1999; Cippitelli et al., 2005; Economidou et al., 2006; Femenía et al., 2010; De Bruin et al., 2011; Getachew et al., 2011), suggesting that CB1 receptor blockade reduces the rewarding value of ethanol. In turn, chronic administration of

ethanol is associated with increased concentrations of endocannabinoids, in accordance with a reduction in the activity of their removal mechanisms, and in CB1 receptor expression, thus affecting the system as a whole (Basavarajappa et al., 1998, 2000, 2003; Vinod et al., 2006).

Given these premises, it is worth focusing on CB1 receptor involvement in ACD self-administration, employing an operant conditioning paradigm which may reliably model the distinct phases of the addiction cycle. Indeed, punishment resistance, which represents the behavioral equivalent of compulsive drug use despite negative consequences (Deroche-Gamonet et al., 2004), is considered as a mandatory component in mirroring the addictive phenotype, besides drug taking, drug seeking and relapse (Marchant et al., 2013). Hence, by the assessment of the capacity of orally self-administered ACD to induced and maintain an operant behavior after forced abstinence, and in the presence of an aversive stimulus, according to a programmed schedule of responding (Cannizzaro et al., 2011; Cacace et al., 2012), the evaluation of the influence of CB1 receptor blockade by AM281 was carried out on ACD-seeking and relapse, and on compulsive-like behavior.

MATERIALS AND METHODS

ANIMALS

Adult male Wistar rats (Harlan, Udine, Italy) weighing 250 to 300 g, were housed two per cage and maintained on a 12-h light/dark cycle, under controlled environmental conditions (temperature 22 ± 2°C; humidity 55 ± 10%) with food and water available *ad-libitum*. During operant behavior experiments they were water-restricted and allowed to drink only 1 h/day at the end of the experimental sessions. Water intake was recorded. All subjects were experimentally naive and randomly assigned to the following groups ($n = 16$): ACD-drinking rats (ACD) which self-administered a solution of ACD (0.9% v/v) and water-drinking rats (CTR) which self-administered water. All experiments were in accordance with the Committee for Use of Experimental Animals of the University of Palermo, the Italian legislation D.L. 116/1992 and the European Union Council Directive 2010/63, dealing with research on experimental animals.

DRUGS

ACD 99.98% (Sigma-Aldrich, Milan, Italy) was diluted in tap water, in order to achieve a final concentration of 0.9% v/v (0.450 ml ACD in 50 ml of water). ACD solution was daily prepared, sealed with Parafilm (American Can Company), stored at 4°C during experiments, aiming at avoiding concentration loss (Cacace et al., 2012).

The CB1 selective cannabinoid antagonist AM281 (Sigma-Aldrich, Milan, Italy) was dissolved in a vehicle of Tween 80 (3%) in saline solution (0.9% NaCl), and administered intraperitoneally (1 mg/kg).

APPARATUS

The experimental sessions were carried out in a custom-built operant-conditioning chamber (30 × 28 × 37 cm), included in a dim-illuminated, ventilated, sound-attenuating cubicle. The chamber was equipped with one active lever and a cup that collected liquid from a corked reservoir, aiming at ACD solution preservation from evaporation, with a solenoid-actuated delivery system. It assured the delivery of 0.05 ml of solution for each lever pressing. Moreover, a foot-shock generator was able to deliver a constant-current, intermittent, inescapable, foot-shock (0.2 mA) to the chamber grid floor. A light-cue above the lever turned on during the punished period, allowing the animals to be aware of the aversive stimulus. Animal performance was recorded on a counter connected to the chamber. The devices were thoroughly cleaned before the introduction of each animal to ensure that the particular rat's behavior was not affected by the detection of another rat's scent.

OPERANT SELF-ADMINISTRATION PROCEDURE

The lever pressing procedure was scheduled into four different periods: Training—rewarded responses under a continuous schedule of reinforcement; Extinction—non rewarded responses; Relapse—reinstatement of the reinforced operant behavior following 1 week-deprivation; Conflict—rewarded responses cyclically associated with a 0.2 mA footshock.

Shaping and training

Animals underwent water deprivation for 23 h and then they were shaped to lever press in order to obtain water on a continuous reinforcement schedule (fixed ratio 1), until they achieved a steady performance. Afterwards, during the *Training* session rats orally self-administered ACD solution or water, according to their group, in the operant chamber, under a fixed ratio 1, along 21 days. For each operant response, the system delivered 0.05 ml of water or 0.90% v/v ACD solution. The number of lever presses was automatically recorded. Animals were tested each day at the same time (9:00 to 14:00), and each trial lasted 20 min.

Extinction

Animals underwent an operant responding session during which reward delivery was suspended. The number of lever presses at the end of the 20 min session was recorded for both ACD and Control groups.

Deprivation

ACD self-administration was suspended for 7 days to achieve a forced abstinence. Rats were left undisturbed in their home cages and received water and food *ad-libitum*.

Relapse

After the deprivation period, rats were exposed again to lever pressing in the operant chamber with a fixed ratio 1 response schedule for 7 days. Responses for ACD or water were recorded during the 20 min-experimental sessions.

Conflict

This protocol represented a modification of the Geller–Seifter paradigm, a procedure in which a positive reinforcement (water or ACD solution) is earned by an operant response; however, delivery of the positive reinforce is paired with an aversive stimulus, as a footshock. In our study, animals underwent alternatively unpunished and punished responses according to "3 minutes–1 minute" schedule. The session started and ended with an

unpunished interval. The punishment was signaled by a cue-light triggered by the response. During the punished response interval, lever presses were rewarded with the solution and coupled with a mild footshock of 0.2 mA. The number of unpunished and punished responses was automatically recorded.

Treatment

CB1 antagonist AM281 was administered to ACD-group 30 min before the experimental sessions, during extinction (1 day), relapse (7 days), and conflict (7 days). Control rats were administered intraperitoneally with vehicle.

Statistical analysis

Operant-drinking behavior. A Two-Way analysis of variance (ANOVA) was conducted on the number of lever presses during the training, extinction-, relapse- and conflict-sessions, as dependent variables, with "ACD self-administration" (treatment) as between-subjects factor, and "days" as repeated measurement factor. When necessary, simple main effects and *post-hoc* comparisons were calculated with Bonferroni post-test ($\alpha = 0.05$). Differences were considered statistically significant if $p < 0.05$. Furthermore, to compare the effect of AM281 treatment on ACD-seeking behavior during extinction, a 2-tailed Student's *t*-test for unpaired measures was employed.

RESULTS

OPERANT SELF-ADMINISTRATION

Training period

The results of a Two-Way ANOVA for repeated measures including ACD treatment as the between-subjects factor and "Days" as within-subjects factor showed a significant effect of time, treatment, and their interaction on the number of responses emitted, $F_{(20,\ 600)} = 19.52$, $p < 0.0001$; $F_{(1,\ 30)} = 5.16$, $p < 0.0304$; $F_{(20,600)} = 26.81$, $p < 0.0001$. Although during the training period both groups showed a similar pattern of operant-drinking behavior, *post-hoc* analysis revealed significant differences along the paradigm (**Figure 1**). In particular, at the beginning, rats exposed for the first time to ACD in the operant paradigm, showed a lower number of lever presses, and consequently a reduced liquid intake, reaching an amount of ACD consumed of 259 ± 68 mg/kg In the second week of the paradigm, ACD rats' drinking behavior increased, displaying higher number of lever presses and greater liquid intake 3 days of 7, displaying an average intake of 325 ± 21 mg/kg. Values from the third week of training were considered as reference measure of ACD baseline operant behavior. In this week, ACD rats' lever presses were increased significantly with respect to controls, 6 days of 7 ($t = 7.32$, $p < 0.001$; $t = 3.106$, $p < 0.05$; $t = 6.359$, $p < 0.001$; $t = 6.906$, $p < 0.001$; $t = 5.843$, $p < 0.001$; $t = 5.745$, $p < 0.001$), for a mean ACD intake of 355 ± 17 mg/kg. When water intake was measured during the free-drinking hour at the end of the experimental sessions, no differences were observed between the two experimental groups.

Extinction

Rats were tested on the operant condition paradigm to assess drug seeking when reward delivery was suspended. The effects of AM281 treatment on ACD seeking behavior in terms of lever presses were analyzed by a two-tailed Student' *t*-test for unpaired measures. Our data indicated that ACD was able to induce a significant increase in the number of lever presses ($t = 5.152$, $df = 30$, $p < 0.001$) compared to control rats. AM281 administration induced a reduction in the number of lever presses ($t = 4.196$, $df = 30$, $p < 0.001$) in ACD group (**Figures 2A,B**). AM281 was ineffective on control rats' operant behavior for water.

Relapse

Following 7 days of abstinence from ACD self-administration, rats were tested again in the operant chamber to assess whether deprivation could influence their drinking behavior. The results of a Two-Way ANOVA for repeated measures including ACD treatment as the between-subjects factor and "Days" as within-subjects factor showed a significant effect of time, treatment, and

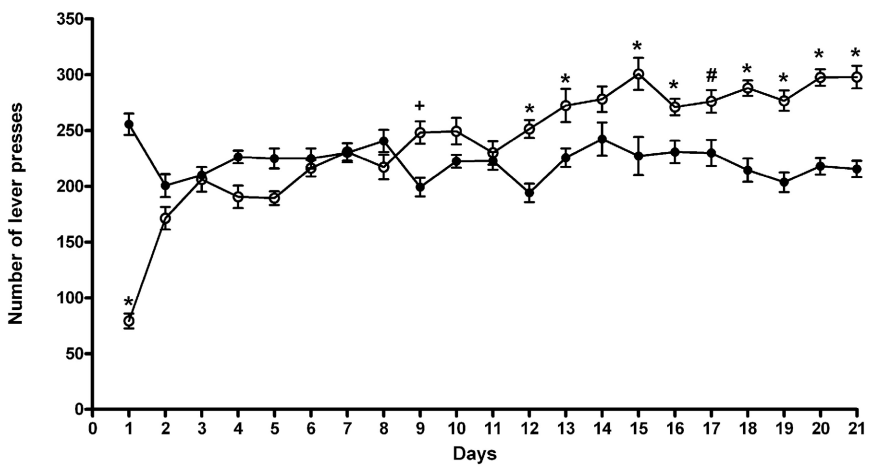


FIGURE 1 | Number of lever presses during the 21 days of training period. Each value represents the means ± S.D. of 16 rats. $^{\#}p < 0.05$; $^{+}p < 0.01$; $^{*}p < 0.001$ vs. control groups. (●) CTR, (○) ACD.

their interaction on the number of responses emitted, $F_{(6,180)} = 14.21, p < 0.0001$; $F_{(1,\ 180)} = 41.14, p < 0.001$; $F_{(6,\ 180)} = 15.79$, $p < 0.0001$. Bonferroni *post-hoc* analysis showed that ACD group displayed an increased number of lever presses on day 1, 2, 4, 6, and 7 ($t = 8.008.$, $p < 0.001$; $t = 4.173$, $p < 0.001$; $t = 6.574$, $p < 0.001$; $t = 8.231$, $p < 0.001$; $t = 4.320$, $p < 0.001$) when compared to controls (**Figure 3A**), reaching an average intake of 409 ± 37 mg/kg. Furthermore, when animals received the selective cannabinoid antagonist AM281, statistical analysis performed by a Two-Way ANOVA for repeated measures, including AM281 treatment as the between-subjects factor and "Days" as within-subjects factor, showed a significant effect of time, treatment, and their interaction on the number of responses emitted, $F_{(6,\ 180)} = 4.61$ $p < 0.0002$; $F_{(1,\ 180)} = 39.57$, $p < 0.001$; $F_{(6,\ 180)} = 7.82$, $p < 0.0001$. Bonferroni *post-hoc* analysis showed that AM281 was able to induce a reduction in the number of lever presses in all days of relapse ($t = 4.763$, $p < 0.001$; $t = 6.836$, $p < 0.001$; $t = 3.028$, $p < 0.05$; $t = 4.522$, $p < 0.001$; $t = 3.944$, $p < 0.001$; $t = 7.936$, $p < 0.001$; $t = 5.448$, $p < 0.001$) in ACD group (**Figure 3B**) leading to a decrease in the amount of ACD consumed (266 ± 16 mg/kg). No significant differences in the number of lever presses were recorded when AM281 was administered to control rats.

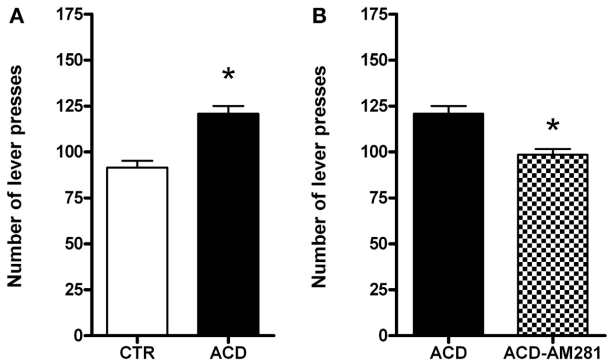


FIGURE 2 | Effects of ACD (A) and AM281 treatment (B) on the number of lever presses during the extinction day. Each value represents the means ± S.D. of 16 rats. $^{*}p < 0.001$ vs. respective control groups.

Conflict

In this set of experiments, rats underwent a cyclic schedule of unpunished and punished rewarded responses. Results of a Two-Way ANOVA for repeated measures, including ACD treatment as the between-subjects factor and "Days" as within-subjects factor on unpunished responses, showed a significant effect of time, treatment, and their interaction on the number of responses emitted, $F_{(6,\ 180)} = 3.80$, $p < 0.0014$; $F_{(1,180)} = 74.32$, $p < 0.0001$; $F_{(6,180)} = 2.35$, $p < 0.0328$. Bonferroni *post-hoc* analysis showed that ACD group displayed an increase in the number of unpunished lever presses on day 1, 2, 3, 4, 5, and 6 ($t = 5.009$, $p < 0.001$; $t = 5.417$, $p < 0.001$; $t = 3.064$, $p < 0.05$; $t = 4.686$, $p < 0.001$; $t = 3.677$, $p < 0.01$; $t = 5.817$, $p < 0.001$) when compared to controls (**Figure 4A**), and greater amount of ACD consumed (377 ± 31 mg/kg). When the parameter "punished responses" was analyzed, a Two-Way ANOVA for repeated measures including ACD treatment as the between-subjects factor and "Days" as within-subjects factor, showed a significant effect of time, treatment, and their interaction on the number of responses emitted, $F_{(6,\ 180)} = 16.62, p < 0.0001$; $F_{(1,\ 180)} = 585.43, p < 0.0001$; $F_{(6,\ 180)} = 3.07, p < 0.0070$. Bonferroni *post-hoc* analysis showed that ACD group displayed an increase in the number of punished lever presses along the conflict period ($t = 7.584$, $p < 0.001$; $t = 5.583$, $p < 0.001$; $t = 6.162$, $p < 0.001$; $t = 6.847$, $p < 0.001$; $t = 8.980$, $p < 0.001$; $t = 9.085$, $p < 0.001$; $t = 10.56$, $p < 0.001$) when compared to controls (**Figure 4B**), reaching an average amount of ACD ingested of 43 ± 23 mg/kg.

When animals received AM281, statistical analysis by a Two-Way ANOVA for repeated measures including AM281 treatment as the between-subjects factor and "Days" as within-subjects factor, showed a significant effect of time, treatment, and their interaction on the number of unpunished responses emitted, $F_{(6,\ 180)} = 32.43$, $p < 0.0001$; $F_{(1,\ 180)} = 38.50$, $p < 0.0001$;

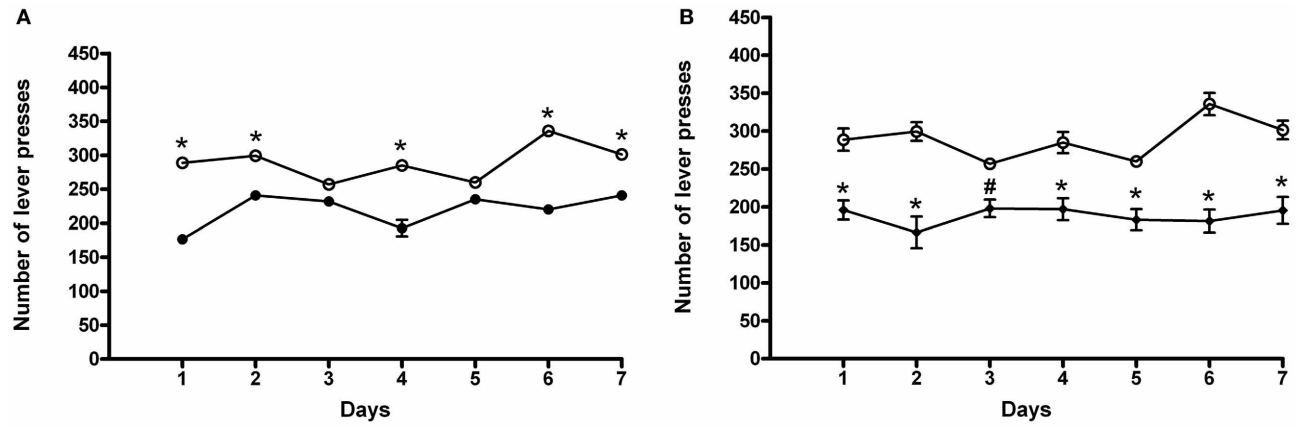


FIGURE 3 | Effects of ACD (A) and AM281 treatment (B) on the number of lever presses during the relapse periods. Each value represents the means ± S.D. of 16 rats. $^{\#}p < 0.05$; $^{*}p < 0.001$ vs. respective controls. (●) CTR, (○) ACD, (◆) (ACD-AM281).

$F_{(6,\ 180)} = 5.09, p < 0.0001$. Bonferroni *post-hoc* analysis showed that AM281 was able to induce a decrease in the number of unpunished lever presses in day 1, 2, 5 ($t = 7.649$, $p < 0.001$; $t = 4.146, p < 0.001$; $t = 4.229, p < 0.001$) in ACD group compared to respective controls (**Figure 5A**), and lower amount of ACD consumed (259 ± 21 mg/kg). When the parameter "punished responses" was analyzed after AM281 administration, the results of a Two-Way ANOVA for repeated measures, including ACD treatment as the between-subjects factor and "Days" as within-subjects factor, showed a significant effect of time, treatment, and their interaction on the number of responses emitted, $F_{(6,\ 180)} = 5.21$, $p < 0.0001$; $F_{(1,\ 180)} = 328.73$, $p < 0.0001$; $F_{(6,\ 180)} = 4.19, p < 0.0001$. Bonferroni *post-hoc* analysis showed that AM281 was able to induce a decrease in the number of punished lever presses along the conflict period ($t = 12.01$, $p < 0.001$; $t = 7.791, p < 0.001$; $t = 6.779, p < 0.001$; $t = 6.203, p < 0.001$; $t = 7.345, p < 0.001$; $t = 9.057, p < 0.001$; $t = 9.280, p < 0.001$) (**Figure 5B**) compared to their respective controls, reaching an average amount of ACD ingested of 12 ± 1 mg/kg. No significant difference in the number of unpunished and punished responses was recorded when AM281 was administered to control rats.

DISCUSSION

The aim of the current study was to evaluate whether ACD could induce and maintain a self-administration drinking behavior in an operant-conditioning paradigm which consisted of training-, extinction-, reinstatement and conflict phases (Cannizzaro et al., 2011; Cacace et al., 2012), in order to demonstrate ACD reinforcing and motivational properties. Afterwards we pointed at exploring the effect of AM281, a CB1 antagonist, on drug-seeking, drug-taking and drug-induced compulsive-like behavior.

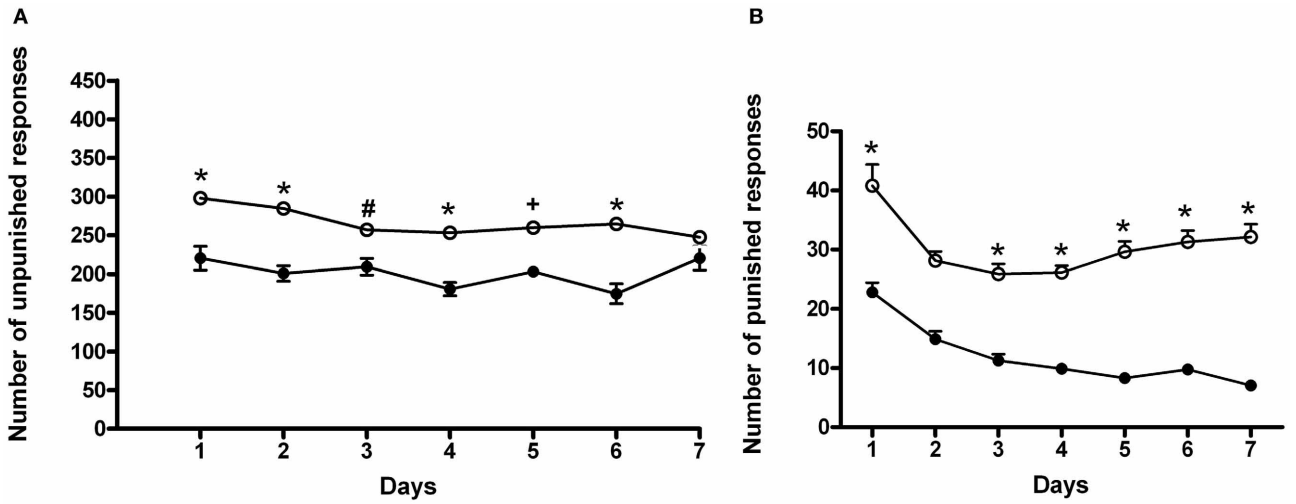


FIGURE 4 | Differences in the number of unpunished (A) and punished (B) responses during the conflict periods in ACD-and in water-drinking rats. Each value represents the means ± S.D. of 16 rats. $^{\#}p < 0.05$; $^{+}p < 0.01$; $^{*}p < 0.001$ vs. control groups. (●) CTR, (○) ACD.

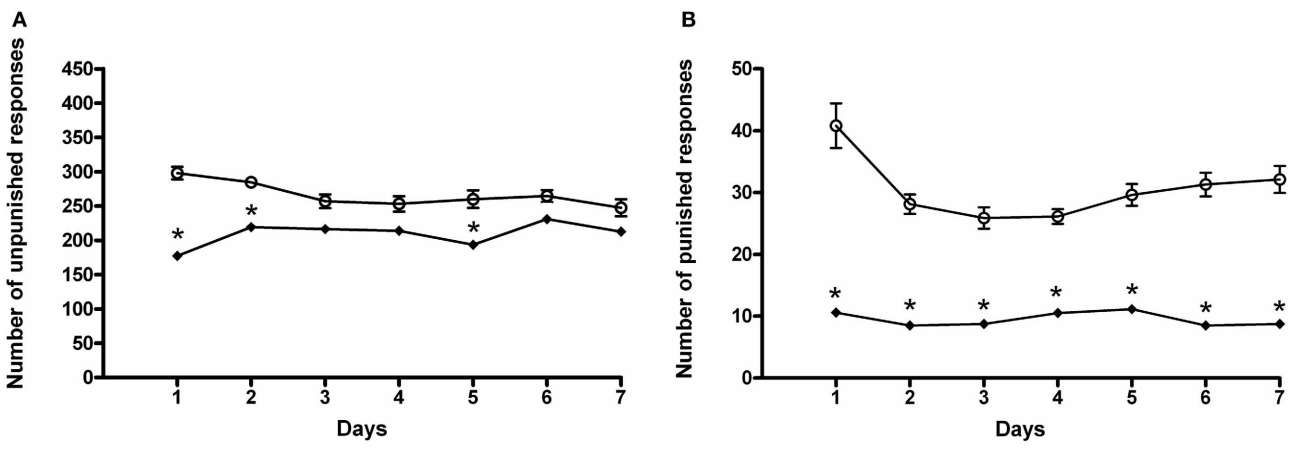


FIGURE 5 | Effects of the administration of AM281 on the number of unpunished (A) and punished (B) responses during the conflict periods in ACD treated rats. Each value represents the means ± S.D. of sixteen rats. $^{*}p < 0.001$ vs. control groups. (○) ACD, (◆) (ACD-AM281).

There is increasing interest in developing animal models that more closely mimic addiction diagnostic criteria (DSM-IV-TR, American Psychiatric Association, 2000) than classical reinstatement models (Vanderschuren and Ahmed, 2012). Based on this awareness, the experimental protocol implemented in the present study aimed at evaluating the co-occurrence of various aspects of the addictive phenotype, such as the increase in drug use over time; difficulty in restricting drug intake or consuming more than intended; perseveration in drug abuse despite its negative consequences. Our data show that ACD exerted motivational and reinforcing activity, since it was able to induce and maintain an operant drinking behavior; it induced drug-seeking during extinction, and a relapse behavior after 1-week forced abstinence; remarkably, ACD rats displayed a higher emission of punished responses with respect to controls, in a modified Geller–Seifter conflict procedure, which may efficaciously model compulsive drug taking despite negative consequences.

In detail, our results confirm data from a previous study (Cacace et al., 2012), showing that rats readily acquire ACD operant self-administration, according to a fixed ratio of reinforcement, during the training sessions. The number of lever presses for ACD increased over the 3 weeks of training, significantly overcoming control group's lever presses for water from the second week. Moreover when animals were allowed to freely access to water, no significant differences in water intake were observed between the two groups, a finding that accounts for ACD specific motivational effects.

Given that escalation of drug intake is a well-known phenomenon in oral ethanol self-administration studies (Wise, 1973), our data clearly show that ACD shares this feature with ethanol and suggest a direct role in the progressive loss of control over drinking behavior. Difficulty in abstaining from drug use can be studied in animals by assessing drug seeking when the drug is no longer available that is to say extinction paradigm (Ahmed, 2012). Our results indicate that ACD induced a drug-seeking behavior, since ACD group emitted a significantly higher number of lever presses in the extinction experiment with respect to controls. ACD-self administering rats persisted in responding in an attempt to earn the rewarding substance, due to the motivational property of ACD; in fact water self-administering rats showed an earlier extinction of responding, due to the lower value of the reward. This finding allows us to speculate that in ACD group the formation of specific ACD-related associations becomes overly salient, thus enhancing craving for ACD. In the operant conditioning paradigm, reinstatement refers to the rapid resumption of drug-reinforced operant response in animals previously extinguished from drug self-administration training (Marchant et al., 2013). This experimental model mirrors the relapse behavior observed in humans, the most troublesome facet of addiction. ACD oral self-administration sustained a reinstatement in animals. Indeed, ACD-rats, previously exposed to extinction and 1-week forced abstinence, displayed a higher number of lever presses with respect to controls, during the relapse phase. Furthermore, in the first relapse day, lever presses for ACD were higher than in the last training day, suggesting that ACD maintained acute reinforcing strength and motivation even after extinction and deprivation (Martin-Fardon and Weiss, 2013). It is well-known that repeated drug use leads enhances the salience attributed to drugs and drug-associated contexts (Robinson and Berridge, 2008), increasing their consumption following periods of abstinence (Hölter et al., 1998; Rodd et al., 2003). Accordingly, ACD-drinking rats showed a ready resumption of the operant drinking behavior, and displayed a significant effect of deprivation, suggesting that ACD might be also involved in alcohol deprivation effect during relapse (Spanagel and Hölter, 1999).

Drug self-administration in the presence of response-contingent shock punishment highlights the motivational properties of substances and it reliably models compulsive drug use despite adverse consequences (Deroche-Gamonet et al., 2004). Moreover, increasing attention is paid to addiction models that focus on punishment-resistance, as a core feature in capturing the addictive phenotype (Vanderschuren and Ahmed, 2012). Given these premises, we developed the operant-conflict procedure in order to assess whether ACD consumption itself was resistant to aversive consequences associated to drug intake. In the operant-conflict procedure, responses were alternatively paired to a footshock, signaled by a light-cue. In the conflict paradigm rats suffer from the "conflict" between the drive to drink and the fear of the shock: this usually leads to a suppression of conditioned responses for reinforcement. The aversive stimulus has a general dissuasive effect in the operant behavior for water, as it strongly decreases the number of lever presses in CTR group. Despite its highly negative value, the contingent punishment less effectively inhibited operant responding for ACD, and a higher number of punished responses was observed, with respect to controls. It seemed that ACD shares ethanol anti-conflict properties (Baldwin et al., 1991), but since ACD-rats showed an increase both in punished and unpunished responses, it is reasonable to interpret our data recalling ACD strong motivational properties, rather than an anti-conflict effect. Indeed, ACD appears to be a 1000-fold more potent reinforcement than ethanol in the posterior VTA (Rodd et al., 2005). Besides, ACD involvement in recruiting the neuroendocrine stress system (Cannizzaro et al., 2010; Escrig et al., 2012) may be crucial in the development of negative emotional states, thus leading to the progressive loss of control in drinking behavior and compulsive alcohol intake (Koob, 2013). A major finding of the present study was the pharmacological probing of ACD reinforcing and motivational properties, addressing the AM281 effect in the distinct addiction-related behaviors explored, namely drug-seeking, relapse and punishment resistance. Recent preclinical and clinical data indicate that CB1 receptor antagonists, such as SR141716A (SR, Rimonabant), can reduce self-administration and craving for several commonly addictive drugs (Colombo et al., 1998; De Vries et al., 2001; Navarro et al., 2001; Cohen et al., 2005; Rigotti et al., 2009). CB1 function is required for ethanol-mediated activation of VTA DA neurons (Cheer et al., 2007), supporting the hypothesis that ethanol rewarding properties are due in part to ECs release, which likely exerts reduction of GABA inhibition onto VTA dopamine neurons (Lupica and Riegel, 2005; Barrot et al., 2012). This effect is quite specific, since the neuroanatomical loci of the SR-mediated reduction in ethanol self-administration involve brain regions typically associated with addiction; indeed SR microinjections into VTA, medial prefrontal cortex and NAcc reduce ethanol self-administration,

whereas injections into the dorsal striatum do not affect the number of responses for ethanol (Caillé et al., 2007; Hansson et al., 2007; Malinen and Hyytiä, 2008). AM281 is structurally related to SR, but displays higher affinity and specificity for CB1 receptor (Gatley et al., 1998), since it does not interact with GPR55 and opioid receptors (Seely et al., 2012). Our results show that AM281 administration was able to decrease ACD-seeking, ACD-relapse after forced abstinence, and ACD-induced resistance to punishment in highly predictive experimental procedures. Indeed, animals receiving the CB1 antagonist emitted a lower number of responses for ACD with respect to vehicle group during extinction. This evidence suggests that ACD positive incentive properties underlie perseveration in lever pressing when reinforce delivery is suspended. No significant effect was recorded in water-administering rats, ruling out an aspecific action on operant responding. AM281 administration induced a significant reduction in lever-pressing during the relapse session, when compared to their respective controls. This finding further suggests a role for CB1 receptor in ACD-induced as well as it has been reported in alcohol-related addictive behavior (Serrano and Parsons, 2011). Ultimately, AM281 administration in the conflict paradigm decreased the number of punished lever presses for ACD, and a similar though less evident effect was observed in unpunished ones. At this regard, an aspecific effect of the CB1 antagonist on motor activity seems unlikely, since AM281 administration was able to affect unpunished responses for ACD discontinuously, while the reduction of punished responses occurred along the whole conflict period. This effect may be related to a decrease in the incentive for lever-pressing due to the pharmacological treatment. The clear influence of CB1 receptor activity on ACD-induced punishment resistance further highlights the involvement of the reward-processing machinery as the intrinsic mechanisms underlying ACD-related behavioral features. As a matter of fact, these data provide evidence of ACD incentive properties, whose contribution must be taken into account in studying and treating ethanol-related behaviors. The neural substrates underpinning rewarding properties of orally self-administer ACD involve CB1 receptors, which are able to indirectly modulate DA mesocorticolimbic pathway. Drugs able to manipulate EC system might represent a useful therapeutic strategy affecting both ethanol and its neuroactive metabolite actions on crucial addiction-related behaviors, such as drug seeking, relapse and drug abuse despite negative consequences. This study aims at representing a step forward in elucidating the complex framework of actors playing a role in maintaining ethanol addiction; nevertheless further efforts are needed to fully characterize the actual contribution of ACD to ethanol's effects.

REFERENCES

Ahmed, S. H. (2012). The science of making drug-addicted animals. *Neuroscience* 211, 107–125. doi: 10.1016/j.neuroscience.2011.08.014

American Psychiatric Association. (2000). *Diagnostic and Statistical Manual of Mental Disorders, IV-TR Edn.* Washington, DC: American Psychiatric Association.

Amit, Z., Brown, Z. W., and Rockman, G. E. (1977). Possible involvement of acetaldehyde, norepinephrine and their tetrahydroisoquinoline derivatives in the regulation of ethanol self-administration. *Drug Alcohol Depend.* 2, 495–500. doi: 10.1016/0376-8716(77)90049-7

Arizzi-LaFrance, M. N., Correa, M., Aragon, C. M. G., and Salamone, J. D. (2006). Motor stimulant effects of ethanol injected into the substantia nigra pars reticulata: importance of catalase-mediated metabolism and the role of acetaldehyde. *Neuropsychopharmacology* 31, 997–1008. doi: 10.1038/sj.npp.1300849

Arnone, M., Maruani, J., Chaperon, F., Thiébot, M. H., Poncelet, M., Soubrié, P., et al. (1997). Selective inhibition of sucrose and ethanol intake by SR 141716, an antagonist of central cannabinoid (CB1) receptors. *Psychopharmacology* 132, 104–106.

Baldwin, H. A., Wall, T. L., Schuckit, M. A., and Koob, G. F. (1991). Differential effects of ethanol on punished responding in the P and NP rats. *Alcohol. Clin. Exp. Res.* 15, 700–704. doi: 10.1111/j.1530-0277.1991.tb00582.x

Barrot, M., Sesack, S. R., Georges, F., Pistis, M., Hong, S., and Jhou, T. C. (2012). Braking dopamine systems: a new GABA master structure for mesolimbic and nigrostriatal functions. *J. Neurosci.* 32, 14094–14101. doi: 10.1523/JNEUROSCI.3370-12.2012

Basavarajappa, B. S., Cooper, T. B., and Hungund, B. L. (1998). Chronic ethanol administration down-regulates cannabinoid receptors in mouse brain synaptic plasma membrane. *Brain Res.* 793, 212–218. doi: 10.1016/S0006-8993(98)00175-9

Basavarajappa, B. S., Saito, M., Cooper, T. B., and Hungund, B. L. (2000). Stimulation of cannabinoid receptor agonist 2-arachidonylglycerol by chronic ethanol and its modulation by specific neuromodulators in cerebellar granule neurons. *Biochim. Biophys. Acta* 1535, 78–86. doi: 10.1016/S0925-4439(00)00085-5

Basavarajappa, B. S., Saito, M., Cooper, T. B., and Hungund, B. L. (2003). Chronic ethanol inhibits the anandamide transport and increases extracellular anandamide levels in cerebellar granule neurons. *Eur. J. Pharmacol.* 466, 73–83. doi: 10.1016/S0014-2999(03)01557-7

Brown, Z. W., Amit, Z., and Rockman, G. E. (1979). Intraventricular self-administration of acetaldehyde, but not ethanol, in naive laboratory rats. *Psychopharmacology (Berl.)* 64, 271–276. doi: 10.1007/BF00427509

Cacace, S., Plescia, F., Barberi, I., and Cannizzaro, C. (2012). Acetaldehyde oral self-administration: evidence from the operant-conflict paradigm. *Alcohol. Clin. Exp. Res.* 36, 1278–1287. doi: 10.1111/j.1530-0277.2011.01725.x

Caillé, S., Alvarez-Jaimes, L., Polis, I., Stouffer, D. G., and Parsons, L. H. (2007). Specific alterations of extracellular endocannabinoid levels in the nucleus accumbens by ethanol, heroin, and cocaine self-administration. *J. Neurosci.* 27, 3695–3702. doi: 10.1523/JNEUROSCI.4403-06.2007

Cannizzaro, C., La Barbera, M., Plescia, F., Cacace, S., and Tringali, G. (2010). Ethanol modulates corticotropin releasing hormone release from the rat hypothalamus: does acetaldehyde play a role? *Alcohol. Clin. Exp. Res.* 34, 588–593. doi: 10.1111/j.1530-0277.2009.01127.x

Cannizzaro, C., Plescia, F., and Cacace, S. (2011). Role of acetaldehyde in alcohol addiction: current evidence and future perspectives. *Malta Med. J.* 23, 27.

Cheer, J. F., Wassum, K. M., Sombers, L. A, Heien, M. L., Ariansen, J. L., Aragona, B. J., et al. (2007). Phasic dopamine release evoked by abused substances requires cannabinoid receptor activation. *J. Neurosci.* 27, 791–795. doi: 10.1523/JNEUROSCI.4152-06.2007

Cippitelli, A., Bilbao, A., Hansson, A. C., Del Arco, I., Sommer, W., Heilig, M., et al. (2005). Cannabinoid CB1 receptor antagonism reduces conditioned reinstatement of ethanol-seeking behavior in rats. *Eur. J. Neurosci.* 21, 2243–2251. doi: 10.1111/j.1460-9568.2005.04056.x

Cohen, C., Kodas, E., and Griebel, G. (2005). CB1 receptor antagonists for the treatment of nicotine addiction. *Pharmacol. Biochem. Behav.* 81, 387–395. doi: 10.1016/j.pbb.2005.01.024

Colombo, G., Agabio, R., Fà, M., Guano, L., Lobina, C., Loche, A., et al. (1998). Reduction of voluntary ethanol intake in ethanol-preferring sP rats by the cannabinoid antagonist SR-141716. *Alcohol Alcohol.* 33, 126–130.

Correa, M., Salamone, J. D., Segovia, K. N., Pardo, M., Longoni, R., Spina, L., et al. (2012). Piecing together the puzzle of acetaldehyde as a neuroactive agent. *Neurosci. Biobehav. Rev.* 36, 404–430. doi: 10.1016/j.neubiorev.2011.07.009

De Bruin, N. M. W. J., Lange, J. H., Kruse, C. G., Herremans, A. H., Schoffelmeer, A. N., Van Drimmelen, M., et al. (2011). SLV330, a cannabinoid CB(1)

receptor antagonist, attenuates ethanol and nicotine seeking and improves inhibitory response control in rats. *Behav. Brain Res.* 217, 408–415. doi: 10.1016/j.bbr.2010.11.013

Deehan, G. A., Brodie, M. S., and Rodd, Z. A. (2013a). What is in that drink: the biological actions of ethanol, acetaldehyde, and salsolinol. *Curr. Top. Behav. Neurosci.* 13, 163–184. doi: 10.1007/7854_2011_198

Deehan, G. A. Jr., Engleman, E. A., Ding, Z. M., McBride W. J., and Rodd. Z. A. (2013b). Microinjections of acetaldehyde or salsolinol into the posterior ventral tegmental area increase dopamine release in the nucleus accumbens shell. *Alcohol. Clin. Exp. Res.* 37, 722–729. doi: 10.1111/acer.12034.

Deroche-Gamonet, V., Belin, D., and Piazza, P. V. (2004). Evidence for addiction-like behavior in the rat. *Science* 305, 1014–1017.

De Vries, T. J., Shaham, Y., Homberg, J. R., Crombag, H., Schuurman, K., Dieben, J., et al. (2001). A cannabinoid mechanism in relapse to cocaine seeking. *Nat. Med.* 7, 1151–1154. doi: 10.1038/nm1001-1151

Economidou, D., Mattioli, L., Cifani, C., Perfumi, M., Massi, M., Cuomo, V., et al. (2006). Effect of the cannabinoid CB1 receptor antagonist SR-141716A on ethanol self-administration and ethanol-seeking behaviour in rats. *Psychopharmacology* 183, 394–403. doi: 10.1007/s00213-005-0199-9

Escrig, M. A., Pardo, M., Aragon, C. M., and Correa, M. (2012). Anxiogenic and stress-inducing effects of peripherally administered acetaldehyde in mice: similarities with the disulfiram-ethanol reaction. *Pharmacol. Biochem. Behav.* 100, 404–412. doi: 10.1016/j.pbb.2011.10.002

Femenía, T., García-Gutiérrez, M. S., and Manzanares, J. (2010). CB1 receptor blockade decreases ethanol intake and associated neurochemical changes in fawn-hooded rats. *Alcohol. Clin. Exp. Res.* 34, 131–141. doi: 10.1111/j.1530-0277.2009.01074.x

Gallate, J. E., and McGregor, I. S. (1999). The motivation for beer in rats: effects of ritanserin, naloxone and SR 141716. *Psychopharmacology* 142, 302–308. doi: 10.1007/s002130050893

Gatley, S. J., Lan, R., Volkow, N. D., Pappas, N., King, P., Wong, C. T., et al. (1998). Imaging the brain marijuana receptor: development of a radioligand that binds to cannabinoid CB1 receptors *in vivo*. *J. Neurochem.* 70, 417–423. doi: 10.1046/j.1471-4159.1998.70010417.x

Getachew, B., Hauser, S. R., Dhaher, R., Katner, S. N., Bell, R. L., Oster, S. M., et al. (2011). CB1 receptors regulate alcohol-seeking behavior and alcohol self-administration of alcohol-preferring (P) rats. *Pharmacol. Biochem. Behav.* 97, 669–675. doi: 10.1016/j.pbb.2010.11.006

Hansson, A. C., Bermúdez-Silva, F. J., Malinen, H., Hyytiä, P., Sanchez-Vera, I., Rimondini, R., et al. (2007). Genetic impairment of frontocortical endocannabinoid degradation and high alcohol preference. *Neuropsychopharmacology* 32, 117–126. doi: 10.1038/sj.npp.1301034

Hölter, S. M., Engelmann, M., Kirschke, C., Liebsch, G., Landgraf, R., and Spanagel, R. (1998). Long-term ethanol self-administration with repeated ethanol deprivation episodes changes ethanol drinking pattern and increases anxiety-related behaviour during ethanol deprivation in rats. *Behav. Pharmacol.* 9, 41–48.

Jamal, M., Ameno, K., Uekita, I., Kumihashi, M., Wang, W., and Ijiri, I. (2007). Catalase mediates acetaldehyde formation in the striatum of free-moving rats. *Neurotoxicology* 28, 1245–1248. doi: 10.1016/j.neuro.2007.05.002

Karahanian, E., Quintanilla, M. E., Tampier, L., Rivera-Meza, M., Bustamante, D., Gonzalez-Lira, V., et al. (2011). Ethanol as a prodrug: brain metabolism of ethanol mediates its reinforcing effects. *Alcohol. Clin. Exp. Res.* 35, 606–612. doi: 10.1111/j.1530-0277.2011.01439.x

Koob, G. F. (2013). Theoretical frameworks and mechanistic aspects of alcohol addiction: alcohol addiction as a reward deficit disorder. *Curr. Top. Behav. Neurosci.* 13, 3–30. doi: 10.1007/7854_2011_129

Lupica, C. R., and Riegel, A. C. (2005). Endocannabinoid release from midbrain dopamine neurons: a potential substrate for cannabinoid receptor antagonist treatment of addiction. *Neuropharmacology* 48, 1105–1116. doi: 10.1016/j.neuropharm.2005.03.016

Malinen, H., and Hyytiä, P. (2008). Ethanol self-administration is regulated by CB1 receptors in the nucleus accumbens and ventral tegmental area in alcohol-preferring AA rats. *Alcohol. Clin. Exp. Res.* 32, 1976–1983. doi: 10.1111/j.1530-0277.2008.00786.x

Marchant, N. J., Li, X., and Shaham, Y. (2013). Recent developments in animal models of drug relapse. *Curr. Opin. Neurobiol.* doi: 10.1016/j.conb.2013.01.003

Martin-Fardon, R., and Weiss, F. (2013). Modeling relapse in animals. *Curr. Top. Behav. Neurosci.* 13, 403–432. doi: 10.1007/7854_2012_202

Melis, M., Enrico, P., Peana, A. T., and Diana, M. (2007). Acetaldehyde mediates alcohol activation of the mesolimbic dopamine system. *Eur. J. Neurosci.* 26, 2824–2833. doi: 10.1111/j.1460-9568.2007.05887.x

Melis, M., Muntoni, A. L., and Pistis, M. (2012). Endocannabinoids and the processing of value-related signals. *Front. Pharmacol.* 3:7. doi: 10.3389/fphar.2012.00007

Myers, W. D., Ng, K. T., and Singer, G. (1982). Intravenous self-administration of acetaldehyde in the rat as a function of schedule, food deprivation and photoperiod. *Pharmacol. Biochem. Behav.* 17, 807–811. doi: 10.1016/0091-3057(82)90364-1

Navarro, M., Carrera, M. R., Fratta, W., Valverde, O., Cossu, G., Fattore, L., et al. (2001). Functional interaction between opioid and cannabinoid receptors in drug self-administration. *J. Neurosci.* 21, 5344–5350.

Peana, A. T., Enrico, P., Assaretti, A. R., Pulighe, E., Muggironi, G., Nieddu, M., et al. (2008). Key role of ethanol-derived acetaldehyde in the motivational properties induced by intragastric ethanol: a conditioned place preference study in the rat. *Alcohol. Clin. Exp. Res.* 32, 249–258. doi: 10.1111/j.1530-0277.2007.00574.x

Peana, A. T., Muggironi, G., and Diana, M. (2010). Acetaldehyde-reinforcing effects: a study on oral self-administration behavior. *Front. Psychiatry* 1:23. doi: 10.3389/fpsyt.2010.00023

Quertemont, E., Tambour, S., and Tirelli, E. (2005). The role of acetaldehyde in the neurobehavioral effects of ethanol: a comprehensive review of animal studies. *Prog. Neurobiol.* 75, 247–274. doi: 10.1016/j.pneurobio.2005.03.003

Rigotti, N. A., Gonzales, D., Dale, L. C., Lawrence, D., and Chang, Y. (2009). A randomized controlled trial of adding the nicotine patch to rimonabant for smoking cessation: efficacy, safety and weight gain. *Addiction* 104, 266–276. doi: 10.1111/j.1360-0443.2008.02454.x

Robinson, T. E., and Berridge, K. C. (2008). Review. The incentive sensitization theory of addiction: some current issues. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 363, 3137–3146. doi: 10.1098/rstb.2008.0093

Rodd, Z. A., Bell, R. L., Kuc, K. A., Murphy, J. M., Lumeng, L., Li, T. K., et al. (2003). Effects of repeated alcohol deprivations on operant ethanol self-administration by alcohol-preferring (P) rats. *Neuropsychopharmacology* 28, 1614–1621. doi: 10.1038/sj.npp.1300214

Rodd, Z. A., Bell, R. L., Zhang, Y., Murphy, J. M., Goldstein, A., Zaffaroni, A., et al. (2005). Regional heterogeneity for the intracranial self-administration of ethanol and acetaldehyde within the ventral tegmental area of alcohol-preferring (P) rats: involvement of dopamine and serotonin. *Neuropsychopharmacology* 30, 330–338. doi: 10.1038/sj.npp.1300561

Seely, K. A., Brents, L. K., Franks, L. N., Rajasekaran, M., Zimmerman, S. M., Fantegrossi, W. E., et al. (2012). AM-251 and rimonabant act as direct antagonists at mu-opioid receptors: implications for opioid/cannabinoid interaction studies. *Neuropharmacology* 63, 905–915. doi: 10.1016/j.neuropharm.2012.06.046

Serrano, A., and Parsons, L. H. (2011). Endocannabinoid influence in drug reinforcement, dependence and addiction-related behaviors. *Pharmacol. Ther.* 132, 215–241. doi: 10.1016/j.pharmthera.2011.06.005

Smith, B. R., Amit, Z., and Splawinsky, J. (1984). Conditioned place preference induced by intraventricular infusions of acetaldehyde. *Alcohol* 1, 193–195. doi: 10.1016/0741-8329(84)90097-1

Spanagel, R., and Hölter, S. M. (1999). Long-term alcohol self-administration with repeated alcohol deprivation phases: an animal model of alcoholism? *Alcohol Alcohol.* 34, 231–243. doi: 10.1093/alcalc/34.2.231

Vanderschuren, L. J. M. J., and Ahmed, S. H. (2012). Animal studies of addictive behavior. *Cold Spring Harb. Perspect. Med.* doi: 10.1101/cshperspect.a011932

Vinod, K. Y., Yalamanchili, R., Xie, S., Cooper, T. B., and Hungund, B. L. (2006). Effect of chronic ethanol exposure and its withdrawal on the endocannabinoid

system. *Neurochem. Int.* 49, 619–625. doi: 10.1016/j.neuint.2006.05.002

Wise, R. A. (1973). Voluntary ethanol intake in rats following exposure to ethanol on various schedules. *Psychopharmacologia* 29, 203–210. doi: 10.1007/BF00414034

Zakhari, S. (2006). Overview: how is alcohol metabolized by the body? *Alcohol Res. Health* 29, 245–254.

Zimatkin, S. M., Liopo, A. V., and Deitrich, R. A. (1998). Distribution and kinetics of ethanol metabolism in rat brain. *Alcohol. Clin. Exp. Res.* 22, 1623–1627. doi: 10.1111/j.1530-0277.1998.tb03958.x

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 20 March 2013; accepted: 22 May 2013; published online: 11 June 2013.

Citation: Plescia F, Brancato A, Marino RAM and Cannizzaro C (2013) Acetaldehyde as a drug of abuse: insight into AM281 administration on operant-conflict paradigm in rats. Front. Behav. Neurosci. 7:64. doi: 10.3389/fnbeh.2013.00064

frontiers in
BEHAVIORAL NEUROSCIENCE

ORIGINAL RESEARCH ARTICLE
published: 10 July 2013
doi: 10.3389/fnbeh.2013.00081

Acetate as an active metabolite of ethanol: studies of locomotion, loss of righting reflex, and anxiety in rodents

Marta Pardo[1], Adrienne J. Betz[2], Noemí San Miguel[1], Laura López-Cruz[1], John D. Salamone[3] and Mercè Correa[1,3]*

[1] Àrea de Psicobiologia, Campus Riu Sec, Universitat Jaume I, Castelló, Spain
[2] Department of Psychology, Quinnipiac University, Hamden, CT, USA
[3] Department of Psychology, University of Connecticut, Storrs, CT, USA

Edited by:
Elio Acquas, University of Cagliari, Italy

Reviewed by:
Fabrício A. Pamplona, Universidade Federal de Santa Catarina, Brazil
Elio Acquas, University of Cagliari, Italy
Sergey M. Zimatkin, Grodno State Medical University, Belarus

***Correspondence:**
Mercè Correa, Àrea de Psicobiologia, Campus Riu Sec, Universitat Jaume I, Avda. Sos Baynat s/n, 12071 Castelló, Spain
e-mail: correa@psb.uji.es

It has been postulated that a number of the central effects of ethanol are mediated via ethanol metabolites: acetaldehyde and acetate. Ethanol is known to produce a large variety of behavioral actions such anxiolysis, narcosis, and modulation of locomotion. Acetaldehyde contributes to some of those effects although the contribution of acetate is less known. In the present studies, rats and mice were used to assess the acute and chronic effects of acetate after central or peripheral administration. Male Sprague-Dawley rats were used for the comparison between central (intraventricular, ICV) and peripheral (intraperitoneal, IP) administration of acute doses of acetate on locomotion. CD1 male mice were used to study acute IP effects of acetate on locomotion, and also the effects of chronic oral consumption of acetate (0, 500, or 1000 mg/l, during 7, 15, 30, or 60 days) on ethanol- (1.0, 2.0, 4.0, or 4.5 g/kg, IP) induced locomotion, anxiolysis, and loss of righting reflex (LORR). In rats, ICV acetate (0.7–2.8 μmoles) reduced spontaneous locomotion at doses that, in the case of ethanol and acetaldehyde, had previously been shown to stimulate locomotion. Peripheral acute administration of acetate also suppressed locomotion in rats (25–100 mg/kg), but not in mice. In addition, although chronic administration of acetate during 15 days did not have an effect on spontaneous locomotion in an open field, it blocked ethanol-induced locomotion. However, ethanol-induced anxiolysis was not affected by chronic administration of acetate. Chronic consumption of acetate (up to 60 days) did not have an effect on latency to, or duration of LORR induced by ethanol, but significantly increased the number of mice that did not achieve LORR. The present work provides new evidence supporting the hypothesis that acetate should be considered a centrally-active metabolite of ethanol that contributes to some behavioral effects of this alcohol, such as motor suppression.

Keywords: ataxia, anxiety, alcohol metabolism, acetaldehyde, acetate, narcosis

INTRODUCTION

Acetate is a short-chain fatty acid formed as the final step in ethanol oxidation. The oxidative metabolism of ethanol into acetaldehyde takes place in several organs, and can involve multiple enzymes, including alcohol dehydrogenase (ADH), cytochrome P450 2E1 (CYP2E1), and catalase. Acetaldehyde is then metabolized mainly by aldehyde dehydrogenase (ALDH) into acetate. Following ethanol ingestion, approximately 70% of the acetate generated through oxidative metabolism is released from the liver into systemic circulation (Busch, 1953; Van den Berg et al., 1966). Acetate can be detected in plasma after ethanol administration, because the portion that has not been metabolized hepatically is released into the blood. Acetate is then redistributed throughout the body, metabolized in extra-hepatic organs (Lundquist et al., 1962), rapidly taken up into the brain by a carrier-mediated process (Oldendorf, 1973), and also is actively metabolized in the brain (Cullen and Carlen, 1992). An alternative central source of acetate is brain ethanol metabolism. It has been demonstrated (Zimatkin et al., 2006) that pharmacological manipulations that reduce catalase activity also reduce the amount of acetate detected in rat and mice brain homogenates. Moreover, when brain homogenates from CYP2E1 KO mice where incubated with ethanol plus a catalase inhibitor, there was a significant reduction of acetate formation, an effect which was not observed in brain homogenates from catalase-deficient mice (Zimatkin et al., 2006). Pharmacological inhibition of CYP2E1 also leads to significant decreases in acetate accumulation in rat brain homogenates. Moreover, enzymatic inhibition of ADH and ALDH also reduced acetate levels (Zimatkin et al., 2006). These results demonstrate that acetate can be formed in the brain via ethanol metabolism and that the enzymatic systems involved in this process are some of the ones required to form acetaldehyde.

Acetate has been demonstrated to have specific effects on behavior. Peripherally administered acetate increased the time off a treadmill, a measure of motor incoordination in rats, and

suppressed locomotion in mice (Carmichael et al., 1991; Israel et al., 1994). In fact, peripherally injected acetate has been demonstrated to be three times more potent than ethanol at suppressing locomotion in mice (Israel et al., 1994). Moreover, acetate injected peripherally or in the brain ventricles also suppressed food-reinforced lever pressing on a FR5 schedule of reinforcement, which generates high levels of performance (Arizzi et al., 2003; McLaughlin et al., 2008). Thus, it has been suggested that acetate is involved mainly in the depressant effects of ethanol (Carmichael et al., 1991; Israel et al., 1994; Arizzi et al., 2003; Correa et al., 2003). Consistent with this idea, acetate can mimic some of the motor suppressant, ataxic, or sedative effects of ethanol. For instance, general anesthesia is potentiated in a dose-dependent fashion by ethanol as well as acetate (Carmichael et al., 1991; Campisi et al., 1997). Acetate seems to mediate tolerance to the loss of the righting reflex (LORR) produced by ethanol. Repeated administration of ethanol [3.5 g/kg, intraperitoneal (IP) during 7 days] to outbred rats, resulted in tolerance to LORR induced by ethanol and to higher concentrations of acetate in different areas of the brain compared to acutely treated animals (Kiselevski et al., 2003). Moreover, higher amounts of acetate are formed in short sleeping (SS) rats, which have an inborn tolerance to the LORR induced by high doses of ethanol, relative to the long sleeping (LS) substrain (Zimatkin et al., 2011).

Because direct administration of ethanol and acetaldehyde seem to have different motor effects depending on the route of administration (for a review see Correa et al., 2012), the present experiments addressed the potential differences between peripheral and central injections of acetate on locomotor activity in rats. We also evaluated the impact of peripherally administered acetate on motor activity in mice at similar low doses. In a second group of experiments, because acetate accumulation after repeated administration of ethanol seems to mediate tolerance to LORR induced by ethanol (Kiselevski et al., 2003), we evaluated the impact of chronic consumption of acetate across multiple time periods on different behaviors modulated by an acute dose of ethanol in mice. Thus, we evaluated the impact of chronic exposure to acetate on ethanol-induced stimulation of locomotion and on ethanol-induced LORR in mice. Moreover, although acutely administered acetate has not shown to have an effect on anxiety measures in mice (Escrig et al., 2007, 2012) and rats (Correa et al., 2003), the anxiolytic actions of ethanol at low doses are well known (Correa et al., 2008). Thus, in the present study we also evaluated the impact of chronic administration of acetate on measures of anxiolysis induced by a low dose of ethanol in mice.

METHODS

SUBJECTS

Male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN), were housed in a colony maintained at 23°C with lights on from 7:00 to 19:00 h. Animals weighed between 350 and 430 g at the time of the experiment. These animals had *ad libitum* access to food and water in their home cages. Before the test day, rats were allowed 2 weeks to acclimate to laboratory conditions, plus 1 week of being handled by the experimenter for 5 min each day. For the IP study, a total of 43 rats ($n = 8$–9 per group) were used and for the intraventricular (ICV) study the number was 38 ($n = 8$–10 per group).

CD1 male mice (30–40 g) were purchased from Harlan-Interfauna Iberica S.A. (Barcelona, Spain). Mice 6–7 weeks old at the beginning of experiments were housed in groups of three per cage, with standard laboratory rodent chow and tap water available *ad libitum*. They were maintained in the colony at 22 ± 1°C with lights on from 8:00 to 20:00 h. Mice were handled and habituated to the test room for 1 week before tests were conducted. For the acute acetate study, 42 mice were used ($n = 10$–11 per group). For the chronic acetate studies, the locomotion experiment included 78 mice ($n = 8$–9 per group), the anxiety experiment included a total of 63 mice ($n = 10$–11 per group), and for the LORR experiments the total number was 218 ($n = 14$ per group).

All experimental procedures were approved by the Institutional Animal Care and Use Committee, and complied with the European Community Council directive (86/609/ECC) for the use of laboratory animal subjects and with the "Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research" (National Research Council, 2003).

DRUGS AND SELECTION OF DOSES

Anhydrous sodium acetate (hereafter referred to as acetate, Fisher Scientific) was dissolved in physiological saline for the IP studies, in artificial cerebrospinal fluid (aCSF) for the ICV studies, and in tap water for the oral chronic studies. These vehicles serve as the control solutions. For IP injections, acetate 10% w/v was used as the stock solution from which the different doses were obtained. ICV acetate doses of 0.7, 1.4, or 2.8 μmoles (0.0, 42.03, 84.07, or 168.14 μg), were administered in 1.0 μl total volume. Chronically administered acetate was prepared dissolving sodium acetate in tap water. Concentration of the solutions were 500 or 1000 mg/l. After recording fluid intake and body weight per animal for 60 days, we calculated that the average dose of acetate consumed for the group exposed to 500 mg/l was 29.9 ± 5.3 mg/kg and for the 1000 mg/l group was 67.6 ± 1.8 mg/kg. Ethanol (96% v/v, Panreac Quimica S. A.) was dissolved in physiological saline in a 20% v/v solution used as the stock solution from which the different doses were obtained. Hydrochloric acid (1N, Panreac Quimica S. A.) was used to bring the sodium acetate solutions for the acute studies to pH 7.4. Xylazine and Ketamine were purchased from Phoenix Pharmaceutical, Inc. (St. Joseph, Mo).

The selection of doses and times was based on pilot studies and on previous studies from our laboratory (Arizzi et al., 2003; Arizzi-LaFrance et al., 2006; Correa et al., 2003; Escrig et al., 2012).

SURGICAL PROCEDURE AND ICV INJECTIONS

For the ICV study, rats were implanted with unilateral guide cannulae (10.0 mm length, 23 ga.). Rats were anesthetized with a solution (1.0 ml/kg, IP) that contained Ketamine (100 mg/ml) and Xylazine (20 mg/ml). The stereotaxic coordinates for the cannulation into the lateral ventricle were as follows: AP −0.5 mm (from bregma), DL +1.3 mm lateral (from midline), and DV −3.0 mm ventral (from the surface of the skull). The incisor bar on the stereotax was set to 0.0 mm above the interaural line. All

animals were single housed following surgery, and were allowed to recover for 7–10 days before behavioral testing. Stainless steel stylets were kept in each guide cannulae to maintain its integrity.

ICV injections were made via 30 ga. stainless steel injection cannulae extending 1.5 mm below the guide cannulae. The injectors were attached to 10.0 μl Hamilton syringes by PE-10 tubing, and were driven by a syringe pump (Harvard Apparatus) at a rate of 0.5 μl/min for a total volume of 1.0 μl. Following the infusion period the injectors were left in place for 1 min to allow for diffusion of the drug, after which the injectors were removed, stylets were replaced, and animals were immediately placed into the behavioral chambers for testing.

HISTOLOGY

For the ICV experiments, the placements of the injectors were verified histologically. After the experiments were completed, all animals were intracardially perfused with heparinized physiological saline. Brains were stored refrigerated in 3.7% formaldehyde solution for at least 5 days prior to slicing. Consecutive 50 micron sections through the relevant brain areas were collected, mounted on slides, and stained with cresyl violet solution to aid in detection of the injector tracts. Coverslipped slides were viewed microscopically to assess accuracy of implantation. Any animal with improper placement, or significant damage around the injection site, was not included in the statistical analyses of behavioral data. A total of 5 animals were rejected due to bad placements.

APPARATUS AND BEHAVIORAL PROCEDURE IN RATS

Enclosed stabilimeter

Locomotor testing was performed in an automated activity chamber (28 × 28 × 28 cm), which was inside a sound-proof shell. The floor of the chamber consisted of two moveable wire mesh panels (27 × 13 cm) mounted 6.0 cm above the chamber floor on a center rod attached at either end to the sides of the chamber; this allowed for slight vertical movement of the floor panels. Movement of the panels was detected by microswitches mounted outside the chamber at the ends of the panels. A depression of a given quadrant (quadrant = 1/2 of each panel) would close the circuit on the microswitch attached to the panel. Each microswitch closure was counted as a single activity count, and activity counts were recorded by a computer in 10 min intervals. Rats were habituated to the chamber and to injections prior to the drug test. This was done to decrease activational effects due to novelty on the test day. On the test day, animals were placed into the activity chamber immediately after IP injections, and for the ICV studies they were placed in the chambers after 1 min to allow for diffusion of the drug, as described above. Locomotion was recorded in 10 min periods. In the ICV studies, after drug injections animals were anesthetized and perfused as described above, and histological analyses of brain sections were performed.

APPARATUS AND BEHAVIORAL PROCEDURES IN MICE

Enclosed activity box

The enclosed locomotion chamber was made of polypropylene and consisted of a square white box divided in two compartments (25 cm W × 25 cm H × 22 cm L), covered with a translucent ceiling. The behavioral test room was illuminated with a soft red light, and external noise was attenuated. As in the stabilimeter, this enclosed two-compartment box was used in order to minimize anxiogenic stimulation of locomotion. Mice were habituated to the chamber and to injections prior to the drug test. This was done to decrease activational effects due to novelty on the test day. Acetate IP was injected 10 min before test started. Locomotion was recorded for 10 min. An activity count was registered by a trained observer, unaware of the experimental condition, each time the animal crossed from one quadrant to another with all four legs.

Open Field (OF)

The OF arena consisted of a Plexiglass cylinder with translucent walls (30 cm in diameter and 30 cm high) and an opaque floor divided into four equal quadrants by two intersecting lines. Mice were handled repeatedly and habituated to the test room before the behavioral test, but were not pre-exposed to the OF. On the test day, ethanol (1.0 or 2.0 g/kg) or saline were administered acutely IP and animals were placed immediately in the OF and locomotor observations started 10 min later. The behavioral test room was illuminated with a soft light, and external noise was attenuated. An activity count was registered by a trained observer, unaware of the experimental condition, each time the animal crossed from one quadrant to another with all four legs.

Dark-light box

The apparatus consisted of a polypropylene chamber divided in two compartments by a partition containing a small opening (5 cm H × 5 cm W). The light compartment (25 cm W × 25 cm H × 25 cm L) was open, painted in white, and illuminated, while the dark compartment (25 cm W × 25 cm H × 18 cm L) was painted in black and enclosed by a removable ceiling. This anxiety paradigm measures the avoidance that rodents show to bright open spaces. Several parameters were recorded during 5 min testing sessions. The dependent variables were: latency for the first entry into the bright compartment from the dark one, latency to go back to the dark compartment, total time spent in the bright compartment, and total crosses between compartments. In the acute study, acetate IP was injected 10 min before the dark-light box test.

LORR

Test of latency and duration of LORR were recorded consecutively. Ethanol (4.0 or 4.5 g/kg) was injected IP, and immediately mice were individually placed in a plexiglass cage. The latency was defined as the time elapsed between ethanol injection and LORR. Mice that did not lose righting reflex were not included in the posterior measurements. After mice lost the righting reflex, they were put on their back in a V-shape bed. The duration of LORR was defined as the time elapsed from LORR to the time that righting reflex was regained. Recovery was determined when mice could right themselves twice in 1 min after being placed on their backs. All the animals recovered the righting reflex. The behavioral room was illuminated with a soft light and external noise was attenuated.

These parameters were chosen based on previous studies (Correa et al., 1999, 2001, 2003, 2008; Arizzi-LaFrance et al., 2006; Chuck et al., 2006; Escrig et al., 2012).

STATISTICAL ANALYSIS

All the experiments used a between-groups design, with each animal only being tested once. Data were analyzed by simple analysis of variance (ANOVA). If there was a significant overall drug effect, the LSD was used to make planned comparisons between each dose and the respective vehicle control condition. A computerized statistical program was used to analyze these data (SPSS 10.0).

RESULTS

EXPERIMENT 1: EFFECT OF ACUTE CENTRAL OR PERIPHERAL ADMINISTRATION OF ACETATE ON LOCOMOTOR ACTIVITY IN RATS

Figure 1A shows the effect of ICV acetate administration (0.0, 0.7, 1.4, or 2.8 μmoles) on locomotor activity in the stabilimeter. Because the pattern of results was the same in the two time periods registered and there was no interaction, separate ANOVAs were performed for the two periods. The One-Way ANOVA for the 0–10 period showed a statistically significant overall treatment effect [$F_{(3,\ 22)} = 7.82$, $p < 0.01$]. Planned comparisons showed all doses of acetate were significantly different from vehicle (0.7 and 2.8 μmoles $p < 0.01$, and 1.4 μmoles $p < 0.05$). The same pattern of results were found for the ANOVA of the second period [$F_{(3,\ 22)} = 8.47$, $p < 0.01$]. The data for the effect of IP acetate administration (0, 12.5, 25, 50, or 100 mg/kg) on locomotor activity in the stabilimeter were analyzed in the same way (see **Figure 1B**). The One-Way ANOVA for the first period of time showed a significant effect of the peripheral dose of acetate [$F_{(4,\ 36)} = 4.90$, $p < 0.01$], and the planned comparisons showed that the three highest doses were significantly different from vehicle ($p < 0.01$). The same results were shown for the second period of time; 10–20 min [$F_{(4,\ 36)} = 4.86$, $p < 0.01$], and for the planned comparisons.

EXPERIMENT 2: EFFECT OF ACUTE IP ADMINISTRATION OF ACETATE ON LOCOMOTOR ACTIVITY IN MICE

The one-way factorial ANOVA for the effect of acetate treatment (0, 50, 100, or 200 mg/kg) did not show significant effects on the number of crossings between the two compartments of the enclosed box [$F_{(3,\ 38)} = 0.63$, n.s.]. These data are shown in **Figure 2**.

EXPERIMENT 3: EFFECT OF 15 DAYS OF ORAL CONSUMPTION OF ACETATE ON ETHANOL-INDUCED LOCOMOTION IN MICE

A two-way factorial ANOVA (concentration of acetate × dose of ethanol) showed no effect of the acetate concentration factor

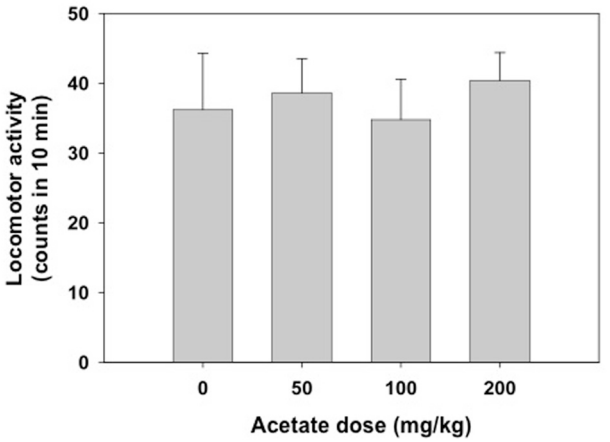


FIGURE 2 | Effect of acetate administered acutely IP on locomotor activity evaluated in mice in the enclosed activity box. Data are expressed as mean + SEM counts in 10 min.

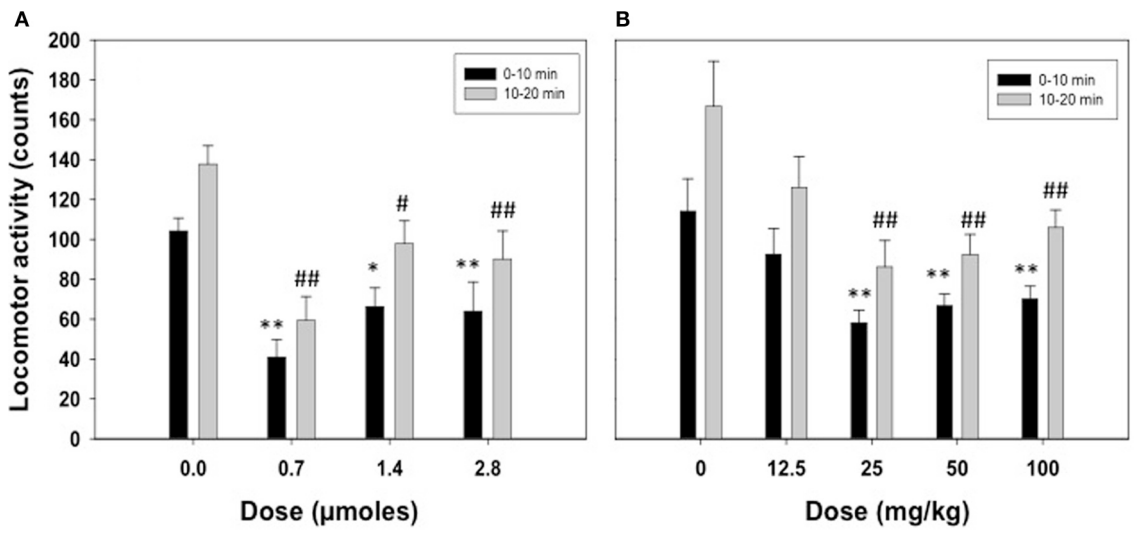


FIGURE 1 | Effect of acetate administered acutely ICV (A) or IP (B) on locomotor activity evaluated in rats in the enclosed stabilimeter. Data are expressed as mean + SEM counts in 10 min periods. $^{**}p < 0.01$, $^{*}p < 0.05$ different from vehicle in the 0–10 min period. $^{\#\#}p < 0.01$, $^{\#}p < 0.05$ different from vehicle in the 10–20 min period.

[$F_{(2, 69)} = 1.42$, n.s.], but a significant effect of the ethanol dose factor [$F_{(2, 69)} = 9.50$, $p < 0.01$], and a significant interaction [$F_{(4, 69)} = 2.76$, $p < 0.05$]. Planned comparison revealed that the two doses of ethanol significantly induced locomotion (1.0 g/kg $p < 0.05$ and 2.0 g/kg $p < 0.01$) compared to vehicle in the water-consuming group. Moreover, these differences disappeared in the acetate consuming groups. These results are depicted in **Figure 3**.

EXPERIMENT 4: EFFECT OF 15 DAYS OF ORAL CONSUMPTION OF ACETATE ON ETHANOL-INDUCED ANXIOLYSIS IN MICE

The four dependent variables (see **Table 1**) were analyzed independently. A two-way factorial ANOVA (concentration of acetate × dose of ethanol) was performed in every case. The results of the ANOVA for the dependent variable latency to enter the bright compartment showed that there was a significant effect of the ethanol dose [$F_{(1, 57)} = 4.72$, $p < 0.05$], but no effect of the acetate treatment [$F_{(2, 57)} = 0.36$, n.s.], and no significant interaction [$F_{(2, 57)} = 0.57$, n.s.]. The same pattern of results for the dependent variable latency to come back to the dark compartment was found: ethanol dose [$F_{(1, 57)} = 5.32$, $p < 0.05$], the concentration of acetate [$F_{(2, 57)} = 0.46$, n.s.], and the interaction [$F_{(2, 57)} = 0.78$, n.s.]. These results demonstrate that ethanol had an anxiolytic effect independently of the acetate treatment. The results for the total time in the bright compartment showed no significant effect: ethanol dose [$F_{(1, 57)} = 2.67$, n.s.], acetate treatment [$F_{(2, 57)} = 0.82$, n.s.], and the interaction [$F_{(2, 57)} = 0.68$, n.s.]. The frequency of crossings between the bright and the dark compartments showed a marginally non-significant effect of the ethanol factor [$F_{(1, 57)} = 3.28$, $p = 0.07$], a significant effect of the acetate treatment [$F_{(2, 57)} = 3.53$, $p < 0.05$], but no significant interaction [$F_{(2,57)} = 0.52$, n.s.].

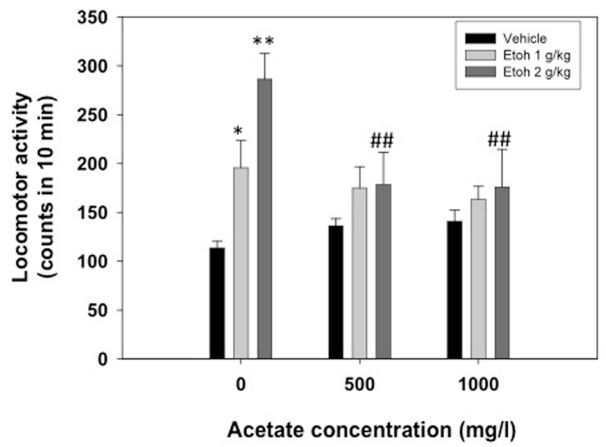


FIGURE 3 | Effect of chronic acetate consumption during 15 days on ethanol-induced locomotor activity in an open field in mice. Data are expressed as mean ± SEM counts in 10 min. $^{**}p < 0.01$, $^{*}p < 0.05$ different from vehicle in the same acetate group. $^{\#\#}p < 0.01$ different from the same dose of ethanol in the 0 mg/l group.

EXPERIMENT 5: EFFECT OF ORAL CONSUMPTION OF ACETATE DURING DIFFERENT PERIODS OF TIME ON ETHANOL-INDUCED LORR IN MICE

Animals received ethanol only once and different measures were assessed. We observed that the lower dose of ethanol (4.0 g/kg) did not produce LORR in some animals that were immediately excluded from the following measures in this experiment (they are not included in the latency and duration analyses). Grouping together the number of animals in the three treatment groups (water, 500 and 1000 mg/l) independently of how many days they had consumed acetate (15, 30, or 60 days, there were no animals in the 7 days groups), the χ^2 test for independence showed a significant effect of the acetate treatment ($\chi^2 = 10.64$, $df = 2$, $p < 0.01$). These data are depicted as percentage of animals not achieving LORR in every treatment group in **Figure 4**.

Among the animals that did achieve LORR, a two-way factorial ANOVA (concentration of acetate × time of consumption) for the latency to reach LORR measure yielded no significant effect of acetate concentration [$F_{(2, 163)} = 0.54$, n.s.], no effect of time of consumption [$F_{(3, 163)} = 1.48$, n.s], and no interaction [$F_{(6, 163)} = 0.79$, n.s.]. The factorial ANOVA for duration of LORR demonstrate no effect of the acetate concentration [$F_{(2, 163)} = 0.07$, n.s.], but a significant effect of the time of consumption [$F_{(3, 163)} = 14.28$, $p < 0.01$]. However, the interaction was not significant [$F_{(6, 163)} = 0.20$, n.s.]. Thus, 4.0 g/kg ethanol produced an increase in duration of LORR in older animals independently of the acetate treatment. The data for the higher dose of ethanol (4.5 g/kg) in animals consuming acetate during 60 days were analyzed separately by means of a One-Way ANOVA. The results show no effect of the concentration on either the latency [$F_{(2, 40)} = 0.43$, n.s.], or the duration of LORR [$F_{(2, 40)} = 0.62$, n.s.]. These data are presented in **Figures 5A,B**.

Table 1 | Effect of chronic acetate consumption during 15 days on vehicle or ethanol (1 g/kg, IP) treated mice in measures of anxiety in the dark/light box.

Acetate (mg/l)	Latency to lit compartment		Latency to go back to dark compartment		Time in lit compartment		Number of crossings into the lit compartment	
	Veh	EtOH	Veh	EtOH	Veh	EtOH	Veh	EtOH
0	11.6 ± 1.8	9.4 ± 1.6	6.6 ± 1.9	14.6 ± 2.9	105.9 ± 10.9	122.8 ± 14.5	27.7 ± 3.1	30.8 ± 3.7
500	11.2 ± 1.9	6.6 ± 1.5	7.5 ± 1.8	9.6 ± 1.4	128.2 ± 10.6	129.3 ± 6.6	27.2 ± 3.3	37.4 ± 2.8
1000	15.3 ± 6.5	7.2 ± 1.3	8.7 ± 1.4	12.4 ± 3.2	110.0 ± 11.2	137.1 ± 11.6	37.2 ± 4.8	40.8 ± 4.1

Data are expressed as the mean ± SEM seconds or counts in 5 min.

EXPERIMENT 6: EFFECT OF ORAL CONSUMPTION OF ACETATE DURING DIFFERENT PERIODS OF TIME ON VOLUME OF WATER CONSUMED AND BODY WEIGHT GAIN

Results from the evolution of body weight and fluid intake in animals for experiment 5 are shown in **Figures 6A,B**. The Two-Way ANOVA for the body weight was analyzed with a within factor for duration of treatment and a between factor for concentration of acetate. There was a significant effect of the duration [$F_{(4,\ 960)} = 437.5$, $p < 0.01$], but no effect of concentration [$F_{(2,\ 10)} = 0.40$, n.s.], and no significant interaction [$F_{(8,\ 2)} = 0.84$, n.s.]. The same pattern of results was shown for the fluid intake variable. The Two-Way ANOVA showed a significant effect of the duration [$F_{(4,\ 11)} = 15.43$, $p < 0.01$], but no effect of concentration [$F_{(2,\ 10)} = 0.11$, n.s.], and no significant interaction [$F_{(8,\ 1)} = 0.58$, n.s.].

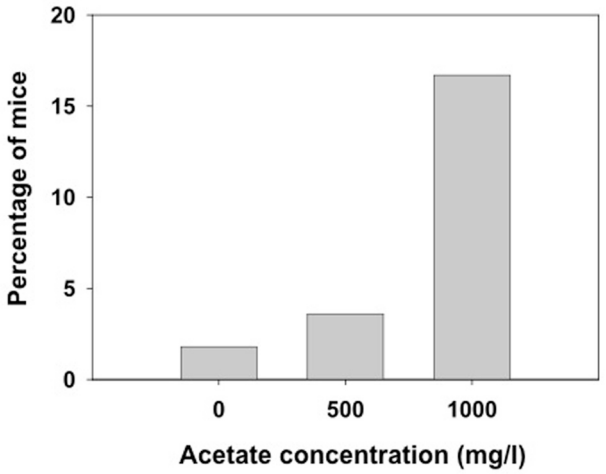


FIGURE 4 | Percentage of mice exposed to different concentrations of acetate that did not achieve LORR after receiving 4.0 g/kg ethanol IP.

DISCUSSION

Studies of the behavioral effects of the ethanol metabolite acetaldehyde have been increasing in number, especially during the last decade; as a result, our knowledge of acetaldehyde's behavioral and neurochemical effects is quite comprehensive (for a recent review see Correa et al., 2012). However, acetate has remained mostly unknown, and only a handful of studies have addressed its behavioral and neurochemical actions (Israel et al., 1994; Correa et al., 2003; Kiselevski et al., 2003; Arizzi-LaFrance et al., 2004; McLaughlin et al., 2008; Zimatkin et al., 2011; Escrig et al., 2012). The present results demonstrate that acute low doses of acetate administered peripherally or into the ventricles reduce spontaneous locomotion in rats at least during 20 min (see **Figures 1A,B**). The present studies measured locomotion in small and enclosed stabilimeter cages. Centrally administered acetate (ICV) has also been shown to produce locomotor suppressant effects in rats in an open field arena (Correa et al., 2003). In that case acetate produced a monotonic decrease in activity (1.4 and 2.8 μmoles) marked by significant decreases in locomotion as well as rearing (Correa et al., 2003). The suppression is more efficacious when using the small stabilimeter cages (0.7 μmoles also suppressed locomotion), possibly because this device is less anxiogenic than the open field, and therefore induces a higher level of locomotion.

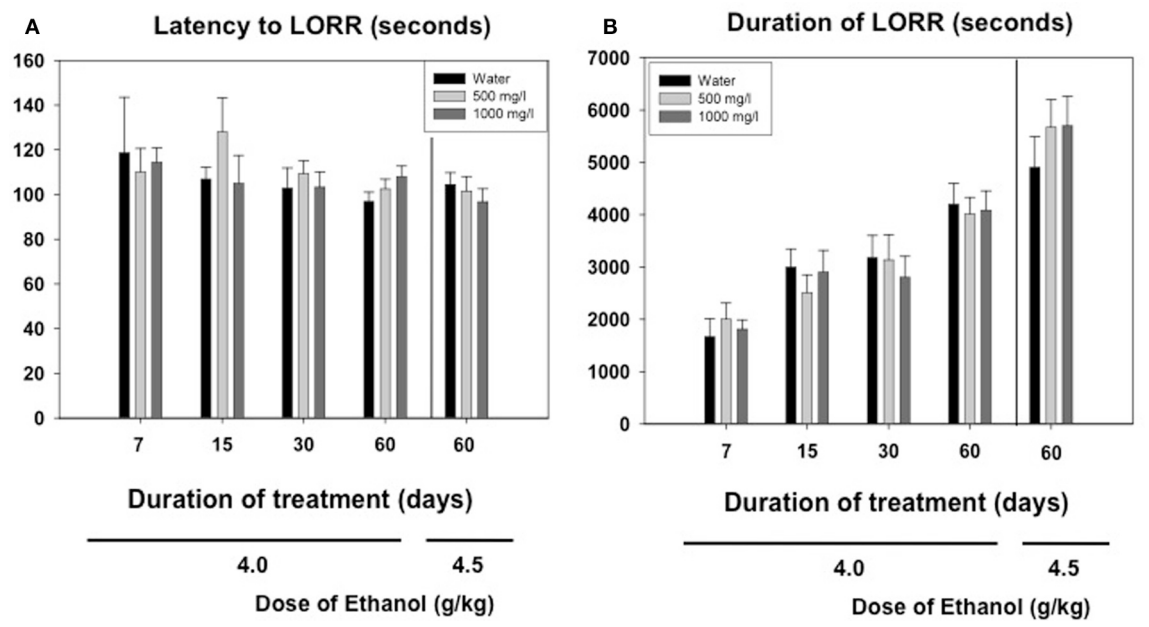


FIGURE 5 | Effect of chronic acetate consumption during different periods of time on latency (A) and duration (B) to LORR induced by an acute administration of ethanol (4.0 or 4.5 g/kg, IP). Data are expressed as mean ± SEM of time in seconds.

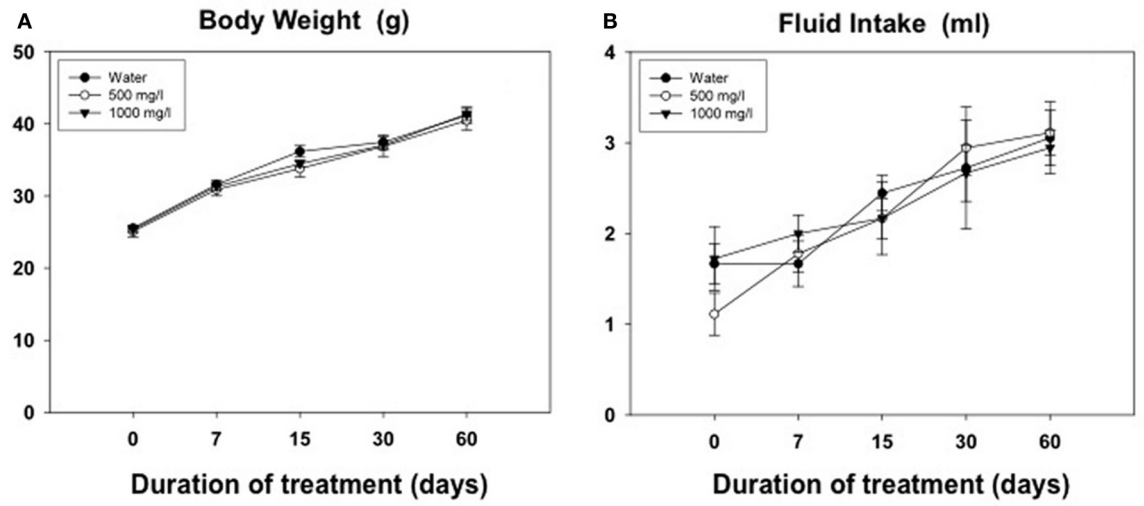


FIGURE 6 | Evolution of body weight (g) (A) and volume of fluid consumed (ml) (B) in animals exposed for 60 days to different concentrations of acetate. Mean ± SEM of grams.

Rats seem to be more sensitive than mice to the suppressant effects of peripherally administered ethanol and acetate. Thus, in the present studies acetate doses between 25 and 100 mg/kg reduced locomotion in rats but not in mice; even the dose of 2.0 mg/kg did not suppress locomotion in mice under the present conditions (enclosed activity box); this dose is much lower than doses used in previous studies in mice (Israel et al., 1994). In mice the minimal dose of acetate effective for suppressing locomotion in an open field was 1.0 g/kg, while the dose of ethanol was 3.0 g/kg (Israel et al., 1994). Thus, acetate seems more potent than ethanol at suppressing locomotion. This difference in drug potency has also been observed in other studies in rats. When injected peripherally, acetate was more potent than ethanol or acetaldehyde for suppressing food-reinforced operant responding (Arizzi et al., 2003; McLaughlin et al., 2008), reducing the number of fast responses and increasing the number of pauses that the animals took during the operant session at doses of 200–400 mg/kg, IP (McLaughlin et al., 2008). Injected into the ventricles, acetate suppressed lever pressing (2.8 and 5.6 μmoles), and also increased the number of pauses at the highest concentration (5.6 μmoles; McLaughlin et al., 2008), while ethanol and acetaldehyde did not. Moreover, acetate at the highest doses (5.6 and 8.8 μmoles) was also the most efficacious of the three substances at suppressing lever pressing in an operant schedule of reinforcement that generates very low rates of response, and thus is very difficult to suppress [i.e., the differential-reinforcement-of-low-rates-of-responding (DRL) 30 s schedule, Arizzi et al., 2003].

While in the present experiments acute administration of acetate was demonstrated to suppress locomotion, at least in rats, chronic administration of acetate in the drinking water for 15 days did not change locomotion on its own. Nevertheless, it did reduce ethanol-induced locomotion in the open field (see **Figure 3**). Thus, chronic pre-exposure to a low dose of acetate made animals more resistant to the stimulating effects of medium doses of ethanol in mice. Acetate, however, does not seem to mediate other ethanol well known effects, such as the anxiolytic response which acetaldehyde has been demonstrated to regulate (Correa et al., 2003, 2008; Escrig et al., 2007, 2012). Acutely administered acetate (50–200 mg/kg, IP) did not alter the behavior of mice in either the elevated plus maze or the dark and light box (Escrig et al., 2007, 2012). The same pattern of effects was observed in the interior part of an OF (Correa et al., 2003). Acutely administered acetate ICV at doses similar to the present ones (0.35–2.8 μmoles) did not modify anxiety measures in the open field in rats, although it reduced locomotion (Correa et al., 2003). Moreover, in the present results, mice exposed to acetate for 15 days did not show changes in the anxiolytic response in the dark/light box after ethanol administration. The dose of ethanol used (1.0 g/kg) has previously been demonstrated to have a potent anxiolytic effect under the present conditions (Correa et al., 2008; Escrig et al., 2012). Unfortunately, that anxiolytic effect was very mild in the present results, thus we cannot rule out this fact as the lack of interaction. In summary, although acetate has been shown to be involved in the locomotor suppressing effects of ethanol in mice (Israel et al., 1994) and rats (Correa et al., 2003; Arizzi-LaFrance et al., 2004; present results), it does not seem to mediate ethanol's anxiolytic actions (Correa et al., 2003), nor does it seem to be involved in the anxiogenic response produced by a bolus injection of acetaldehyde in the periphery (Escrig et al., 2012).

The higher levels of acetate that accumulate in the brain after repeated administration of ethanol (3.5 g/kg, IP, during 7 days) seem to mediate tolerance to LORR induced by an acute dose of ethanol (3.5 g/kg) in outbred rats (Kiselevski et al., 2003). Moreover, there is evidence that higher amounts of acetate are formed in SS rats that have an inborn tolerance to hypnotic doses of ethanol compared to the LS substrain (Zimatkin et al., 2011). In the present studies with mice, the doses achieved

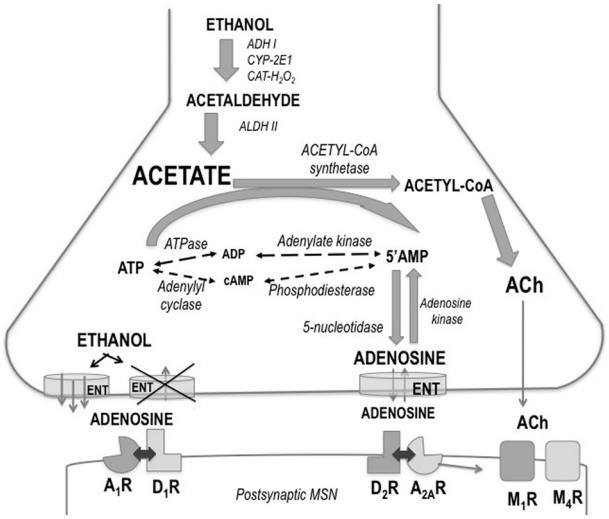


FIGURE 7 | Schematic drawing showing ethanol regulation of adenosine production, release, and uptake in striatum. Abbreviations: A_1R and $A_{2A}R$, adenosine receptors; ACh, acetylcholine; ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; ATP, adenosine triphosphate; AMP, adenosine monophosphate; CAT-H_2O_2, catalase; CYP-2E1, cytochrome P4502E1; D_1R and D_2R, dopamine receptors; ENT, equilibrative nucleoside transporters; M_1R and M_4R, muscarinic receptors; MSN, medium spiny neuron.

after consuming water with acetate concentrations of 500 and 1000 mg/l are significantly lower (around 30 and 65 mg/kg per day, respectively). Thus, the lack of effects in latency and duration of LORR after acute administration of the high doses of ethanol (4.0 and 4.5 g/kg) could be due to the fact that the doses achieved after consuming these concentrations of acetate are significantly lower than the ones used in other studies. Also, these discrepancies in results could be due to species differences; mice been shown to be more resistant than rats to the suppressive effects of ethanol and acetate. However, our results on number of animals achieving LORR (**Figure 4**) indicate that chronic acetate provides some sort of resistance in mice to the hypnotic effects of ethanol.

The precise brain areas and neural mechanisms through which acetate produces its potent suppression of motor activity are not known. A potential neuroanatomical locus for the locomotor actions of acetate, ethanol and acetaldehyde was previously found (Arizzi-LaFrance et al., 2004, 2006). Acetate injected into the substantia nigra pars reticulata of the mesencephalon produced a slight locomotor suppression (Arizzi-LaFrance et al., 2004) in contrast to the clear stimulation demonstrated for ethanol and acetaldehyde (Arizzi-LaFrance et al., 2006). Concentrations of several neurotransmitters such as acetylcholine (ACh) and adenosine seem to be modulated by the production of acetate. These hypothetical mechanisms are summarized in **Figure 7**. Acetate has demonstrated to increase the formation of adenosine (Dar et al., 1983; Phillis et al., 1992; Carmichael et al., 1993; Israel et al., 1994; Kiselevski et al., 2003). Ethanol increases adenosine levels by acting as a precursor through the production of acetate (Orrego et al., 1988; Carmichael et al., 1991). High doses of sub-chronically administered ethanol have been demonstrated to increase acetate, adenosine, and ACh, as well as several other biochemical factors responsible of acetate, in several areas of the brain (Kiselevski et al., 2003). It has also been suggested that ethanol as well as acetate can block adenosine uptake into the neuron (Fredholm and Wallman-Johansson, 1996; Kiselevski et al., 2003; Correa and Font, 2008), thus increasing extra-synaptic adenosine levels. Adenosine has been implicated in multiple behaviors including sleep, arousal, and motor activity (Huston et al., 1996; Iversen et al., 2009). There is evidence that adenosine may contribute to some behavioral effects of ethanol such as sedation, and motor suppression or incoordination (Proctor et al., 1985; Clark and Dar, 1988, 1989; Dar, 1990, 1993, 2000; Carmichael et al., 1991; Meng and Dar, 1995; Campisi et al., 1997; Barwick and Dar, 1998). Motor incoordination induced by ethanol is controlled by adenosine in the striatum and cerebellum (Dar, 1993; Meng and Dar, 1995). Studies also indicate that adenosine receptor activation provides a major contribution to motor suppressant effects of low concentrations of ethanol when the production of acetate is near maximal (Carmichael et al., 1993; Israel et al., 1994). At higher doses of ethanol, such as the ones used in LORR studies, the role of the acetate–adenosine system is proportionately reduced (Israel et al., 1994). As the acetate level increases after high doses of ethanol, the activation of acetyl–CoA synthetase would be expected and the formation of ACh is then potentiated (Kiselevski et al., 2003). Acetate induced increases in ACh in cerebral cortex have been associated to tolerance to ethanol-induced LORR (Zimatkin et al., 2011). Thus, the present results suggest that an increase in ACh/adenosine content may be responsible for the effects of acetate on locomotor suppression, and for blocking the stimulation of locomotion induced by ethanol and increasing resistance to achieve LORR. Further studies about the involvement of ACh, adenosine, and their subtype-receptors in these actions of acetate are warranted.

The relevance of the present acetate results (i.e., suppression of locomotion, blockade of ethanol stimulation) is related to the suggestion that two pharmacological effects that may be particularly relevant for alcohol consumption are behavioral stimulation and sedation (King et al., 2002, 2011). In general, doses of ethanol that produce more stimulation are more likely to be consumed. Subjects report that their typical drinking bout is in the dose range that was considered as having activating or disinhibiting effects (King et al., 2002, 2011). Sedative or suppressing effects on activation may also influence drinking behavior; anticipated sedative effects vary inversely with alcohol consumption (Earleywine and Martin, 1993) and heavier drinkers anticipate fewer sedative effects of alcohol than lighter drinkers (O'Malley and Maisto, 1984). Thus, sedative effects seem to prevent self-administration of ethanol, and stimulant effects can foster consumption of this drug. In agreement with these hypotheses, rats do not self-administer acetate ICV under the same conditions that lead to ethanol or acetaldehyde self-administration (Rodd-Henricks et al., 2002), and acetate does not stimulate locomotion under the same conditions that ethanol and acetaldehyde do (Correa et al., 2003).

ACKNOWLEDGMENTS

This research was supported by a grant to Mercè Correa from Plan Nacional de Drogas (2010I024), Spain, and a grant to John D. Salamone from NIH/NIMH (MH078023-01A1), USA. Marta Pardo was funded by a fellowship from Fundació Bancaixa-UJI (PREDOC2007-43), Laura López-Cruz was funded by a fellowship from Ministerio de Educación FPU (AP2010-3793), and Noemí San Miguel was supported by Plan Nacional de Drogas (2010I024).

REFERENCES

Arizzi-LaFrance, M. N., Correa, M., Aragon, C. M., and Salamone, J. D. (2006). Motor stimulant effects of ethanol objected into the substantia nigra pars reticulata: importance of catalase-mediated metabolism and the role of acetaldehyde. *Neuropsychpharmacology* 31, 997–1008. doi: 10.1038/sj.npp.1300849

Arizzi-LaFrance, M. N., Correa, M., and Salamone, J. D. (2004). Locomotor activating effects of ethanol and ethanol metabolites after injection into the substantia nigra pars reticulata: effects of catalase blockade. *Alcohol. Clin. Exp. Res.* 28, 61A.

Arizzi, M. N., Correa, M., Betz, A. J., Wisniecki, A., and Salamone, J. D. (2003). Behavioral effects of intraventricular injections of low doses of ethanol, acetaldehyde, and acetate in rats: studies with low and high rate operant schedules. *Behav. Brain Res.* 147, 203–210. doi: 10.1016/S0166-4328(03)00158-X

Barwick, V. S., and Dar, M. S. (1998). Adenosinergic modulation of ethanol-induced motor incoordination in the rat motor cortex. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 22, 587–607. doi: 10.1016/S0278-5846(98)00025-6

Busch, H. (1953). Studies on the metabolism of acetate-1-C in tissues of tumor-bearing rats. *Cancer. Res.* 13, 789–794.

Campisi, P., Carmichael, F. J., Crawford, M., Orrego, H., and Khanna, J. M. (1997). Role of adenosine in the ethanol-induced potentiation of the effects of general anesthetics in rats. *Eur. J. Pharmacol.* 325, 165–172. doi: 10.1016/S0014-2999(97)00124-6

Carmichael, F. J., Israel, Y., Crawford, M., Minhas, K., Saldivia, V., Sandrin, S., et al. (1991). Central nervous system effects of acetate: contribution to the central effects of ethanol. *J. Pharmacol. Exp. Ther.* 259, 403–408.

Carmichael, F. J., Orrego, H., and Israel, Y. (1993). Acetate-induced adenosine mediated effects of ethanol. *Alcohol Alcohol.* 2, 411–418.

Chuck, T. L., McLaughlin, P. J., Arizzi-LaFrance, M. N., Salamone, J. D., and Correa, M. (2006). Comparison between multiple behavioral effects of peripheral ethanol administration in rats: sedation, ataxia, and bradykinesia. *Life Sci.* 79, 154–161. doi: 10.1016/j.lfs.2005.12.045

Clark, M., and Dar, M. S. (1988). Mediation of acute ethanol-induced motor disturbances by cerebellar adenosine in rats. *Pharmacol. Biochem. Behav.* 30, 155–161. doi: 10.1016/0091-3057(88)90439-X

Clark, M., and Dar, M. S. (1989). Effect of acute ethanol on release of endogenous adenosine from rat cerebellar synaptosomes. *J. Neurochem.* 52, 1859–1865. doi: 10.1111/j.1471-4159.1989.tb07268.x

Correa, M., Chuck, T., Arizzi, M. N., Betz, A., McLaughlin, P. J., and Salamone, J. D. (2003). Motor effects of ethanol and ethanol metabolites after intracranial or peripheral injections in rats. *Behav. Pharmacol.* 14, 76–77.

Correa, M., and Font, L. (2008). Is there a major role of A2A adenosine receptor in anxiety? *Front. Biosci.* 1, 4058–4070. doi: 10.2741/2994

Correa, M., Manrique, H. M., Font, L., Escrig, M. A., and Aragon, C. M. G. (2008). Reduction in the anxiolytic effects of ethanol by centrally formed acetaldehyde: the role of catalase inhibitors and acetaldehyde-sequestering agents. *Psychopharmacol.* 200, 455–464. doi: 10.1007/s00213-008-1219-3.

Correa, M., Miquel, M., Sanchis-Segura, C., and Aragon, C. M. (1999). Acute lead acetate administration potentiates ethanol-induced locomotor activity in mice: the role of brain catalase. *Alcohol. Clin. Exp. Res.* 23, 799–805.

Correa, M., Salamone, J. D., Segovia, K. N., Pardo, M., Longoni, R., Spina, L., et al. (2012). Piecing together the puzzle of acetaldehyde as a neuroactive agent. *Neurosci. Biobehav. Rev.* 36, 404–430. doi: 10.1016/j.neubiorev.2011.07.009

Correa, M., Sanchis-Segura, C., and Aragon, C. M. (2001). Influence of brain catalase on ethanol-induced loss of righting reflex in mice. *Drug. Alcohol. Depend.* 65, 9–15. doi: 10.1016/S0376-8716(01)00142-9

Cullen, N., and Carlen, P. L. (1992). Electrophysiological actions of acetate, a metabolite of ethanol, on hippocampal dentate granule neurons: interactions with adenosine. *Brain Res.* 588, 49–57. doi: 10.1016/0006-8993(92)91343-D

Dar, M. S. (1990). Central adenosinergic system involvement in ethanol-induced motor incoordination in mice. *J. Pharmacol. Exp. Ther.* 255, 1202–1209.

Dar, M. S. (1993). Brain adenosinergic modulation of acute ethanol-induced motor impairment. *Alcohol Alcohol. Suppl.* 2, 425–429.

Dar, M. S. (2000). Cerebellar CB(1) receptor mediation of Delta(9)-THC-induced motor incoordination and its potentiation by ethanol and modulation by the cerebellar adenosinergic A(1) receptor in the mouse. *Brain Res.* 864, 186–194. doi: 10.1016/S0006-8993(00)02103-X

Dar, M. S., Mustafa, S. J., and Wooles, W. R. (1983). Possible role of adenosine in the CNS effects of ethanol. *Life. Sci.* 33, 1363–1374. doi: 10.1016/0024-3205(83)90819-6

Earleywine, M., and Martin, C. S. (1993). Anticipated stimulant and sedative effects of alcohol vary with dosage and limb of the blood alcohol curve. *Alcohol. Clin. Exp Res.* 17, 135–139. doi: 10.1111/j.1530-0277.1993.tb00738.x

Escrig, M. A., Pardo, M., Aragon, C. M., and Correa, M. (2007). Anxiogenic effects in mice of peripheral acetaldehyde accumulation. *Behav. Pharmacol.* 18, 77.

Escrig, M. A., Pardo, M., Aragon, C. M., and Correa, M. (2012) Anxiogenic and stress-inducing effects of peripherally administered acetaldehyde in mice: similarities with the disulfiram-ethanol reaction. *Pharmacol. Biochem. Behav.* 100, 404–412. doi: 10.1016/j.pbb.2011.10.002

Fredholm, B. B., and Wallman-Johansson, A. (1996). Effects of ethanol and acetate on adenosine production in rat hippocampal slices. *Pharmacol. Toxicol.* 79, 120–123. doi: 10.1111/j.1600-0773.1996.tb00254.x

Huston, J. P., Haas, H. L., Boix, F., Pfister, M., Decking, U., Schrader, J., et al. (1996). Extracellular adenosine levels in neostriatum and hippocampus during rest and activity periods of rats. *Neuroscience* 73, 99–107. doi: 10.1016/0306-4522(96)00021-8

Israel, Y., Orrego, H., and Carmichael, F. J. (1994). Acetate-mediated effects of ethanol. *Alcohol. Clin. Exp. Res.* 18, 144–148. doi: 10.1111/j.1530-0277.1994.tb00894.x

Iversen, L. L., Iversen, S. D., Bloom, F. E., and Roth, R. H. (2009). *Introduction to Neuropsychopharmacology*. New York, NY: Oxford University Press.

King, A. C., de Wit, H., McNamara, P. J., and Cao, D. (2011). Rewarding, stimulant, and sedative alcohol responses and relationship to future binge drinking. *Arch. Gen. Psychiatry* 68, 389–399. doi: 10.1001/archgenpsychiatry.2011.26

King, A. C., Houle, T., de Wit, H., Holdstock., L., and Schuster, A. (2002). Biphasic alcohol response differs in heavy versus light drinkers. *Alcohol. Clin. Exp. Res.* 26, 827–835. doi: 10.1111/j.1530-0277.2002.tb02611.x

Kiselevski, Y., Oganesian, N., Zimatkin, S., Szutowicz, A., Angielski, S., Niezabitowski, P., et al. (2003). Acetate metabolism in brain mechanisms of adaptation to ethanol. *Med. Sci. Monit.* 9, 178–182.

Lundquist, F., Tygstrup, N., Winkler, K., Mellemgaard, K., and Munck-Petersen, S. (1962). Ethanol metabolism and production of free acetate in the human liver. *J. Clin. Invest.* 41, 955–961. doi: 10.1172/JCI104574

McLaughlin, P. J., Chuck, T. L., Arizzi-LaFrance, M. N., Salamone, J. D., and Correa, M. (2008). Central vs. peripheral administration of ethanol, acetaldehyde and acetate in rats: effects on lever pressing and response initiation. *Pharmacol. Biochem. Behav.* 89, 304–313. doi: 10.1016/j.pbb.2008.01.002

Meng, Z. H., and Dar, M. S. (1995). Possible role of striatal adenosine in the modulation of acute

ethanol-induced motor incoordination in rats. *Alcohol. Clin. Exp. Res.* 19, 892–901. doi: 10.1111/j.1530-0277.1995.tb00964.x

O'Malley, S. S., and Maisto, S. A. (1984) Factors affecting the perception of intoxication: dose, tolerance, and setting. *Addict. Behav.* 9, 111–120. doi: 10.1016/0306-4603(84)90049-2

Oldendorf, W. H. (1973). Carrier-mediated blood-brain barrier transport of short-chain monocarboxylic organic acids. *Am J. Physiol.* 224, 1450–1453.

Orrego, H., Carmichael, F. J., and Israel, Y. (1988). New insights on the mechanism of the alcohol-induced increase in portal blood flow. *Can. J. Physiol. Pharmacol.* 66, 1–9. doi: 10.1139/y88-001

Phillis, J. W., O'Regan, M. H., and Perkins, L. M. (1992). Actions of ethanol and acetate on rat cortical neurons: ethanol/adenosine interactions. *Alcohol* 9, 541–546. doi: 10.1016/0741-8329(92)90094-Q

Proctor, W. R., Baker, R. C., and Dunwiddie, T. V. (1985). Differential CNS sensitivity to PIA and theophylline in long-sleep and short-sleep mice. *Alcohol* 2, 387–391. doi: 10.1016/0741-8329(85)90061-8

Rodd-Henricks, Z. A., Bell, R. L., Kuc, K. A., Murphy, J. M., McBride, W. J., Lumeng, L., et al. (2002). Effects of ethanol exposure on subsequent acquisition and extinction of ethanol self-administration and expression of alcohol-seeking behavior in adult alcohol-preferring (P) rats: II. Adult exposure. *Alcohol. Clin. Exp. Res.* 26, 1642–1652. doi: 10.1111/j.1530-0277.2002.tb02466.x

Van den Berg, C. J., Mela, P., and Waelsch, H. (1966). On the contribution of the tricarboxylic acid cycle to the synthesis of glutamate, glutamine and aspartate in brain. *Biochem. Biophys. Res. Commun.* 23, 479–484. doi: 10.1016/0006-291X(66)90753-4

Zimatkin, S. M., Oganesian, N. A., Kiselevski, Y. V., and Deitrich, R. A. (2011). Acetate-dependent mechanisms of inborn tolerance to ethanol. *Alcohol Alcohol.* 46, 233–238. doi: 10.1093/alcalc/agr014

Zimatkin, S. M., Pronko, S. P., Vasiliou, V., Gonzalez, F. J., and Deitrich, R. A. (2006). Enzymatic mechanisms of ethanol oxidation in the brain. *Alcohol. Clin. Exp. Res.* 30, 1500–1505. doi: 10.1111/j.1530-0277.2006.00181.x

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 10 April 2013; accepted: 18 June 2013; published online: 10 July 2013.

Citation: Pardo M, Betz AJ, San Miguel N, López-Cruz L, Salamone JD and Correa M (2013) Acetate as an active metabolite of ethanol: studies of locomotion, loss of righting reflex, and anxiety in rodents. Front. Behav. Neurosci. 7:81. doi: 10.3389/fnbeh.2013.00081

frontiers in
BEHAVIORAL NEUROSCIENCE

ORIGINAL RESEARCH ARTICLE
published: 17 June 2013
doi: 10.3389/fnbeh.2013.00069

Acetaldehyde reinforcement and motor reactivity in newborns with or without a prenatal history of alcohol exposure

Samanta M. March[1,2]*, Marcela E. Culleré[1], Paula Abate[1,2], José I. Hernández[1], Norman E. Spear[3] and Juan C. Molina[1,2]*

[1] Laboratorio de Alcohol, Ontogenia y Desarrollo, Instituto de Investigación Médica Mercedes y Martín Ferreyra, Córdoba, Argentina
[2] Facultad de Psicología, Universidad Nacional de Córdoba, Cátedra Psicobiología Experimental, Córdoba, Argentina
[3] Center for Developmental Psychobiology, Department of Psychiatry, Binghamton University, Binghamton, NY, USA

Edited by:
Merce Correa, University Jaume I, Spain

Reviewed by:
Merce Correa, University Jaume I, Spain
Mariana Pereira, Rutgers, The State University of New Jersey, USA

***Correspondence:**
Samanta M. March and Juan C. Molina, Laboratorio de Alcohol, Ontogenia y Desarrollo, Instituto de Investigación Médica Mercedes y Martín Ferreyra, PO Box 389, Friuli 2434, 5016 Córdoba, Argentina
e-mail: smarch@immf.uncor.edu; juancmolina2003@yahoo.com

Animal models have shown that early ontogeny seems to be a period of enhanced affinity to ethanol. Interestingly, the catalase system that transforms ethanol (EtOH) into acetaldehyde (ACD) in the brain, is more active in the perinatal rat compared to adults. ACD has been found to share EtOH's behavioral effects. The general purpose of the present study was to assess ACD motivational and motor effects in newborn rats as a function of prenatal exposure to EtOH. Experiment 1 evaluated if ACD (0.35 μmol) or EtOH (0.02 μmol) supported appetitive conditioning in newborn pups prenatally exposed to EtOH. Experiment 2 tested if prenatal alcohol exposure modulated neonatal susceptibility to ACD's motor effects (ACD dose: 0, 0.35 and 0.52 μmol). Experiment 1 showed that EtOH and ACD supported appetitive conditioning independently of prenatal treatments. In Experiment 2, latency to display motor activity was altered only in neonates prenatally treated with water and challenged with the highest ACD dose. Prenatal EtOH experience results in tolerance to ACD's motor activity effects. These results show early susceptibility to ACD's appetitive effects and attenuation of motor effects as a function of prenatal history with EtOH, within a stage in development where brain ACD production seems higher than later in life.

Keywords: prenatal ethanol exposure, acetaldehyde, associative learning, reinforcement, ontogeny, neonatal learning, motor activity, tolerance

INTRODUCTION

Epidemiological studies have shown an association between prenatal exposure to ethanol (EtOH) and later development of EtOH drinking problems (Baer et al., 2003; Alati et al., 2006). Animal studies have extensively agreed with this observation (for a review see Abate et al., 2008). The effect of prenatal exposure to EtOH upon later responsiveness to the drug has been observed soon after birth (March et al., 2009; Miranda-Morales et al., 2010), during infancy (Arias and Chotro, 2005), adolescence (Chotro and Arias, 2003), and adulthood (Barbier et al., 2008).

Several mechanisms have been proposed to explain this effect. One possibility relies on fetal associative learning and memory capabilities. The odor, taste and somatosensory stimulation provided by EtOH in the amniotic fluid is perceived by the fetus (Domínguez et al., 1996). The fetus can also experience EtOH's postabsorptive pharmacological effects (Domínguez et al., 1996; Abate et al., 2000), originating the opportunity for the development of an association between EtOH's chemosensory properties and its pharmacological effects (Abate et al., 2000, 2001, 2002). Additionally, it has been proposed that exposure to EtOH during early ontogeny involves learning about positive effects of the drug, since at this developmental stage rats seem more susceptible to EtOH's effects than later in life (Molina et al., 2007b; Pautassi et al., 2009). In other words, there seems to be an ontogenetic switch in the way organisms perceive EtOH's positive effects. For example, EtOH intoxication on postnatal days 7–8 increases EtOH intake and enhances EtOH's palatability. On the contrary, intoxication during postnatal days 10–11 decreases EtOH intake and increases aversive responses to the drug (Arias and Chotro, 2006).

These changes in EtOH consumption and reinforcement occur during a period of rapid brain maturation (Pautassi et al., 2009). Among the several changes occurring in the neural system during early ontogeny are those concerning EtOH metabolism. Although some sources of EtOH metabolism are slow to develop, brain catalase activity and acetaldehyde (ACD) production are significantly higher in rat pups than adults (Gill et al., 1992). Catalase activity progressively drops during the first weeks of life (Del Maestro and McDonald, 1987). This is not a minor fact, since central catalase oxidizes EtOH into ACD in the brain (Aragon et al., 1991). Additionally, in adult rodents ACD has been shown to have an active role in EtOH postabsorptive effects, such as motor stimulation, anxiolysis, appetitive, and aversive properties (Correa et al., 2003a,b, 2009; Escarabajal et al., 2003; Quertemont et al., 2003). In spite of this evidence, few studies have analyzed ACD effects during infancy (Nizhnikov et al., 2007; Pautassi et al., 2011). These studies show that appetitive memories related to EtOH can be blocked by sequestering ACD (Pautassi et al., 2011; March

et al., 2013) or by the inhibition of the catalase system (Nizhnikov et al., 2007).

An important issue in studying the effects of EtOH (or ACD) during early ontogeny is the accommodation of research tools to the age-specific behavioral repertoire of the animal. Suckling is an age-specific, complex and organized behavior, essential for the newborn's survival (Petrov et al., 2003). The artificial nipple technique has provided valuable information about the reinforcing properties of fluids during early ontogeny (Nizhnikov et al., 2002). Neonates readily self-administer milk as well as sucrose and EtOH solutions through the surrogate nipple. Exposure to an artificial nipple providing EtOH increases subsequent attachment to an empty surrogate nipple (Cheslock et al., 2001). Additionally, 2–5-h old pups are also capable of robust olfactory conditioning when an odor cue (conditioned stimulus, CS) is associated with a natural reinforcer such as milk (Cheslock et al., 2000). For tests of EtOH reinforcement, presenting a surrogate nipple providing water (CS) in close temporal contiguity with an i.p. administration of very low EtOH doses (0.125 and 0.25 g/kg) results in increased attachment to an empty surrogate nipple (Petrov et al., 2003). Although prenatal exposure to EtOH increases later acceptance for the drug (Arias and Chotro, 2005; March et al., 2009), it has not been established if this experience alters the acute effects induced by EtOH's first metabolite (ACD).

EtOH motivational and motor effects have been linked (Arias et al., 2010). Moreover, motor activating effects of EtOH seems to be mediated by ACD, in adult and infant rodents (Correa et al., 2003a,b; Pautassi et al., 2011). Additionally, infants are likely to exhibit motor conditioned responses resulting from the association between chemosensory stimuli and drugs of abuse, such as EtOH that alter frequency and duration of different behaviors. These learned responses may overshadow other specific behaviors (such as suckling) indicative of the drug's motivational properties.

The general purpose of the present study was to analyze ACD's reinforcement capability as well as its motor effects in newborn pups, as a function of prenatal exposure to EtOH. In Experiment 1, this goal was addressed using the artificial nipple technique, an animal model developed to assess drug and natural reinforcement in neonatal pups (Petrov et al., 2003). Yet, the use of EtOH (or ACD) as an unconditioned stimulus (US), can also lead to motor conditioned responses which can confound interpretations in tests related with drug reinforcement (Molina et al., 2007a). For this reason, Experiment 2 was performed to directly assess the possibility that ACD may induce motor effects that can alter pups' responsiveness to the artificial nipple.

METHODS

SUBJECTS

Wistar derived rats were born and reared at the vivarium of the Instituto de Investigaciones Médicas Mercedes y Martín Ferreyra. Temperature was kept at 22–24°C with a 12-h light/12-h dark cycle (light onset at 0800 h). Vaginal smears of female rats (pregnancy weight: 230–300 g) were microscopically analyzed on a daily basis. On the day of Proestrus, females were housed with males (3 females per male) overnight. The next morning, females were withdrawn and this day was considered to be gestational day 0 (GD 0). From DG 0, pregnant females were housed in group of 3 in standard maternity cages. These dams had continuous access to rat chow (Cargill, Buenos Aires, Argentina) and tap water. From GD 17 to 21, dams were individually housed.

Animals were maintained and treated in compliance with guidelines for animal care established by the Institute of Laboratory Animal Resources, National Research Council, U.S.A. (Institute of Laboratory Animal Research, 1996) and were approved by the Animal Care and Use committee at INIMEC-CONICET.

PRENATAL TREATMENTS

During GD 17–20 pregnant females received a daily intragastric administration of either 0 or 2 g/kg EtOH. Control dams (Prenatal Water) received 0.015 ml per g of body weight of tap water. The 2 g/kg EtOH dose was achieved using a similar volume (0.015 ml/g) of a 16.8% v/v EtOH solution (190 proof alcohol, Porta Hnos.). This EtOH dose and timing of exposure were chosen according to previous data showing that, at this age, the fetus can process EtOH chemosensory as well as its postabsorptive effects and form an associative memory comprising these stimuli (Abate et al., 2002; Chotro and Arias, 2003). During GD 21, pups were born by cesarean delivery procedure. For a more detailed description of procedures involved in prenatal treatment and cesarean delivery see (Domínguez et al., 1996; Abate et al., 2002). This procedure was performed to avoid suckling experiences with the dam. It has been shown that appetitive behavior toward the surrogate nipple increases over the first 3 h after birth (Smotherman et al., 1997) and that suckling from an artificial nipple is more vigorous when pups lack experience with the maternal nipple (Petrov et al., 2001). In Experiment 1, 12 pregnant females received water (group Prenatal Water) and 13 received EtOH (group Prenatal EtOH). For Experiment 2, prenatal pups from water treated dams were derived from 10 pregnant females and pups in the prenatal EtOH group were derived from 9 dams.

CENTRAL DRUG ADMINISTRATION PROCEDURE

Drugs were administered into the cisterna magna (intra-cisterna magna administration –IC-) using a 30-gauge hypodermic needle attached to transparent polyethylene tubing (PE 10, Clay Adams, Parsippany, NJ). EtOH and ACD were prepared using phosphate buffer as a vehicle (PB). For Experiment 1, vehicle (PB 0.1 M), EtOH (0.02 μmol) or ACD (0.35 μmol) were administered. The dosage of each particular drug was selected in accordance with previous literature (Arizzi-LaFrance et al., 2006; Nizhnikov et al., 2006c, 2007; Correa et al., 2009; March et al., 2013). For Experiment 2, the following ACD doses were administered: 0.0, 0.35 or 0.52 μmol. Administration procedure has been described elsewhere (Nizhnikov et al., 2006c; March et al., 2013). Briefly, the needle was inserted under visual guidance into the foramen magnum between the occipital bone and the first cervical vertebra (Petrov et al., 1998). Successful placement of the needle into the target site was confirmed by the appearance of cerebrospinal fluid in the tubing. The corresponding solution (1 μl) was injected during a period of 10 s using a micrometer gastight syringe (Hewlett Packard, U.S.A.). Following each IC administration, the needle remained in position during

30 s and then removed to minimize leaking of cerebrospinal fluid. It has been observed that an inert substance administered into the cisterna magna (inulin) follows a caudal-to-rostral and ventral-to-dorsal pattern of distribution and preferred entry of tracer from ventral surfaces of the ventral forebrain—particularly hypothalamus—and brainstem (Proescholdt et al., 2000).

APPARATUS

In Experiment 1 and 2, neonates were kept in a heated incubator (32–34°C; Fábrica Eléctrica Delver, La Plata, Argentina) until commencement of experimental manipulations. Responsiveness to a surrogate nipple (Experiment 1) and motor reactivity (Experiment 2) were assessed 3–5 h after cesarean delivery. The evaluation procedure took place in a transparent Plexiglas (63 × 50 × 25 cm) glove box equipped with a fan system for ventilation and two holes in the front section that allowed access to the neonate. For facilitating presentation of the nipple or aromatic cues, newborns were individually placed in this conditioning chamber equipped with a heated Styrofoam container (internal base diameter: 9 cm; volume capacity: 750 cc) maintained at 35 ± 0.5°C via a temperature controller (Model 40-90B; Frederick Haer Co., Brunswick, ME).

ASSESSMENT OF ACD MOTIVATIONAL EFFECTS BY THE ARTIFICIAL NIPPLE TECHNIQUE (EXPERIMENT 1)

Two hours after delivery, pups received an intracisternal (IC) administration of one of the following drugs: vehicle (PB 0.1 M), EtOH (0.02 μmol), or ACD (0.35 μmol). Thirty seconds later (see drug administration procedure), pups were suited in a restriction vest and taken into the conditioning chamber. One minute after placement in the conditioning chamber, lemon odor (CS) was presented using a cotton applicator scented with 0.1 cc of lemon oil (Montreal, Argentina) during 5 min. Pups returned to the incubator where they remained for 1 h until commencement of the nipple attachment test.

During testing, pups were presented with an artificial nipple scented with lemon oil. Carved soft vinyl was used to shape a 25-mm long surrogate nipple also characterized by a rounded tip with a diameter of 1 mm. The base of the nipple was attached to the end of an angled dental probe, which served to allow precise control of the position of the nipple in relation to the pup and to establish physical distance between the newborn and the experimenter. Polyethylene tubing (PE 10, 0.58 mm inner diameter; Clay Adams, Sparks, MD) was inserted through the nipple. This tubing was attached to a syringe that contained distilled water. The tubing and the syringe, once filled with the distilled water, represented an open hydraulic system because the end of the tubing was opened and the syringe had a hole located in its upper body surface. Slight negative pressure produced by pups while attached to the nipple was necessary and sufficient to extract fluid from this device. Water availability through the nipple seems to facilitate attachment behavior but water does not induce conditioning in these circumstances (Smotherman et al., 1993). Before testing, pups were voided and body weights were registered. Immediately after termination of the test, body weights were also registered. An experimenter, blind to pre- and postnatal treatments, tested the pups via the artificial nipple technique. Exposure to the surrogate nipple involved gentle contact between the tip of the nipple and the oral area of the test subject. No attempt was made to force the tip of the nipple into the mouth of the pup (Petrov et al., 1997). Attachment was confirmed by periodic (every 30 s) gentle attempts to withdraw the nipple from the pup. The pup's active release of the nipple was considered to be a disengagement from the nipple (Nizhnikov et al., 2006c). Video records were obtained during conditioning and testing sessions.

ASSESSMENT OF MOTOR REACTIVITY FOLLOWING AN IC ADMINISTRATION OF ACD IN NEWBORNS (EXPERIMENT 2)

Two hours after delivery, pups prenatally exposed to 0 or 2 g/Kg EtOH, were IC administered with 0, 0.35 or 0.52 μmol ACD. One minute following ACD central administration, pups were placed in the conditioning chamber and were given five discrete presentations of lemon odor. Each odor exposure lasted for 5 s. One group of animals was presented with this odorant five times during the first 5 min of testing (1–5 min). These pups remained in the heated container for 5 additional minutes without further olfactory stimulation (Olfactory Treatment: Odor/No Odor). The remaining pups were placed in the heated container without lemon odor for the first 5 min, then were given five presentations of lemon odor distributed in 5 min (Olfactory Treatment: No Odor/Odor). Motor activity of all animals was videotaped.

EXPERIMENTAL DESIGN AND DATA ANALYSIS

Experiment 1 was a 2 (prenatal treatment: Water or EtOH) × 3 (US: vehicle, EtOH or ACD) between-subject factorial design. No more than one subject from a given litter was assigned to the same treatment condition (Holson and Pearce, 1992). Efforts were made to maintain an equivalent number of males and females per group. Number of pups per group was as follows: prenatal water/postnatal vehicle, $n = 12$; prenatal water/postnatal EtOH, $n = 10$; prenatal water/postnatal ACD, $n = 8$; prenatal EtOH/postnatal vehicle, $n = 11$; prenatal EtOH/postnatal EtOH, $n = 10$; and, prenatal EtOH/postnatal ACD, $n = 8$. During conditioning, duration and frequency of forelimb and hind limb movements were registered (results are shown in **Table 2**). During testing, the suckling response was further delineated separated into two components consisting of (1) measures of latency to grasp the nipple, total time spent on the nipple (referred as total attachment duration, calculated as the sum of the duration of all grasps), and mean grasp duration (total time divided by number of grasps), and (2) percentage of body weight gain (measure of fluid intake). Additionally, latency to perform limb movements during testing was also registered. All of these measures served as dependent variables.

Experiment 2 was defined by orthogonal variation in prenatal treatment (water or EtOH), postnatal ACD administration (0, 0.35, or 0.52 μmol), and order of odor presentation (groups No Odor/Odor and Odor/No Odor). Temporal block of testing (1–5 and 6–10 min) served as a within factor. Each group was composed by 7–9 pups. A total of 99 pups were utilized. Frequency of crawling, rolling, turning on side, probing and stretching were registered during the first 10 s of each minute of testing. Probing was registered when the neonate touched the

rounded wall of the heated container with its nose. Stretching was considered as coordinated extension of both hindlimbs, often accompanied by dorsoflexion of the back and elevation of the head. Overall motor activity was considered as the sum of the frequency of the mentioned behaviors. Latency to show any of these behaviors was also registered (we will refer to this variable as latency to exert an overt behavior). Separated mixed ANOVAs were used to analyze motor activity and latency to exhibit an overt behavior.

Data were evaluated using separate between-groups ANOVA procedures. Significant interactions were further analyzed using Tukey's HSD tests with a probability of Type I error set at 0.05. In this and prior studies, it was observed that sex systematically failed to exert significant effects or to interact with EtOH reinforcement (Nizhnikov et al., 2012; Pautassi et al., 2012a,b,c). For this reason, inferential processing of data were performed by collapsing sex across treatments.

RESULTS

EFFECTS OF PRENATAL EtOH EXPOSURE UPON MATERNAL AND NEONATAL PHYSICAL PARAMETERS

In Experiment 1, percentage of maternal body weight gained (%BWG) during gestational days 17–20, number of pups delivered alive and pup's body weight at birth were evaluated. Percentage of dam's %BWG during late pregnancy was calculated as follows: {[(maternal body weight at GD20—maternal body weight at GD17)/maternal body weight at GD17] × 100}. A One-Way ANOVA showed that prenatal treatments had no effect upon this index [$F_{(1,\ 23)} = 0.10$; $p = 0.75$]. Number of pups born alive was not affected by prenatal exposure to EtOH [$F_{(1,\ 23)} = 0.93$, $p = 0.35$]. On the other hand, pups' body weights at birth (averaged within each litter), were affected by prenatal treatment [$F_{(1,\ 23)} = 5.95$; $p < 0.025$]. Pups prenatally exposed to EtOH had lower body weights than control pups. These data have been summarized in **Table 1**.

In Experiment 2, neither percentage of maternal body weight gain during GDs 17–20, number of pups per litter, nor average litter body weight were significantly affected by prenatal treatment. Data have been summarized in **Table 1**.

Table 1 | Maternal and neonatal physical parameters registered in Experiments 1 and 2, as a function of prenatal treatments.

	Treatment during GD 17–20			
	Experiment 1		Experiment 2	
	Water	EtOH	Water	EtOH
Percentage of MBW gain (g.)	7.78 ± 0.49	7.97 ± 0.36	8.22 ± 0.87	7.15 ± 0.92
Number of pups per litter	8.42 ± 0.79	9.46 ± 0.74	8.50 ± 0.91	9.77 ± 0.96
Litter's average weight (g.)	5.02 ± 0.11	4.74* ± 0.04	5.05 ± 0.13	4.70 ± 0.14

Data are presented as mean ± SEM. Asterisk represent statistically different from water controls in the same experiment ($p < 0.05$).

ASSESSMENT OF ACD MOTIVATIONAL EFFECTS BY THE ARTIFICIAL NIPPLE TECHNIQUE (EXPERIMENT 1)

Conditioning session

Limb activity was registered during conditioning. Neither frequency [$F_{(2,\ 53)} = 0.84$, $p = 0.44$] of limb movement nor duration [$F_{(2,\ 53)} = 0.13$, $p = 0.87$] differed across treatments. These data have been summarized in **Table 2**.

Attachment behavior

Conditioned reinforcing effects of EtOH and ACD were observed in the analysis of total attachment duration [main effect of US drug: $F_{(2,\ 53)} = 8.27$; $p < 0.001$]. This dependent variable was significantly higher in neonates conditioned with EtOH ($p < 0.01$) or ACD ($p < 0.001$) compared to control pups. A similar profile was found when analyzing mean grasping duration [main effect of US drug: $F_{(2,\ 53)} = 3.65$; $p < 0.05$]. In this case, EtOH-treated pups displayed intermediate levels of mean grasping duration, whereas those pups treated with ACD differed significantly from the control group ($p < 0.05$). Additionally, latency to grasp the nipple varied as a function of drug utilized as US [$F_{(2,\ 53)} = 5.16$, $p < 0.01$]. Neonates from the control group had longer delays to grasp the artificial nipple than did EtOH ($p < 0.05$) or ACD ($p < 0.025$) treated pups. These results have been summarized in **Figure 1**.

Latency to initiate hindlimb or forelimb movements were recorded during testing. Latency to initiate limb movement was not affected by prenatal treatment or by the drug given during conditioning [$F_{(2,\ 53)} = 0.65$, $p = 0.53$]. Means and S.E. of the mean were as follow: for neonates prenatally treated with water and postnatally administered with vehicle: 26.73 ± 4.86, EtOH: 15.51 ± 5.52 and ACD: 25.01 ± 5.95 and for neonates prenatally exposed to EtOH and postnatally administered with vehicle: 19.27 ± 5.07, EtOH: 16.45 ± 5.32, and ACD: 13.67 ± 5.95.

Intake

%BWG varied as a function of drug administered during conditioning [main effect: $F_{(2,\ 53)} = 6.45$, $p < 0.01$]. *Post-hoc* testing indicated that neonates conditioned with EtOH, achieved higher %BWG than control siblings ($p < 0.001$). Neonates conditioned with ACD had intermediate levels of water consumption (means and S.E. were as follows: 0.17 ± 0.07 g; 0.51 ± 0.07 g and 0.38 ± 0.08 g for the control, EtOH and ACD groups, respectively). Prenatal treatment did not exert any significant effect

Table 2 | Data summarize mean + SEM duration and frequency of limb movements registered during the conditioning phase in Experiment 1.

US drug	Prenatal treatment			
	Water		EtOH	
	Frequency	Duration	Frequency	Duration
Vehicle	12.08 ± 2.06	144.85 ± 27.09	9.90 ± 2.15	95.06 ± 28.30
EtOH	11.90 ± 2.26	140.47 ± 29.68	12.50 ± 2.26	118.00 ± 29.68
ACD	14.25 ± 2.53	135.16 ± 33.18	8.62 ± 2.53	86.92 ± 33.18

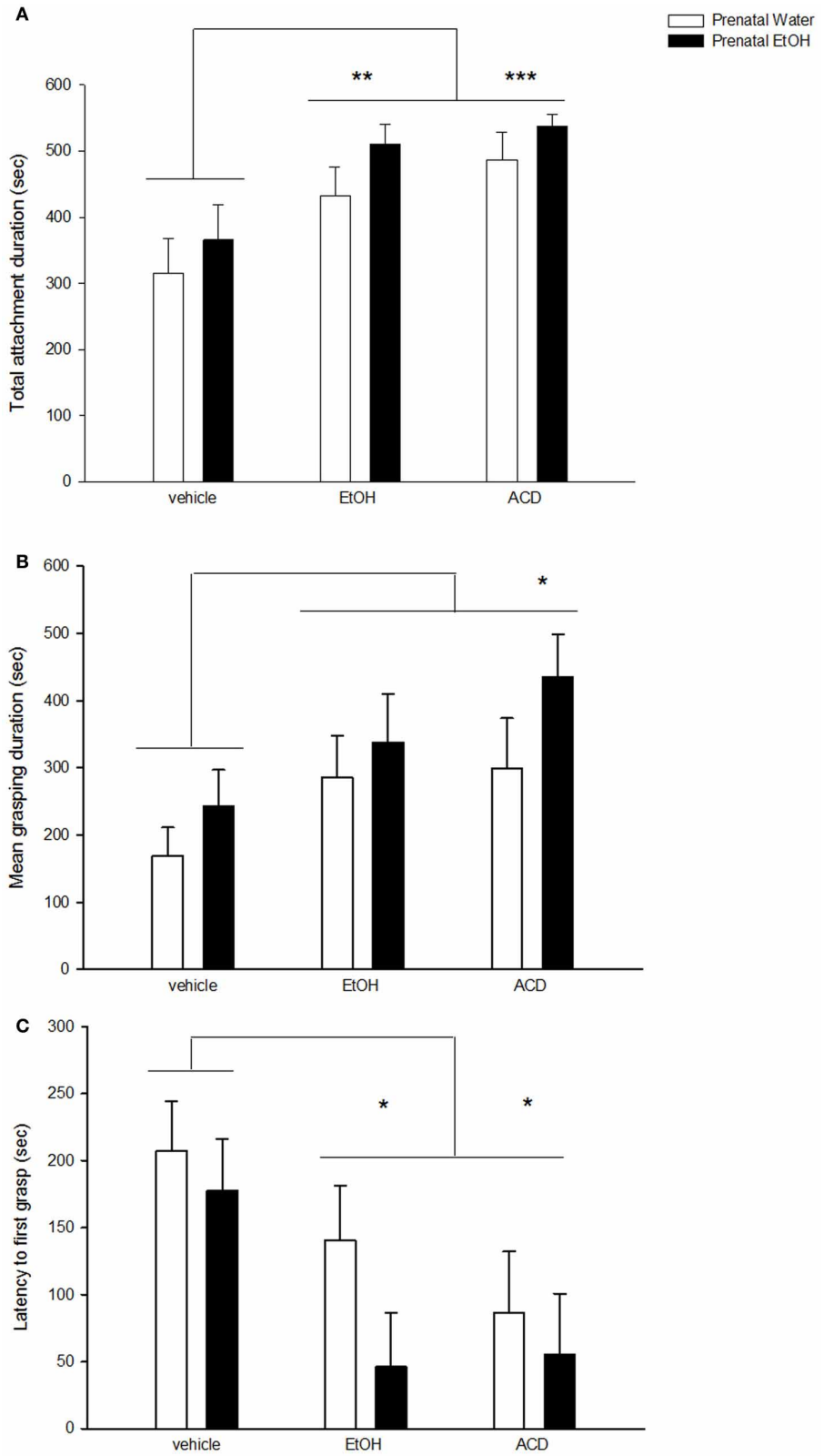


FIGURE 1 | Effect of prenatal exposure to EtOH on attachment behavior to a surrogate nipple in the presence of an odor conditioned to EtOH or ACD. Data represent mean + SEM in seconds of **(A)** total attachment duration, **(B)** grasp duration, and **(C)** latency to first grasp. Asterisk (*$p < 0.05$; **$p < 0.01$; and ***$p < 0.001$) depicts a significant difference from vehicle control group.

upon this dependent variable nor did it interact with postnatal treatment.

MOTOR REACTIVITY FOLLOWING CENTRAL ADMINISTRATION OF ACD AS A FUNCTION OF PRENATAL TREATMENT (EXPERIMENT 2)

A Four-Way ANOVA (prenatal treatment × ACD dose × olfactory treatment × time block) showed that frequency of motor activity was significantly higher during the first time block of testing relative to the second block [$F_{(1,\ 86)} = 10.21$, $p < 0.01$]. Interestingly, this effect was tempered by the interaction involving block and ACD treatment [$F_{(2,\ 86)} = 8.58$, $p < 0.001$]. This results have been depicted in **Figure 2A**. *Post-hoc* analysis indicated that while levels of activity during the first, relative to the second block of testing, were significantly higher in pups administered 0 or 0.35 μmol ACD (both p's < 0.01). This difference was not significant in neonates that received the highest ACD dose (0.52 μmol). In this group, levels of activity remained low across testing. This significant interaction is depicted in **Figure 2B**. When analyzing this dependent variable, prenatal treatment did not exert statistically significant effects.

A significant main effect of olfactory treatment was found in the analysis of latency to exert an overt behavior. Specifically, pups exposed to lemon odor at commencement of testing (Odor/No Odor group: 70 ± 8 s) exhibited significantly lower latency scores than pups initially exposed to a non-olfactory testing context (No Odor/Odor group: 96 ± 8 s). Presentation of lemon odorant rapidly recruited behavioral responsiveness. A significant interaction between prenatal and ACD treatments [$F_{(2,\ 86)} = 3.19$, $p < 0.05$] was found. *Post-hoc* comparisons indicated that neonates treated with the highest ACD dose (0.52 μmol) and with no prior EtOH experience exhibited significantly longer latencies ($p < 0.025$) than pups treated with a similar ACD dose but with a positive history of prenatal EtOH exposure (**Figure 3**).

DISCUSSION

As mentioned, EtOH exposure during gestation has been linked to later EtOH acceptance and drinking. In previous studies, we have found that EtOH exposure during GD 17–20 increases postnatal operant responsiveness for an intraorally infused EtOH solution (3% v/v) (March et al., 2009; Miranda-Morales et al., 2010). Nizhnikov et al. (2006a) found that prenatal exposure to the drug also increased the range of doses capable of sustaining appetitive conditioning when EtOH is administered into the peritoneum. This route of administration minimizes the possibility of recruiting the chemosensory properties of the drug. Nevertheless, some detection of such cues can be present due to hematopoietic

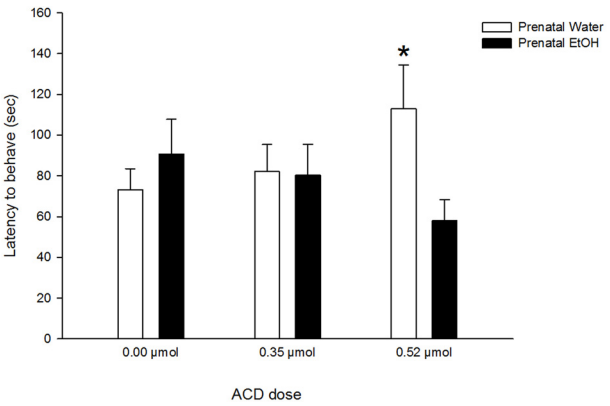


FIGURE 3 | Effect of prenatal exposure to EtOH on latency to exert a given overt behavior after central ACD administration. Data represent mean + SEM in seconds. Asterisk (*$p < 0.025$) depicts significant differences between prenatal treatments in the same ACD dose.

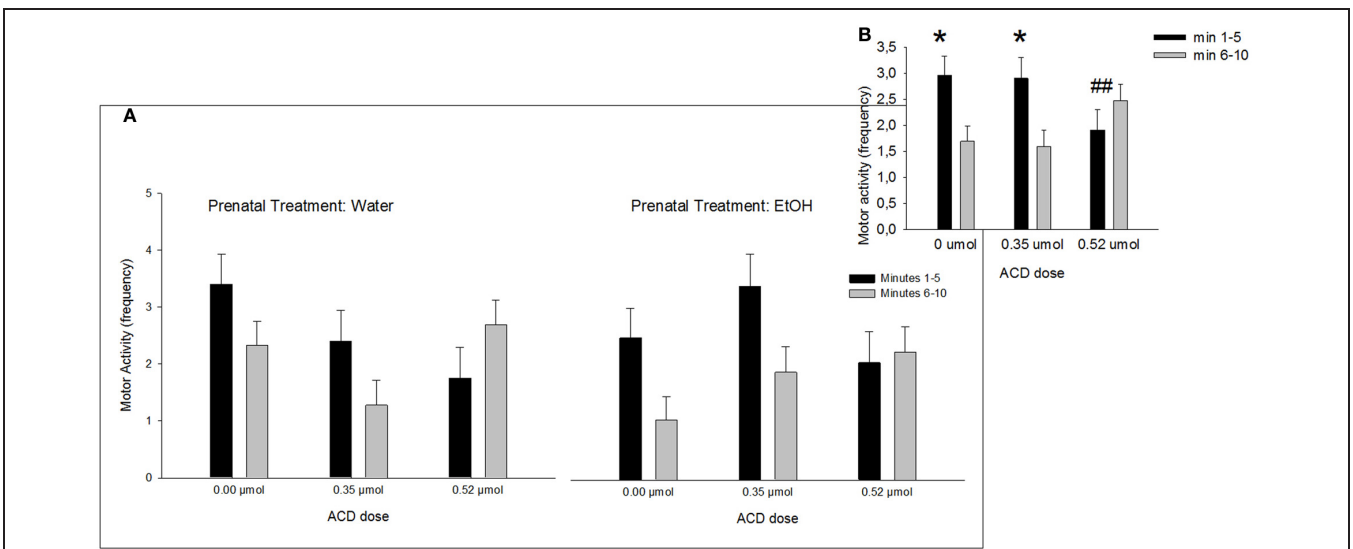


FIGURE 2 | Effect of central ACD administration on motor activity. Data represent mean + SEM of motor behavior (frequency) in consecutive 5 min blocks during testing. **(A)** Motor activity after central ACD administration as a function of prenatal exposure to EtOH. **(B)** Motor activity collapsed across prenatal treatments. Asterisk (*$p < 0.01$) depicts a significant difference between time intervals in the same ACD dose. Double numeral ($^{\#\#}p < 0.01$) indicates a significant difference between doses during minutes in block 1–5 min.

stimulation (Nizhnikov et al., 2006a). In Experiment 1, when EtOH and ACD were directly administered in the cisterna magna, differences in drug induced appetitive conditioning as a function of prenatal exposure to EtOH were not observed. However, only one dose of each substance was tested in the mentioned experiment. It is possible that an effect of prenatal exposure to EtOH upon central reinforcement of ACD might emerge when using a wider range of doses.

It could be objected that in the present study we did not employ untreated dams during gestation, since prenatal stress by itself can alter later responsiveness to EtOH (Van Waes et al., 2011a,b). However, in a recent study (March et al., 2013) we analyzed central EtOH and ACD appetitive conditioning in neonates using the artificial nipple technique. This study was conducted without any prior prenatal treatment and the results in terms of conditioned responses, promoted by the different pharmacological treatments, is analogous to those reported in the present article.

It can be argued that differences in suckling from the artificial nipple in neonates administered with EtOH or ACD compared to vehicle-administered siblings, can be explained either by pseudo-conditioning or by the effects of these substances upon motor activity. Regarding the first possibility, previous studies in which unpaired and US-only control groups have been included have consistently supported the notion that an associative learning mechanism underlies later increases in responses to lemon-nipple-water CS when EtOH is used as the US (Petrov et al., 2003; Nizhnikov et al., 2006b,c). The second alternative explanation can arise from the observation that in preweanlings, motor conditioned responses arising from the use of EtOH as a US can confound interpretations in tests related with drug reinforcement (Molina et al., 2007a). In Experiment 1 we directly assessed limb activity during conditioning and found no differences between groups. Additionally, in Experiment 2 we explicitly examined in freely moving neonates if ACD had specific motor effects in a time frame similar to the post-administration time during which conditioning occurred in Experiment 1. Groups administered with 0.35 μmol of ACD did not show any modifications in motor activity (whereas a higher dose did alter motor activity). This observation argues against the possibility that the behavioral expression of EtOH- or ACD-derived associative learning was due to motor effects of these pharmacological agents rather than their motivational properties.

Experiment 2 suggested that 0.52 μmol ACD had a sedative effect expressed as increased latency to display an overt behavior as well as a reduction in motor activity. These effects were not present in newborns prenatally exposed to EtOH, perhaps due to the development of tolerance to ACD effects. In adult rats and mice, increased locomotion has been induced by acute challenges with central ACD (in rats; Correa et al., 2003a,b, 2009; Sanchez-Catalan et al., 2009); in mice: Correa et al., 1999, 2001a. Sedative effects of ACD have been found in adult mice (Holtzman and Schneider, 1974; Correa et al., 2001b; Quertemont et al., 2004; Tambour et al., 2006, 2007) and, to a minor extent, in adult rats (Myers et al., 1987). In infant rats, ACD seems to stimulate motor activity (Pautassi et al., 2011). Current discrepancies concerning stimulatory vs. sedative effects in adults are difficult to reconcile due to differences in methodologies for inducing ACD, including direct administration of ACD vs. alteration of EtOH metabolism, as well as differences in route of administration and strain of rodent.

In summary, the present study found that very early in ontogeny (1) classical olfactory conditioning occurs when either EtOH or ACD are used as US, (2) this conditioning is expressed as an increase in suckling and in water ingestion from an artificial nipple scented with the odorant previously paired with intracisternal ACD (0.35 μmol) or EtOH and (3) motor activity, when the animals are allowed to move freely in the conditioning context, is decreased after an acute challenge with a high dose of ACD (0.52 μmol). This later effect emerged in animals without a prenatal history of EtOH exposure, whereas for animals prenatally exposed to EtOH, no sedative effect upon motor activity was observed. It is important to note that the emergence of sedative effects appears to coincide with the perception of aversive interoceptive effects of the drug while resistance to this effects has been linked to higher susceptibility to EtOH reinforcement (Arias et al., 2009). If prenatal EtOH exposure reduces sedative effects of the drug it may also ameliorate its aversive properties. In turn, this might help explain the heightened disposition to consume EtOH observed in prenatally exposed subjects and their sensitivity to alternative positive effects of the drug.

There is some controversy in the literature regarding the effect of central ACD in mediating EtOH effects. Uncertainty originates from the observation that very low ACD is detected in the brain after EtOH administration (Gill et al., 1992; Hunt, 1996). However, the participation of ACD has been shown not only when directly administering this substance in the brain (Rodd-Henricks et al., 2002; Correa et al., 2003b, 2009) but also by manipulating EtOH metabolism (Arizzi-LaFrance et al., 2006; Font et al., 2006; Correa et al., 2008; Pastor and Aragon, 2008; Enrico et al., 2009). In the present study, from a behavioral perspective, EtOH and ACD exerted a similar magnitude of appetitive conditioning. Also, we have previously observed that EtOH and ACD reinforcement is similarly inhibited by sequestering ACD trough d-penicillamine (March et al., 2013).

The role of ACD and acetate in mediating EtOH postabsortive effects has been widely studied in adult animals. However, we should be cautious in extrapolating findings in adult animals to expected results in newborns since there are marked differences in metabolic systems (both peripheral and central) between these ontogenetic stages. Catalase concentrations in cerebellum, striatum, cerebral hemispheres, and brain stem of the newborn rat are about eight times higher than those observed in the adult organism (Del Maestro and McDonald, 1987). Additionally, pre-weanlings have slower rates of EtOH blood metabolism after systemic administration compared to older animals (Silveri and Spear, 2000). In newborn and infantile rats, ACD-mediated positive reinforcement and behavioral activation has been observed (Nizhnikov et al., 2007; Pautassi et al., 2011). Nevertheless, the profile of behavioral effects derived from ACD has not been extensively studied during early ontogeny.

For example, negative reinforcement, a property that is believed to play an important role in EtOH use and abuse, has yet to be directly assessed. The gap in contemporary knowledge of the role of ACD in EtOH's postabsortive effects at this developmental period emphasizes the importance of the present study as well as the need for further tests of ontogenetic differences in EtOH acceptance and the role of its metabolites in these differences.

ACKNOWLEDGMENTS

This work was supported by grants from Agencia Nacional de Promoción Científica y Tecnológica (FONCyT, PICT 05-254) awarded to Juan C. Molina, by the Secretaría de Ciencia y Técnica (SECyT) from Universidad Nacional de Córdoba awarded to Samanta M. March, and by grants from NIAAA (AA11960, AA013098, AA015992) and NIMH (MH035219) awarded to Norman E. Spear.

REFERENCES

Abate, P., Pepino, M. Y., Dominguez, H. D., Spear, N. E., and Molina, J. C. (2000). Fetal associative learning mediated through maternal alcohol intoxication. *Alcohol. Clin. Exp. Res.* 24, 39–47. doi: 10.1111/j.1530-0277.2000.tb04551.x

Abate, P., Pueta, M., Spear, N. E., and Molina, J. C. (2008). Fetal learning about ethanol and later ethanol responsiveness: evidence against "safe" amounts of prenatal exposure. *Exp. Biol. Med. (Maywood)* 233, 139–154. doi: 10.3181/0703-MR-69

Abate, P., Spear, N. E., and Molina, J. C. (2001). Fetal and infantile alcohol-mediated associative learning in the rat. *Alcohol.Clin. Exp. Res.* 25, 989–998. doi: 10.1111/j.1530-0277.2001.tb02307.x

Abate, P., Varlinkaya, E. I., Cheslock, S. J., Spear, N. E., and Molina, J. C. (2002). Neonatal activation of alcohol-related prenatal memories: impact on the first suckling response. *Alcohol.Clin. Exp. Res.* 26, 1512–1522. doi: 10.1111/j.1530-0277.2002.tb02450.x

Alati, R., Al Mamun, A., Williams, G. M., O'callaghan, M., Najman, J. M., and Bor, W. (2006). *In utero* alcohol exposure and prediction of alcohol disorders in early adulthood: a birth cohort study. *Arch. Gen. Psychiatry* 63, 1009–1016. doi: 10.1001/archpsyc.63.9.1009

Aragon, C. M. G., Stotland, L. M., and Amit, Z. (1991). Studies on ethanol-brain catalase interaction: evidence for central ethanol oxidation. *Alcohol. Clin. Exp. Res.* 15, 158–164. doi: 10.1111/j.1530-0277.1991.tb01848.x

Arias, C., and Chotro, M. G. (2005). Increased preference for ethanol in the infant rat after prenatal ethanol exposure, expressed on intake and taste reactivity tests. *Alcohol. Clin. Exp. Res.* 29, 337–346. doi: 10.1097/01.ALC.0000156115.35817.21

Arias, C., and Chotro, M. G. (2006). Ethanol-induced preferences or aversions as a function of age in preweanlings rats. *Behav. Neurosci.* 120, 710–718. doi: 10.1037/0735-7044.120.3.710

Arias, C., Mlewski, E. C., Hansen, C., Molina, J. C., Paglini, M. G., and Spear, N. E. (2010). Dopamine receptors modulate ethanol's locomotor-activating effects in preweanling rats. *Dev. Psychobiol.* 52, 13–23. doi: 10.1002/dev.20407

Arias, C., Molina, J. C., and Spear, N. E. (2009). Ethanol-mediated aversive learning as a function of locomotor activity in a novel environment in infant Sprague-Dawley rats. *Pharmacol. Biochem. Behav.* 92, 621–628. doi: 10.1016/j.pbb.2009.03.001

Arizzi-LaFrance, M. N., Correa, M., Aragon, C. M., and Salamone, J. D. (2006). Motor stimulant effects of ethanol injected into the substantia nigra pars reticulata: importance of catalase-mediated metabolism and the role of acetaldehyde. *Neuropsychopharmacology* 31, 997–1008. doi: 10.1038/sj.npp.1300849

Baer, J. S., Sampson, P. D., Barr, H. M., Connor, P. D., and Streissguth, A. P. (2003). A 21-year longitudinal analysis of the effects of prenatal alcohol exposure on young adult drinking. *Arch. Gen. Psychiatry* 60, 377–385. doi: 10.1001/archpsyc.60.4.377

Barbier, E., Pierrefiche, O., Vaudry, D., Vaudry, H., Daoust, M., and Naassila, M. (2008). Long-term alterations in vulnerability to addiction to drugs of abuse and in brain gene expression after early life ethanol exposure. *Neuropharmacology* 55, 1199–1211. doi: 10.1016/j.neuropharm.2008.07.030

Correa, M., Arizzi, M. N., Betz, A., Mingote, S., and Salamone, J. D. (2003a). Locomotor stimulant effects of intraventricular injections of low doses of ethanol in rats: acute and repeated administration. *Psychopharmacology (Berl.)* 170, 368–375. doi: 10.1007/s00213-003-1557-0

Correa, M., Arizzi, M. N., Betz, A., Mingote, S., and Salamone, J. D. (2003b). Open field locomotor effects in rats after intraventricular injections of ethanol and the ethanol metabolites acetaldehyde and acetate. *Brain Res. Bull.* 62, 197–202. doi: 10.1016/j.brainresbull.2003.09.013

Correa, M., Arizzi-Lafrance, M. N., and Salamone, J. D. (2009). Infusions of acetaldehyde into the arcuate nucleus of the hypothalamus induce motor activity in rats. *Life Sci.* 84, 321–327. doi: 10.1016/j.lfs.2008.12.013

Correa, M., Manrique, H. M., Font, L., Escrig, M. A., and Aragon, C. M. (2008). Reduction in the anxiolytic effects of ethanol by centrally formed acetaldehyde: the role of catalase inhibitors and acetaldehyde-sequestering agents. *Psychopharmacology (Berl.)* 200, 455–464. doi: 10.1007/s00213-008-1219-3

Correa, M., Miquel, M., Sanchis-Segura, C., and Aragon, C. M. (1999). Effects of chronic lead administration on ethanol-induced locomotor and brain catalase activity. *Alcohol* 19, 43–49. doi: 10.1016/S0741-8329(99)00023-3

Correa, M., Sanchis-Segura, C., and Aragon, C. M. (2001a). Brain catalase activity is highly correlated with ethanol-induced locomotor activity in mice. *Physiol. Behav.* 73, 641–647. doi: 10.1016/S0031-9384(01)00511-X

Correa, M., Sanchis-Segura, C., and Aragon, C. M. (2001b). Influence of brain catalase on ethanol-induced loss of righting reflex in mice. *Drug Alcohol Depend.* 65, 9–15. doi: 10.1016/S0376-8716(01)00142-9

Cheslock, S. J., Varlinskaya, E. I., Petrov, E. S., Silveri, M. M., Spear, L. P., and Spear, N. E. (2001). Ethanol as a reinforcer in the newborn's first suckling experience. *Alcohol. Clin. Exp. Res.* 25, 391–402. doi: 10.1097/00000374-200103000-00011

Cheslock, S. J., Varlinskaya, E. I., Petrov, E. S., and Spear, N. E. (2000). Rapid and robust olfactory conditioning with milk before suckling experience: promotion of nipple attachment in the newborn rat. *Behav. Neurosci.* 114, 484–495. doi: 10.1037/0735-7044.114.3.484

Chotro, M. G., and Arias, C. (2003). Prenatal exposure to ethanol increases ethanol consumption: a conditioned response? *Alcohol* 30, 19–28. doi: 10.1016/S0741-8329(03)00037-5

Del Maestro, R., and McDonald, W. (1987). Distribution of superoxide dismutase, glutathione peroxidase and catalase in developing rat brain. *Mech. Ageing Dev.* 41, 29–38. doi: 10.1016/0047-6374(87)90051-0

Domínguez, H. D., López, M. F., Chotro, M. G., and Molina, J. C. (1996). Perinatal responsiveness to alcohol's chemosensory cues as a function of prenatal alcohol administration during gestational days 17-20 in the rat. *Neurobiol. Learn. Mem.* 65, 103–112. doi: 10.1006/nlme.1996.0012

Enrico, P., Sirca, D., Mereu, M., Peana, A. T., Lintas, A., Golosio, A., et al. (2009). Acetaldehyde sequestering prevents ethanol-induced stimulation of mesolimbic dopamine transmission. *Drug Alcohol Depend.* 100, 265–271. doi: 10.1016/j.drugalcdep.2008.10.010

Escarabajal, M. D., De Witte, P., and Quertemont, E. (2003). Role of acetaldehyde in ethanol-induced conditioned taste aversion in rats. *Psychopharmacology (Berl.)* 167, 130–136. doi: 10.1007/s00213-003-1427-9

Font, L., Aragon, C. M., and Miquel, M. (2006). Ethanol-induced conditioned place preference, but not aversion, is blocked by treatment with D -penicillamine, an inactivation agent for acetaldehyde. *Psychopharmacology (Berl.)* 184, 56–64. doi: 10.1007/s00213-005-0224-z

Gill, K., Menez, J. F., Lucas, D., and Deitrich, R. A. (1992). Enzymatic production of acetaldehyde from ethanol in rat brain tissue. *Alcohol. Clin. Exp. Res.* 16, 910–915. doi: 10.1111/j.1530-0277.1992.tb01892.x

Holson, R. R., and Pearce, B. (1992). Principles and pitfalls in the analysis of prenatal treatment effects in

multiparous species. *Nuerotoxicol. Teratol.* 14, 221–228. doi: 10.1016/0892-0362(92)90020-B

Holtzman, S. G., and Schneider, F. H. (1974). Comparison of acetaldehyde and ethanol: depression of motor activity in mice. *Life Sci.* 14, 1243–1250. doi: 10.1016/0024-3205(74)90432-9

Hunt, W. A. (1996). Role of acetaldehyde in the actions of ethanol on the brain–a review. *Alcohol* 13, 147–151. doi: 10.1016/0741-8329(950)2026-8

Institute of Laboratory Animal Research, C.O.L.S., National Research Council. (1996). *Guide for the Care and Use of Laboratory Animals.* Washington, DC: The National Academies Press.

March, S. M., Abate, P., Spear, N. E., and Molina, J. C. (2009). Fetal exposure to moderate ethanol doses: heightened operant responsiveness elicited by ethanol-related reinforcers. *Alcohol. Clin. Exp. Res.* 33, 1981–1993. doi: 10.1111/j.1530-0277.2009.01037.x

March, S. M., Abate, P., Spear, N. E., and Molina, J. C. (2013). The role of acetaldehyde in ethanol reinforcement assessed by pavlovian conditioning in newborn rats. *Psychopharmacology (Berl)* 226, 491–499. doi: 10.1007/s00213-012-2920-2929

Miranda-Morales, R. S., Molina, J. C., Spear, N. E., and Abate, P. (2010). Participation of the endogenous opioid system in the acquisition of a prenatal ethanol-related memory: effects on neonatal and preweanling responsiveness to ethanol. *Physiol. Behav.* 101, 153–160. doi: 10.1016/j.physbeh.2010.04.033

Molina, J. C., Pautassi, R. M., Truxell, E., and Spear, N. (2007a). Differential motivational properties of ethanol during early ontogeny as a function of dose and postadministration time. *Alcohol* 41, 41–55. doi: 10.1016/j.alcohol.2007.01.005

Molina, J. C., Spear, N. E., Spear, L. P., Mennella, J. A., and Lewis, M. J. (2007b). The International society for developmental psychobiology 39th annual meeting symposium: alcohol and development: beyond fetal alcohol syndrome. *Dev. Psychobiol.* 49, 227–242. doi: 10.1002/dev.20224

Myers, W. D., Gibson, S., Ng, K. T., and Singer, G. (1987). Sex differences in acetaldehyde on body temperature and open-field performance in the rat. *Drug Alcohol Depend.* 19, 1–6. doi: 10.1016/0376-8716(87)90081-0

Nizhnikov, M. E., Molina, J. C., and Spear, N. E. (2007). Central reinforcing effects of ethanol are blocked by catalase inhibition. *Alcohol* 41, 525–534. doi: 10.1016/j.alcohol.2007.08.006

Nizhnikov, M. E., Molina, J. C., Varlinskaya, E. I., and Spear, N. E. (2006a). Prenatal ethanol exposure increases ethanol reinforcement in neonatal rats. *Alcohol. Clin. Exp. Res.* 30, 34–45. doi: 10.1111/j.1530-0277.2006.00009.x

Nizhnikov, M. E., Varlinskaya, E. I., Petrov, E. S., and Spear, N. E. (2006b). Reinforcing properties of ethanol in neonatal rats: involvement of the opioid system. *Behav. Neurosci.* 120, 267–280. doi: 10.1037/0735-7044.120.2.267

Nizhnikov, M. E., Varlinskaya, E. I., and Spear, N. E. (2006c). Reinforcing effects of central ethanol injections in newborn rat pups. *Alcohol. Clin. Exp. Res.* 30, 2089–2096. doi: 10.1111/j.1530-0277.2006.00253.x

Nizhnikov, M. E., Pautassi, R. M., Varlinskaya, E. I., Rahmani, P., and Spear, N. E. (2012). Ontogenetic differences in ethanol's motivational properties during infancy. *Alcohol* 46, 225–234. doi: 10.1016/j.alcohol.2011.09.026

Nizhnikov, M. E., Petrov, E. S., Varlinskaya, E. I., and Spear, N. E. (2002). Newborn rats' first suckling experience: taste differentiation and suckling plasticity. *Physiol. Behav.* 76, 181–198. doi: 10.1016/S0031-9384(01)00672-2

Pastor, R., and Aragon, C. M. (2008). Ethanol injected into the hypothalamic arcuate nucleus induces behavioral stimulation in rats: an effect prevented by catalase inhibition and naltrexone. *Behav. Pharmacol.* 19, 698–705. doi: 10.1097/FBP.0b013e328315ecd7

Pautassi, R. M., Nizhnikov, M. E., Acevedo, M. B., and Spear, N. E. (2012a). Early role of the kappa opioid receptor in ethanol-induced reinforcement. *Physiol. Behav.* 105, 1231–1241. doi: 10.1016/j.physbeh.2012.01.003

Pautassi, R. M., Nizhnikov, M. E., Fabio, M. C., and Spear, N. E. (2012b). Early maternal separation affects ethanol-induced conditioning in a nor-BNI insensitive manner, but does not alter ethanol-induced locomotor activity. *Pharmacol. Biochem. Behav.* 100, 630–638. doi: 10.1016/j.pbb.2011.11.005

Pautassi, R. M., Nizhnikov, M. E., Spear, N. E., and Molina, J. C. (2012c). Prenatal ethanol exposure leads to greater ethanol-induced appetitive reinforcement. *Alcohol* 46, 585–593. doi: 10.1016/j.alcohol.2012.05.004

Pautassi, R. M., Nizhnikov, M. E., Fabio, M. C., and Spear, N. E. (2011). An acetaldehyde-sequestering agent inhibits appetitive reinforcement and behavioral stimulation induced by ethanol in preweanling rats. *Pharmacol. Biochem. Behav.* 97, 462–469. doi: 10.1016/j.pbb.2010.10.005

Pautassi, R. M., Nizhnikov, M. E., and Spear, N. E. (2009). Assessing appetitive, aversive, and negative ethanol-mediated reinforcement through an immature rat model. *Neurosci. Biobehav. Rev.* 33, 953–974. doi: 10.1016/j.neubiorev.2009.03.008

Petrov, E. S., Varlinskaya, E. I., Becker, L. A., and Smotherman, W. P. (1998). Endogenous mu opioid systems and suckling in the neonatal rat. *Physiol. Behav.* 65, 591–599. doi: 10.1016/S0031-9384(98)00228-5

Petrov, E. S., Varlinskaya, E. I., and Smotherman, W. P. (1997). The newborn rat ingests fluids through a surrogate nipple: a new technique for the study of early suckling behavior. *Physiol. Behav.* 62, 1155–1158. doi: 10.1016/S0031-9384(97)00310-7

Petrov, E. S., Varlinskaya, E. I., and Spear, N. E. (2001). Self-administration of ethanol and saccharin in newborn rats: effects on suckling plasticity. *Behav. Neurosci.* 115, 1318–1331. doi: 10.1037/0735-7044.115.6.1318

Petrov, E. S., Varlinskaya, E. I., and Spear, N. E. (2003). Reinforcement from pharmacological effects of ethanol in newborn rats. *Alcohol. Clin. Exp. Res.* 27, 1583–1591. doi: 10.1097/01.ALC.0000089960.62640.58

Proescholdt, M. G., Hutto, B., Brady, L. S., and Herkenham, M. (2000). Studies of cerebrospinal fluid flow and penetration into brain following lateral ventricle and cisterna magna injections of the tracer [^{14}C]inulin in rat. *Neuroscience* 95, 577–592.

Quertemont, E., Escarabajal, M. D., and De Witte, P. (2003). Role of catalase in ethanol-induced conditioned taste aversion: a study with 3-amino-1, 2, 4-triazole. *Drug Alcohol Depend.* 70, 77–83. doi: 10.1016/S0376-8716(02)00341-1

Quertemont, E., Tambour, S., Bernaerts, P., Zimatkin, S. M., and Tirelli, E. (2004). Behavioral characterization of acetaldehyde in C57BL/6J mice: locomotor, hypnotic, anxiolytic and amnesic effects. *Psychopharmacology (Berl.)* 177, 84–92. doi: 10.1007/s00213-004-1911-x

Rodd-Henricks, Z. A., Melendez, R. I., Zaffaroni, A., Goldstein, A., McBride, W. J., and Li, T. K. (2002). The reinforcing effects of acetaldehyde in the posterior ventral tegmental area of alcohol-preferring rats. *Pharmacol. Biochem. Behav.* 72, 55–64. doi: 10.1016/S0091-3057(01)00733-X

Sanchez-Catalan, M. J., Hipolito, L., Zornoza, T., Polache, A., and Granero, L. (2009). Motor stimulant effects of ethanol and acetaldehyde injected into the posterior ventral tegmental area of rats: role of opioid receptors. *Psychopharmacology (Berl.)* 204, 641–653. doi: 10.1007/s00213-009-1495-6

Silveri, M. M., and Spear, L. P. (2000). Ontogeny of ethanol elimination and ethanol-induced hypothermia. *Alcohol* 20, 45–53. doi: 10.1016/S0741-8329(99)00055-5

Smotherman, W. P., Arnold, H. M., and Robinson, S. R. (1993). Responses to ecologically relevant stimuli in the rat fetus: interactive effects of milk and an artificial nipple. *Dev. Psychobiol.* 26, 359–374. doi: 10.1002/dev.420260606

Smotherman, W. P., Goffman, D., Petrov, E. S., and Varlinskaya, E. I. (1997). Oral grasping of a surrogate nipple by the newborn rat. *Dev. Psychobiol.* 31, 3–17. doi: 10.1002/(SICI)1098-2302(199707)31:1<3::AID-DEV2>3.0.CO;2-Q

Tambour, S., Closon, C., Tirelli, E., and Quertemont, E. (2007). Effects of cyanamide and acetaldehyde accumulation on the locomotor stimulant and sedative effects of ethanol in mice. *Behav. Pharmacol.* 18, 777–784. doi: 10.1097/FBP.0b013e3282f158ca

Tambour, S., Didone, V., Tirelli, E., and Quertemont, E. (2006). Locomotor effects of ethanol and acetaldehyde after peripheral and intraventricular injections in Swiss and C57BL/6J mice. *Behav. Brain Res.* 172, 145–154. doi: 10.1016/j.bbr.2006.05.010

Van Waes, V., Darnaudery, M., Marrocco, J., Gruber, S. H., Talavera, E., Mairesse, J., et al. (2011a). Impact of early life stress on alcohol consumption and on the short- and long-term responses to alcohol in adolescent female rats. *Behav. Brain. Res.* 221, 43–49. doi: 10.1016/j.bbr.2011.02.033

Van Waes, V., Enache, M., Berton, O., Vinner, E., Lhermitte, M., Maccari, S., et al. (2011b). Effect of prenatal stress on alcohol

preference and sensitivity to chronic alcohol exposure in male rats. *Psychopharmacology (Berl.)* 214, 197–208. doi: 10.1007/s00213-009-1765-3

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 20 March 2013; accepted: 30 May 2013; published online: 17 June 2013.

Citation: March SM, Culleré ME, Abate P, Hernández JI, Spear NE and Molina JC (2013) Acetaldehyde reinforcement and motor reactivity in newborns with or without a prenatal history of alcohol exposure. Front. Behav. Neurosci. 7:69. doi: 10.3389/fnbeh.2013.00069

frontiers in
BEHAVIORAL NEUROSCIENCE

MINI REVIEW ARTICLE
published: 19 June 2013
doi: 10.3389/fnbeh.2013.00070

Acetaldehyde involvement in ethanol's postabsortive effects during early ontogeny

Samanta M. March[1,2]*, P. Abate[1,2] and Juan C. Molina[1,2]*

[1] Laboratorio de Alcohol, Ontogenia y Desarrollo, Instituto de Investigación Médica Mercedes y Martín Ferreyra, Córdoba, Argentina
[2] Department de Psicología, Facultad de Psicología, Universidad Nacional de Córdoba, Córdoba, Argentina

Edited by:
Merce Correa, Universitat Jaume, Spain

Reviewed by:
Merce Correa, Universitat Jaume, Spain
Sergey M. Zimatkin, Grodno State Medical University, Belarus
Marta Y. Pepino, Washington University School of Medicine, USA

***Correspondence:**
Samanta M. March and Juan C. Molina, Laboratorio de Alcohol, Ontogenia y Desarrollo, Instituto de Investigación Médica Mercedes y Martín Ferreyra, Friuli 2434, PO Box 389, Córdoba, 5016, Argentina
e-mail: smarch@immf.uncor.edu; juancmolina2003@yahoo.com

Clinical and biomedical studies sustains the notion that early ontogeny is a vulnerable window to the impact of alcohol. Experiences with the drug during these stages increase latter disposition to prefer, use or abuse ethanol. This period of enhanced sensitivity to ethanol is accompanied by a high rate of activity in the central catalase system, which metabolizes ethanol in the brain. Acetaldehyde (ACD), the first oxidation product of ethanol, has been found to share many neurobehavioral effects with the drug. Cumulative evidence supports this notion in models employing adults. Nevertheless very few studies have been conducted to analyze the role of ACD in ethanol postabsorptive effects, in newborns or infant rats. In this work we review recent experimental literature that syndicates ACD as a mediator agent of reinforcing aspects of ethanol, during early ontogenetic stages. We also show a meta-analytical correlational approach that proposes how differences in the activity of brain catalase across ontogeny, could be modulating patterns of ethanol consumption.

Keywords: early ethanol exposure, acetaldehyde, ontogeny, learning, appetitive response, suckling, newborns, infants

WHY SHOULD WE STUDY EARLY ETHANOL EXPOSURE EFFECTS?

Epidemiological and preclinical research indicate that prenatal and/or early postnatal ethanol experiences are associated with later responsiveness and affinity to the drug (for review see: Spear and Molina, 2005; Abate et al., 2008; Pautassi et al., 2009). Early initiation in ethanol drinking constitutes a risk factor for the development of later ethanol related problems. Adolescents who begin drinking at age 15 are four-times more likely to become alcohol-dependent than those who start at age 21 (Grant and Dawson, 1997). Ethanol intake usually begins during adolescence, with a decrease in the average age of initiation in the United States from 17.8 years in 1987 to 15.9 years in 1996 (Windle, 2003). Faden (2006) proposed that the peak year for alcohol initiation is even earlier (13–14 years). Heavy drinking in this population is also widespread; with 30% of 12th graders reporting that they had been drunk at least once in the last 30 days (Johnston et al., 2009). Finally, epidemiological data clearly reflects the high prevalence of alcohol use and abuse in both children and adolescents worldwide (e.g., Ahlstrom and Osterberg, 2005). Yet, ethanol exposure can occur involuntarily earlier in ontogeny (Spear and Molina, 2005). In some cultures alcohol soaked gauze pads are employed to avoid infections derived from the remainder of the umbilical cord or to treat stomach spasms (e.g., Dalt et al., 1991; Mancini, 2004). Transdermal absorption of alcohol and inhalation of alcohol vapors can lead to high infantile blood alcohol concentrations (Choonara, 1994). Maternal alcohol drinking during pregnancy can derive in severe damage, such as Fetal Alcohol Syndrome (West, 1994) but also in more subtle effects (i.e., increases the likelihood of ethanol drinking during adolescence: Baer et al., 1998). Ethanol drinking during breastfeeding is still highly prevalent and implies an alternative mode of early exposure to the drug (e.g., Mennella and Beauchamp, 1991; Mennella, 1999; Pepino and Mennella, 2004; Giglia and Binns, 2006). These epidemiological studies highlight the need for developing experimental animal models for understanding the above mentioned effects of early ethanol exposure.

WHAT DO WE KNOW ABOUT ETHANOL PHARMACOLOGICAL EFFECTS IN EARLY ONTOGENY?

Ethanol exerts a wide array of effects. It is rich in calories (7 kcal/g), has a distinctive taste characterized by a combination of sweet and bitter qualities (Molina et al., 2007b). These sensory features can serve as signals conditioned stimulus (CS) that predict biologically relevant events (unconditioned stimulus, US: Molina et al., 1986). Contingent experiences with the scent of alcohol and aversive stimulation result in conditioned avoidance towards the ethanol odor and reduce ethanol intake in 21-day-old rats (Serwatka et al., 1986). Ethanol can also act as an interoceptive context that, when present during the acquisition and retrieval phases of a learning situation, regulates the storage and expression of memories. State dependency mediated by ethanol has been reported in infant, adolescent, and adult rats (Fernandez-Vidal et al., 2003).

Ethanol's sensory features have been proposed to constitute a "taste barrier," precluding substantial intake of the drug (Pautassi et al., 2008a). Ethanol intake decreases sharply as ethanol concentration increases. If faced with a forced choice between water and a relatively low concentration of ethanol (1–5%), adult heterogeneous rats may show a modest preference for the drug, but

ethanol consumption decreases dramatically as higher concentrations are employed (Kiefer et al., 1987; Samson et al., 1988; Kiefer and Morrow, 1991). In contrast, naïve infant rats ingest surprisingly high quantities of ethanol—in concentrations as high as 30%—without initiation procedures (Truxell and Spear, 2004; Sanders and Spear, 2007; Truxell et al., 2007). Early acceptance for highly concentrated ethanol solutions seems to be mediated by the drug's pharmacological properties (Kozlov et al., 2008).

Ethanol induced reinforcement has been documented in neonates and infant rats. Newborn pups rapidly acquire a conditioned response towards an artificial nipple that signals pharmacological effects of very low doses of the drug (Petrov et al., 2003). Ethanol's central injection also promotes positive reinforcement at this age (Nizhnikov et al., 2006c). In infants, first and second order appetitive conditioning has been observed when using low- to high ethanol doses (0.5–2.5 g/kg: Molina et al., 2007a; Pautassi et al., 2008b). Both, locomotor stimulation and reinforcement have been observed during the raising limb of ethanol blood accumulation curve (Petrov et al., 2006; Nizhnikov et al., 2007).

In adult rodents, ethanol induces dopamine release in striatum and nucleus accumbens (Di Chiara and Imperato, 1986; Imperato and Di Chiara, 1986; Di Chiara and Imperato, 1988). The mesolimbic dopaminergic system is involved in ethanol induced motor activity, since D1-like or D2-like receptor antagonists reduce this effect in mice (Pastor et al., 2005). The dopaminergic system seems to modulate ethanol's activating effects also during early ontogeny, with D1 and D2-like receptors antagonist suppressing locomotor stimulation, as shown in adults (Arias et al., 2009b, 2010). However, compared to infants, adult rats seem more prone to ethanol-induced sedation. Locomotor stimulation has been mostly observed in mice or in selectively breed rat strains (Waller et al., 1986; Risinger et al., 1994; Colombo et al., 1998; Agabio et al., 2001). Infant rats show clear biphasic motor effects in response to ethanol, even in response to high doses (2.5 g/kg: Arias et al., 2009a, 2010).

Neurochemical effects of ethanol also include the opiate system as a mediator for DA release (Gianoulakis, 2009; Bodnar, 2012). For example, naltrexone suppresses ethanol self-administration and prevents ethanol-induced increases in dialysate dopamine levels (Gonzales and Weiss, 1998). In relation to early exposure to ethanol, when opiate antagonists are presented with ethanol administration during late gestation, the usual pattern of later enhancement of ethanol ingestion, appetitive orofacial responses and self-administration is prevented (Chotro and Arias, 2003; Arias and Chotro, 2005b; Miranda-Morales et al., 2010). Yet, the opiate system has been found to function differently in neonates compared to adults. Whereas kappa receptor stimulation has aversive effects in adults (Walker and Koob, 2008; Wee and Koob, 2010), newborn rats found it reinforcing (Smotherman and Robinson, 1992, 1994; Nizhnikov et al., 2012). In addition, ethanol reinforcement during this developmental stage requires the joint activation of mu and kappa receptors (Nizhnikov et al., 2006b). During the second postnatal week, a fully functional opioid system is needed to promote ethanol reinforcement. Disruption by either, naloxone or specific opioid antagonists (mu, delta, kappa) is sufficient for substantial reduction in consummatory and seeking behaviors associated with ethanol reinforcement (Miranda-Morales et al., 2012a,b).

Ethanol-derived aversive effects have been easily detected in adult rats, when pairing a taste stimulus (Chester and Cunningham, 1999) or an external context (Philpot et al., 2003) with stages of peak blood ethanol concentrations. In 11-day old infants, an administration of ethanol (3 g/kg) induces conditioned aversions (Molina and Chotro, 1989; Molina et al., 1989). However, the same ethanol dose does not induce conditioned aversion in younger organisms (8 day-old infants: Arias and Chotro, 2006). This developmental switch in ethanol motivational effects is not explained by a deficit in aversive learning capabilities since conditioned aversions are found using lithium chloride as an US (Smotherman, 1982a,b; Miller et al., 1990; Gruest et al., 2004).

The literature reviewed in the present section, along with the biomedical research discussed above, allow us to propose early ontogeny as a sensitive window during which contact with ethanol increases latter disposition to prefer, use or abuse ethanol.

THE ACETALDEHYDE HYPOTHESIS

As mentioned, during early ontogeny organisms show a high affinity towards ethanol positive effects, and these early experiences facilitate latter ethanol drinking. Even more, the increasing number of studies showing the role of ethanol metabolites on its postabsortive effects during adulthood (Quertemont et al., 2005), show a profound gap in the literature regarding its effects during early ontogeny; specially when considering that developmental changes in ethanol metabolism have been observed. Following systemic administration of ethanol, higher blood ethanol levels as well as a lower rate of clearance are observed in younger organisms compared to adults (Kelly et al., 1987). Central ethanol metabolism also differs across ontogeny. The catalase system activity, which oxidizes ethanol in the brain, is higher in pups compared to adults (Gill et al., 1992; Hamby-Mason et al., 1997). Thus, ethanol metabolism during early ontogeny seems to derive in high ACD levels in the brain (due to high catalase activity) along with slow ACD formation in the periphery (due to slow EtOH clearance). In addition, aldehyde dehydrogenase –ALDH- activity (acetaldehyde is used as a substrate) in the barrier structures of the brain makes only 10–30% during the antenatal period and increases gradually, reaching the activity specific for mature animals by PD 20–40 (Zimatkin and Lis, 1990).

It is interesting to note that the rate of central/peripheral accumulation of ACD has been involved in the perception of appetitive/aversive effects of the drug. Peripheral accumulation of ACD induces aversive effects (Quertemont, 2004). In fact, aversive reactions induced by ethanol drinking in patients treated with disulfiram [which increases ACD peripheral accumulation and allows peripheral ACD to cross the blood brain barrier, by blocking ALDH activity (Escrig et al., 2012) is the basis of its use in alcoholism's treatment (Kristenson, 1995)]. On the other hand, central ACD formation has been mainly linked to ethanol reinforcing effects (Wall et al., 1992; Hahn et al., 2006). The balance between brain and peripheral formation of ACD can determine the amount of ethanol intake (Chao, 1995). Taking

into account these considerations, it is possible to speculate that early ethanol acceptance may be due to high ACD generation in the brain, along with low ACD generation in the periphery.

In spite of cumulative evidence showing that ACD shares most of ethanol effects, such as hypothermic (Closon et al., 2009), locomotive (Correa et al., 2003; Arizzi-Lafrance et al., 2006; Correa et al., 2009; Sanchez-Catalan et al., 2009), sedative (Tambour et al., 2006, 2007), reinforcing (Quertemont and De Witte, 2001; Font et al., 2006a, 2008; Peana et al., 2008), anxiolytic (Correa et al., 2008) and aversive effects (Aragon et al., 1986) in adult rodents, very few studies have analyzed ACD's effects during early ontogeny. In the next section, evidence regarding its involvement in ethanol postabsortive effects in newborn and infant rats will be discussed.

BEHAVIORAL EFFECTS OF ACETALDEHYDE DURING EARLY ONTOGENY

Recently, ACD has been found to have a crucial role in ethanol reinforcement in newborns. Intracisternal administration of ethanol in close temporal contiguity with an odor cue (conditioned stimuli –CS-) derives in increased suckling response to an artificial nipple aromatized with the CS. Yet, this effect is blocked when ACD is inhibited by blocking catalase activity with sodium azide. This effect was specific to ethanol reinforcement since when an alternative central reinforcer was administered (dynorphin), catalase inhibition did not alter subsequent attachment to the scented nipple (Nizhnikov et al., 2007). However, the utilization of catalase inhibitors obstructs certain data interpretation since, along with inhibition of ACD formation, an accumulation of EtOH levels may also take place. Additionally, most catalase inhibitors have unspecific effects such as an impairment of learning produced by sodium azide (Lalonde et al., 1997). Considering this possibility, a study was conducted to corroborate ACD involvement in ethanol reinforcement. Once again, when ACD was neutralized, by inactivating ACD with d-penicillamine, ethanol reinforcement was blocked. Moreover, direct central administration of ACD (0.35 μmol) induced sustained suckling response to an odorized artificial nipple (March et al., 2013a). These studies have been pioneer in showing that ACD has in fact appetitive effects in newborn pups.

ACD reinforcement was also observed by March et al. (2013b), who replicated and extended previous results. Central ethanol or ACD administration induced appetitive conditioning in pups with or without prenatal exposure to ethanol. Pregnant rats received a daily i.g. administration of ethanol (2 g/kg, GDs 17–20). This pattern of prenatal alcohol administration increases appetitive responsiveness to ethanol in newborns (Nizhnikov et al., 2006a; March et al., 2009; Miranda-Morales et al., 2010), infants (Arias and Chotro, 2005a,b), and adolescent rats (Chotro and Arias, 2003). Results showed that ACD induced appetitive conditioning regardless of prior fetal experience with the drug. The conditioned appetitive response to an aromatized artificial nipple was similar to the results reported by March et al., 2013a. The explicit comparison between these studies is represented in **Figure 1**.

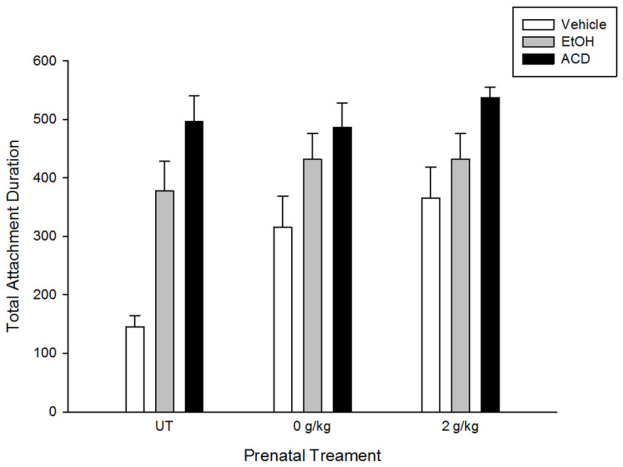


FIGURE 1 | Total attachment duration in naive newborns (untreated—UT—adapted from March et al., 2013a) and newborns prenatally exposed to water or ethanol (a dayly dose of 2 g/kg during gestational days 17–20; adapted from March et al., 2013b). Newborns were trained in a clasical conditioning paradigm in which an aromitic cue was associated to a central administration of vehicle, ethanol, or acetaldehyde. Testing took place 1 h later and consisted of presenting an artificial nipple aromatized with the conditioned cue.

Even though differences in appetitive effects induced by central ethanol (or ACD) administration as a function of prenatal treatments have not been observed, when a higher dose of ACD (0.52 μmol) is centrally administered, motor reactivity is differentially altered. Newborns lacking previous exposure to ethanol showed longer latencies to display motor activity following ACD administration than newborns prenatally exposed to ethanol. Considering this evidence, it is possible that prenatal ethanol exposure may not only induce conditioned appetitive response to the drug's chemosensory properties, but also lead to the development of tolerance to aversive effects induced either by ethanol or its metabolites.

The role of ACD in ethanol's motivational effects were also assessed in 2-week old infant rats (Pautassi et al., 2011). These subjects developed a tactile conditioned preference to a CS (sandpaper) previously paired with ethanol (1 g/kg, i.g.). Conditioning took place during the raising limb of the blood ethanol curve. At this early postadministration time, motor activation was also induced by ethanol. When the authors inactivated ACD by d-penicillamine administration, motivational and locomotive effects of ethanol were inhibited.

Ethanol reinforcing and stimulatory properties appear to be strongly related (Arias et al., 2009a). Interestingly, the ACD dose found to exert appetitive effects in newborns (0.35 μmol) induces motor stimulation in adults (Correa et al., 2003, 2009; Arizzi-Lafrance et al., 2006). The neurochemical bases of these effects have been studied during adulthood. It has been observed that, as well as ethanol, ACD activates the mesolimbic dopamine system (Melis et al., 2007; Diana et al.,

2008; Melis et al., 2009). Interestingly, sequestering of central ACD by d-penicillamine prevents ethanol-induced stimulation of the mesolimbic dopamine transmission (Enrico et al., 2009). The opiate system is also involved in ACD reinforcement. Naloxone produced a decrement in schedule-induced ACD self-administration (Myers et al., 1984). Additionally, enhancement of locomotor activity induced by administration of ACD or EtOH into the ventral tegmental area is reduced in animals previously given naltrexone, or β-funaltrexamine (Sanchez-Catalan et al., 2009). The involvement of dopaminergic and opiate activity in ethanol behavioral effects during early ontogeny has been previously discussed. To the extent in which these effects are due to ethanol or to its metabolic products still needs to be determined.

Is there additional support linking early ethanol affinity with high levels of central catalase activity and hence, heightened bioavailability of ACD? Can we find evidence in the literature establishing at least a correlation between levels of catalase activity and ethanol appetitiveness across ontogeny? To our knowledge, not in a specific article. But from a meta analytical perspective, the answer appears positive. Let's explain ourselves in this approach. First, we took into account developmental changes in catalase activity based on average scores (U/mg protein) observed in cerebral hemispheres, striatum, cerebellum and brain stem (Del Maestro and Mcdonald, 1987). There is a gradual decrease in these levels as a function of increasing age. In accordance with the developmental catalase curves shown by these authors, we extrapolated the corresponding values for postnatal days 12, 18, 22, 25, 28, 30, and 60. These values were linearly correlated with those reported by (Truxell and Spear, 2004; Truxell et al., 2007) in terms of blood ethanol levels obtained in alcohol consumption tests, performed at similar ages. Spontaneous ethanol intake also decreases gradually across development. These tests were conducted with relatively high ethanol concentrations (either 15% or 30% v/v ethanol). In both cases the correlations (Pearson's correlation coefficients) were significantly positive. The values of the correlation indexes were as follows: when employing 15% v/v ethanol, $r = 0.82$ ($p < 0.025$) and when utilizing 30% v/v ethanol, $r = 0.93$ ($p < 0.01$). These results have been depicted in **Figure 2**. From a meta-analytical correlational approach, the hypothesis that differential levels of brain catalase during ontogeny modulates patterns of ethanol affinity, seems to receive support.

Ethanol behavioral and motivational effects (appetitive, aversive and anxyolitic) have been extensively studied in adults and infants. The capability of ACD to induce similar effects has not been comprehensively studied during early ontogeny. For example, negative reinforcement, a property that is believed to play an important role in alcohol use and abuse, has yet to be directly assessed. The gap in contemporary knowledge of the role of ACD in ethanol's postabsortive effects at this developmental period emphasizes the importance of the studies discussed in this section as well as the need for further tests of ontogenetic differences in alcohol acceptance and consumption and the role of EtOH's metabolites in these differences.

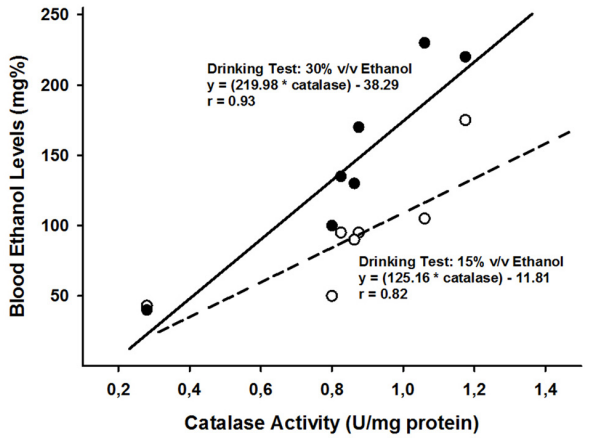


FIGURE 2 | The figure illustrates regression lines and linear correlations when taking into account catalase activity levels (U/mg protein; data derived from the study of -Del Maestro and Mcdonald, 1987-) and blood ethanol levels attained in alcohol drinking tests performed with either 15 or 30% v/v ethanol (data derived from Truxell and Spear, 2004; Truxell et al., 2007). In order to perform these correlations, catalase and blood ethanol levels were calculated for the following postnatal days: 12, 18, 22, 25, 28, 30, and 60.

CONCLUSION

The literature revised here does show that the developing organism can be exposed to ethanol unwillingly. During early ethanol exposure, the organism can learn about ethanol effects (or ACD-mediated effects) and modify its latter responsiveness to the drug (or to associated sensory cues) as a function of these experiences. Compared to the growing body of evidence regarding the modulation of ACD in ethanol effects in adults, little is known about its effects in very young organisms. Even more, to our knowledge, there are none studies addressing acetate effects in newborns or infants.

Until now, ACD levels produced following ethanol administration have not been assessed in newborn or infant rats. The methodological difficulties regarding its measurement *in vivo* and *in vitro* have been discussed elsewhere (Correa et al., 2012) and exceed the purpose of the present review. Yet, studies performed in adult subjects shows compelling evidence signaling ACD's involvement in ethanol effects, since blocking its production (Aragon et al., 1985; Sanchis-Segura et al., 1999; Font et al., 2008; Pastor and Aragon, 2008) or sequestering it (Font et al., 2005, 2006b; Peana et al., 2008; Enrico et al., 2009), inhibits some ethanol behavioral effects. Additionally, potentiating ACD by inducing catalase activity (Correa et al., 1999, 2001) or by administering it directly into the brain (Rodd-Henricks et al., 2002; Correa et al., 2003, 2009; Rodd et al., 2005; Diana et al., 2008; Sanchez-Catalan et al., 2009) mimics the effects typically observed after ethanol administration. Even more, some experimental studies have already started to analyze underlying neurochemical mechanism (Rodd et al., 2003; Hipolito et al., 2009, 2010). We have provided evidence that during early ontogeny ACD has also a role in ethanol reinforcement. Future studies including ontogenetical comparisons are certainly needed.

REFERENCES

Abate, P., Pueta, M., Spear, N. E., and Molina, J. C. (2008). Fetal learning about ethanol and later ethanol responsiveness: evidence against "safe" amounts of prenatal exposure. *Exp. Biol. Med.* 233, 139–154. doi: 10.3181/0703-MR-69

Agabio, R., Carai, M. A., Lobina, C., Pani, M., Reali, R., Vacca, G., et al. (2001). Alcohol stimulates motor activity in selectively bred Sardinian alcohol-preferring (sP), but not in Sardinian alcohol-nonpreferring (sNP), rats. *Alcohol* 23, 123–126. doi: 10.1016/S0741-8329(00)00144-0

Ahlstrom, S. A., and Osterberg, E. S. (2005). International perspectives on adolescent and young adult drinking. *Alcohol. Res. Health* 28, 258–268.

Aragon, C. M., Abitbol, M., and Amit, Z. (1986). Acetaldehyde may mediate reinforcement and aversion produced by ethanol. An examination using a conditioned taste-aversion paradigm. *Neuropharmacology* 25, 79–83. doi: 10.1016/0028-3908(86)90062-6

Aragon, C. M., Spivak, K., and Amit, Z. (1985). Blockade of ethanol induced conditioned taste aversion by 3-amino-1,2,4-triazole: evidence for catalase mediated synthesis of acetaldehyde in rat brain. *Life Sci.* 37, 2077–2084. doi: 10.1016/0024-3205(85)90579-X

Arias, C., and Chotro, M. G. (2005a). Increased palatability of ethanol after prenatal ethanol exposure is mediated by the opioid system. *Pharmacol. Biochem. Behav.* 82, 434–442. doi: 10.1016/j.pbb.2005.09.015

Arias, C., and Chotro, M. G. (2005b). Increased preference for ethanol in the infant rat after prenatal ethanol exposure, expressed on intake and taste reactivity tests. *Alcohol. Clin. Exp. Res.* 29, 337–346. doi: 10.1097/01.ALC.0000156115.35817.21

Arias, C., and Chotro, M. G. (2006). Ethanol-induced preferences or aversions as a function of age in preweanlings rats. *Behav. Neurosci.* 120, 710–718. doi: 10.1037/0735-7044.120.3.710

Arias, C., Mlewski, E. C., Hansen, C., Molina, J. C., Paglini, M. G., and Spear, N. E. (2010). Dopamine receptors modulate ethanol's locomotor-activating effects in preweanling rats. *Dev. Psychobiol.* 52, 13–23. doi: 10.1002/dev.20407

Arias, C., Mlewski, E. C., Molina, J. C., and Spear, N. E. (2009a). Ethanol induces locomotor activating effects in preweanling Sprague-Dawley rats. *Alcohol* 43, 13–23. doi: 10.1016/j.alcohol.2008.09.002

Arias, C., Mlewski, E. C., Molina, J. C., and Spear, N. E. (2009b). Naloxone and baclofen attenuate ethanol's locomotor-activating effects in preweanling Sprague-Dawley rats. *Behav. Neurosci.* 123, 172–180. doi: 10.1037/a0014049

Arizzi-Lafrance, M. N., Correa, M., Aragon, C. M., and Salamone, J. D. (2006). Motor stimulant effects of ethanol injected into the substantia nigra pars reticulata: importance of catalase-mediated metabolism and the role of acetaldehyde. *Neuropsychopharmacology* 31, 997–1008. doi: 10.1038/sj.npp.1300849

Baer, J. S., Barr, H. M., Bookstein, F. L., Sampson, P. D., and Streissguth, A. P. (1998). Prenatal alcohol exposure and family history of alcoholism in the ethiology of adolescent alcohol problems. *J. Stud. Alcohol.* 59, 533–543.

Bodnar, R. J. (2012). Endogenous opiates and behavior: 2011. *Peptides* 38, 463–522. doi: 10.1016/j.peptides.2012.09.027

Closon, C., Didone, V., Tirelli, E., and Quertemont, E. (2009). Acetaldehyde and the hypothermic effects of ethanol in mice. *Alcohol. Clin. Exp. Res.* 33, 2005–2014. doi: 10.1111/j.1530-0277.2009.01039.x

Colombo, G., Agabio, R., Lobina, C., Reali, R., Vacca, G., and Gessa, G. L. (1998). Stimulation of locomotor activity by voluntarily consumed ethanol in Sardinian alcohol-preferring rats. *Eur. J. Pharmacol.* 357, 109–113. doi: 10.1016/S0014-2999(98)00560-3

Correa, M., Arizzi-Lafrance, M. N., and Salamone, J. D. (2009). Infusions of acetaldehyde into the arcuate nucleus of the hypothalamus induce motor activity in rats. *Life Sci.* 84, 321–327. doi: 10.1016/j.lfs.2008.12.013

Correa, M., Arizzi, M. N., Betz, A., Mingote, S., and Salamone, J. D. (2003). Open field locomotor effects in rats after intraventricular injections of ethanol and the ethanol metabolites acetaldehyde and acetate. *Brain Res. Bull.* 62, 197–202. doi: 10.1016/j.brainresbull.2003.09.013

Correa, M., Manrique, H. M., Font, L., Escrig, M. A., and Aragon, C. M. (2008). Reduction in the anxiolytic effects of ethanol by centrally formed acetaldehyde: the role of catalase inhibitors and acetaldehyde-sequestering agents. *Psychopharmacology (Berl.)* 200, 455–464. doi: 10.1007/s00213-008-1219-3

Correa, M., Miquel, M., Sanchis-Segura, C., and Aragon, C. M. (1999). Acute lead acetate administration potentiates ethanol-induced locomotor activity in mice: the role of brain catalase. *Alcohol. Clin. Exp. Res.* 23, 799–805. doi: 10.1111/j.1530-0277.1999.tb04186.x

Correa, M., Salamone, J. D., Segovia, K. N., Pardo, M., Longoni, R., Spina, L., et al. (2012). Piecing together the puzzle of acetaldehyde as a neuroactive agent. *Neurosci. Biobehav. Rev.* 36, 404–430. doi: 10.1016/j.neubiorev.2011.07.009

Correa, M., Sanchis-Segura, C., and Aragon, C. M. (2001). Brain catalase activity is highly correlated with ethanol-induced locomotor activity in mice. *Physiol. Behav.* 73, 641–647. doi: 10.1016/S0031-9384(01)00511-X

Chao, H. M. (1995). Alcohol and the mystique of flushing. *Alcohol. Clin. Exp. Res.* 19, 104–109. doi: 10.1111/j.1530-0277.1995.tb01477.x

Chester, J. A., and Cunningham, C. L. (1999). Baclofen alters ethanol-stimulated activity but not conditioned place preference or taste aversion in mice. *Pharmacol. Biochem. Behav.* 63, 325–331. doi: 10.1016/S0091-3057(98)00253-6

Choonara, I. (1994). Percutaneous drug absorption and administration. *Arch. Dis. Child* 71, F73–F74. doi: 10.1136/fn.71.2.F73

Chotro, M. G., and Arias, C. (2003). Prenatal exposure to ethanol increases ethanol consumption: a conditioned response? *Alcohol* 30, 19–28. doi: 10.1016/S0741-8329(03)00037-5

Dalt, L., Dall'Amico, R., Laverda, A., Chemollo, C., and Chiandetti, L. (1991). Percutaneous ethyl alcohol intoxication in a one-month-old infant. *Pediatr. Emerg. Care* 7, 343–344. doi: 10.1097/00006565-199112000-00005

Del Maestro, R., and Mcdonald, W. (1987). Distribution of superoxide dismutase, glutathione peroxidase and catalase in developing rat brain. *Mech. Ageing Dev.* 41, 29–38. doi: 10.1016/0047-6374(87)90051-0

Di Chiara, G., and Imperato, A. (1986). Preferential stimulation of dopamine release in the nucleus accumbens by opiates, alcohol, and barbiturates: studies with transcerebral dialysis in freely moving rats. *Ann. N.Y. Acad. Sci.* 473, 367–381. doi: 10.1111/j.1749-6632.1986.tb23629.x

Di Chiara, G., and Imperato, A. (1988). Opposite effects of mu and kappa opiate agonists on dopamine release in the nucleus accumbens and in the dorsal caudate of freely moving rats. *J. Pharmacol. Exp. Therap.* 244, 1067–1080.

Diana, M., Peana, A. T., Sirca, D., Lintas, A., Melis, M., and Enrico, P. (2008). Crucial role of acetaldehyde in alcohol activation of the mesolimbic dopamine system. *Ann. N.Y. Acad. Sci.* 1139, 307–317. doi: 10.1196/annals.1432.009

Enrico, P., Sirca, D., Mereu, M., Peana, A. T., Lintas, A., Golosio, A., et al. (2009). Acetaldehyde sequestering prevents ethanol-induced stimulation of mesolimbic dopamine transmission. *Drug Alcohol Depend.* 100, 265–271. doi: 10.1016/j.drugalcdep.2008.10.010

Escrig, M. A., Pardo, M., Aragon, C. M., and Correa, M. (2012). Anxiogenic and stress-inducing effects of peripherally administered acetaldehyde in mice: similarities with the disulfiram-ethanol reaction. *Pharmacol. Biochem. Behav.* 100, 404–412. doi: 10.1016/j.pbb.2011.10.002

Faden, V. B. (2006). Trends in initiation of alcohol use in the United States 1975 to 2003. *Alcohol. Clin. Exp. Res.* 30, 1011–1022. doi: 10.1111/j.1530-0277.2006.00115.x

Fernandez-Vidal, J. M., Spear, N. E., and Molina, J. C. (2003). Adolescent rats discriminate a mild state of ethanol intoxication likely to act as an appetitive unconditioned stimulus. *Alcohol* 30, 45–60. doi: 10.1016/S0741-8329(03)00093-4

Font, L., Aragon, C. M., and Miquel, M. (2006a). Ethanol-induced conditioned place preference, but not aversion, is blocked by treatment with D -penicillamine, an inactivation agent for acetaldehyde. *Psychopharmacology (Berl.)* 184, 56–64. doi: 10.1007/s00213-005-0224-z

Font, L., Aragon, C. M., and Miquel, M. (2006b). Voluntary ethanol consumption decreases after the inactivation of central acetaldehyde by d-penicillamine. *Behav. Brain Res.* 171, 78–86. doi: 10.1016/j.bbr.2006.03.020

Font, L., Miquel, M., and Aragon, C. M. (2005). Prevention of ethanol-induced behavioral stimulation by D-penicillamine: a sequestration agent for acetaldehyde. *Alcohol Clin. Exp. Res.* 29, 1156–1164. doi: 10.1097/01.ALC.0000171945.30494.AF

Font, L., Miquel, M., and Aragon, C. M. (2008). Involvement of

brain catalase activity in the acquisition of ethanol-induced conditioned place preference. *Physiol. Behav.* 93, 733–741. doi: 10.1016/j.physbeh.2007.11.026

Gianoulakis, C. (2009). Endogenous opioids and addiction to alcohol and other drugs of abuse. *Curr. Top. Med. Chem.* 9, 999–1015. doi: 10.2174/156802609789630956

Giglia, R., and Binns, C. (2006). Alcohol and lactation: a systematic review. *Nutr. Dietet.* 63, 103–116. doi: 10.1111/j.1747-0080.2006.00056.x

Gill, K., Menez, J. F., Lucas, D., and Deitrich, R. A. (1992). Enzymatic production of acetaldehyde from ethanol in rat brain tissue. *Alcohol. Clin. Exp. Res.* 16, 910–915. doi: 10.1111/j.1530-0277.1992.tb01892.x

Gonzales, R. A., and Weiss, F. (1998). Suppression of ethanol-reinforced behavior by naltrexone is associated with attenuation of the ethanol-induced increase in dialysate dopamine levels in the nucleus accumbens. *J. Neurosci.* 18, 10663–10671.

Grant, B. F., and Dawson, D. A. (1997). Age at onset of alcohol use and its association with DSM-IV alcohol abuse and dependence: results from the National Longitudinal Alcohol Epidemiologic Survey. *J. Subst. Abuse.* 9, 103–110. doi: 10.1016/S0899-3289(97)90009-2

Gruest, N., Richer, P., and Hars, B. (2004). Emergence of long-term memory for conditioned aversion in the rat fetus. *Dev. Psychobiol.* 44, 189–198. doi: 10.1002/dev.20004

Hahn, C. Y., Huang, S. Y., Ko, H. C., Hsieh, C. H., Lee, I. H., Yeh, T. L., et al. (2006). Acetaldehyde involvement in positive and negative alcohol expectancies in han Chinese persons with alcoholism. *Arch. Gen. Psychiatry* 63, 817–823. doi: 10.1001/archpsyc.63.7.817

Hamby-Mason, R., Chen, J. J., Schenker, S., Perez, A., and Henderson, G. I. (1997). Catalase mediates acetaldehyde formation from ethanol in fetal and neonatal rat brain. *Alcohol. Clin. Exp. Res.* 21, 1063–1072. doi: 10.1111/j.1530-0277.1997.tb04255.x

Hipolito, L., Sanchez-Catalan, M. J., Granero, L., and Polache, A. (2009). Local salsolinol modulates dopamine extracellular levels from rat nucleus accumbens: shell/core differences. *Neurochem. Int.* 55, 187–192. doi: 10.1016/j.neuint.2009.02.014

Hipolito, L., Sanchez-Catalan, M. J., Zornoza, T., Polache, A., and Granero, L. (2010). Locomotor stimulant effects of acute and repeated intrategmental injections of salsolinol in rats: role of mu-opioid receptors. *Psychopharmacology (Berl.)* 209, 1–11. doi: 10.1007/s00213-009-1751-9

Imperato, A., and Di Chiara, G. (1986). Preferential stimulation of dopamine release in the nucleus accumbens of freely moving rats by ethanol. *J. Pharmacol. Exp. Therap.* 239, 219–228.

Johnston, L. D., O'Malley, P. M., Bachman, J. G., and Schulenberg, J. E. (2009). *Monitoring the Future: National Results on Adolescent Drug Use-Overview of Key Findings 2008.* Bethesda: National Institute on Drug Abuse.

Kelly, S. J., Bonthius, D. J., and West, J. R. (1987). Developmental changes in alcohol pharmacokinetics in rats. *Alcohol. Clin. Exp. Res.* 11, 281–286. doi: 10.1111/j.1530-0277.1987.tb01308.x

Kiefer, S. W., Lawrence, G. J., and Metzler, C. W. (1987). Alcohol preference in rats lacking gustatory neocortex. *Alcohol* 4, 37–43. doi: 10.1016/0741-8329(87)90058-9

Kiefer, S. W., and Morrow, N. S. (1991). Odor cue mediation of alcohol aversion learning in rats lacking gustatory neocortex. *Behav. Neurosci.* 105, 25–32. doi: 10.1037/0735-7044.105.1.25

Kozlov, A. P., Varlinskaya, E. I., and Spear, N. E. (2008). Ethanol, saccharin, and quinine: early ontogeny of taste responsiveness and intake. *Alcohol. Clin. Exp. Res.* 32, 294–305. doi: 10.1111/j.1530-0277.2007.00581.x

Kristenson, H. (1995). How to get the best out of antabuse. *Alcohol Alcohol.* 30, 775–783.

Lalonde, R., Joyal, C. C., and Beaudin, S. (1997). Effects of sodium azide on motor activity, motor coordination, and learning. *Pharmacol. Biochem. Behav.* 56, 67–71. doi: 10.1016/S0091-3057(96)00158-X

Mancini, A. J. (2004). Skin. *Pediatrics* 113, 1114–1119.

March, S. M., Abate, P., Spear, N. E., and Molina, J. C. (2009). Fetal exposure to moderate ethanol doses: heightened operant responsiveness elicited by ethanol-related reinforcers. *Alcohol. Clin. Exp. Res.* 33, 1981–1993. doi: 10.1111/j.1530-0277.2009.01037.x

March, S. M., Abate, P., Spear, N. E., and Molina, J. C. (2013a). The role of acetaldehyde in ethanol reinforcement assessed by Pavlovian conditioning in newborn rats. *Psychopharmacology (Berl.)* 226, 491–499. doi: 10.1007/s00213-012-2920-9

March, S. M., Culleré, M. E., Abate, P., Hernández, J., Spear, N. E., and Molina, J. C. (2013b). Acetaldehyde reinforcement and motor reactivity in newborns with or without a prenatal history of alcohol exposure. *Front. Behav. Neurosci.* 7:69. doi: 10.3389/fnbeh.2013.00069

Melis, M., Diana, M., Enrico, P., Marinelli, M., and Brodie, M. S. (2009). Ethanol and acetaldehyde action on central dopamine systems: mechanisms, modulation, and relationship to stress. *Alcohol* 43, 531–539. doi: 10.1016/j.alcohol.2009.05.004

Melis, M., Enrico, P., Peana, A. T., and Diana, M. (2007). Acetaldehyde mediates alcohol activation of the mesolimbic dopamine system. *Eur. J. Neurosci.* 26, 2824–2833. doi: 10.1111/j.1460-9568.2007.05887.x

Mennella, J. A. (1999). "The transfer of alcohol to human milk: sensory implications and effects on mother-infant interaction," in *Alcohol and Alcoholism: Brain and Development,* eds N. E. Spear, L. P. Spear, H. J. Hannigan, and C. R. Goodlett (Hillsdale, NJ: Lawrence Erlbaum Associates), 177–198.

Mennella, J. A., and Beauchamp, G. K. (1991). The transfer of alcohol to human milk. Effects on flavor and the infant's behavior. *N. Engl. J. Med.* 325, 981–985. doi: 10.1056/NEJM199110033251401

Miller, J. S., Molina, J. C., and Spear, N. E. (1990). Ontogenetic differences in the expression of odor-aversion learning in 4- and 8-day-old rats. *Dev. Psychobiol.* 23, 319–330. doi: 10.1002/dev.420230404

Miranda-Morales, R. S., Molina, J. C., Spear, N. E., and Abate, P. (2010). Participation of the endogenous opioid system in the acquisition of a prenatal ethanol-related memory: effects on neonatal and preweanling responsiveness to ethanol. *Physiol. Behav.* 101, 153–160. doi: 10.1016/j.physbeh.2010.04.033

Miranda-Morales, R. S., Molina, J. C., Spear, N. E., and Abate, P. (2012a). Naloxone attenuation of ethanol-reinforced operant responding in infant rats in a re-exposure paradigm. *Psychopharmacology (Berl.)* 219, 235–246. doi: 10.1007/s00213-011-2402-5

Miranda-Morales, R. S., Spear, N. E., Nizhnikov, M. E., Molina, J. C., and Abate, P. (2012b). Role of mu, delta and kappa opioid receptors in ethanol-reinforced operant responding in infant rats. *Behav. Brain Res.* 234, 267–277. doi: 10.1016/j.bbr.2012.07.002

Molina, J. C., Chotro, G., and Spear, N. E. (1989). Early (preweanling) recognition of alcohol's orosensory cues resulting from acute ethanol intoxication. *Behav. Neural Biol.* 51, 307–325. doi: 10.1016/S0163-1047(89)90961-8

Molina, J. C., and Chotro, M. G. (1989). Acute alcohol intoxication paired with appetitive reinforcement: effects upon ethanol intake in infant rats. *Behav. Neural Biol.* 51, 326–345. doi: 10.1016/S0163-1047(89)90974-6

Molina, J. C., Pautassi, R. M., Truxell, E., and Spear, N. (2007a). Differential motivational properties of ethanol during early ontogeny as a function of dose and postadministration time. *Alcohol* 41, 41–55. doi: 10.1016/j.alcohol.2007.01.005

Molina, J. C., Spear, N. E., Spear, L. P., Mennella, J. A., and Lewis, M. J. (2007b). The international society for developmental psychobiology 39th annual meeting symposium: alcohol and development: beyond fetal alcohol syndrome. *Dev. Psychobiol.* 49, 227–242. doi: 10.1002/dev.20224

Molina, J. C., Serwatka, J., and Spear, N. E. (1986). Alcohol drinking patterns of young adult rats as a function of infantile aversive experiences with alcohol odor. *Behav. Neural Biol.* 46, 257–271. doi: 10.1016/S0163-1047(86)90191-3

Myers, W. D., Ng, K. T., and Singer, G. (1984). Effects of naloxone and buprenorphine on intravenous acetaldehyde self-injection in rats. *Physiol. Behav.* 33, 449–455. doi: 10.1016/0031-9384(84)90168-9

Nizhnikov, M. E., Molina, J. C., and Spear, N. E. (2007). Central reinforcing effects of ethanol are blocked by catalase inhibition. *Alcohol* 41, 525–534. doi: 10.1016/j.alcohol.2007.08.006

Nizhnikov, M. E., Molina, J. C., Varlinskaya, E. I., and Spear, N. E. (2006a). Prenatal ethanol exposure increases ethanol reinforcement in neonatal rats. *Alcohol. Clin. Exp. Res.* 30, 34–45. doi: 10.1111/j.1530-0277.2006.00009.x

Nizhnikov, M. E., Varlinskaya, E. I., Petrov, E. S., and Spear, N. E. (2006b). Reinforcing properties of ethanol in neonatal rats: involvement of the opioid system. *Behav. Neurosci.* 120, 267–280. doi: 10.1037/0735-7044.120.2.267

Nizhnikov, M. E., Varlinskaya, E. I., and Spear, N. E. (2006c). Reinforcing

effects of central ethanol injections in newborn rat pups. *Alcohol. Clin. Exp. Res.* 30, 2089–2096. doi: 10.1111/j.1530-0277.2006.00253.x

Nizhnikov, M. E., Pautassi, R. M., Varlinskaya, E. I., Rahmani, P., and Spear, N. E. (2012). Ontogenetic differences in ethanol's motivational properties during infancy. *Alcohol* 46, 225–234. doi: 10.1016/j.alcohol.2011.09.026

Pastor, R., and Aragon, C. M. (2008). Ethanol injected into the hypothalamic arcuate nucleus induces behavioral stimulation in rats: an effect prevented by catalase inhibition and naltrexone. *Behav. Pharmacol.* 19, 698–705. doi: 10.1097/FBP.0b013e328315ecd7

Pastor, R., Miquel, M., and Aragon, C. M. (2005). Habituation to test procedure modulates the involvement of dopamine D2- but not D1-receptors in ethanol-induced locomotor stimulation in mice. *Psychopharmacology (Berl.)* 182, 436–446. doi: 10.1007/s00213-005-0115-3

Pautassi, R. M., Molina, J. C., and Spear, N. (2008a). Infant rats exhibit aversive learning mediated by ethanol's orosensory effects but are positively reinforced by ethanol's post-ingestive effects. *Pharmacol. Biochem. Behav.* 88, 393–402. doi: 10.1016/j.pbb.2007.09.012

Pautassi, R. M., Myers, M., Spear, L. P., Molina, J. C., and Spear, N. E. (2008b). Adolescent but not adult rats exhibit ethanol-mediated appetitive second-order conditioning. *Alcohol. Clin. Exp. Res.* 32, 2016–2027. doi: 10.1111/j.1530-0277.2008.00789.x

Pautassi, R. M., Nizhnikov, M. E., Fabio, M. C., and Spear, N. E. (2011). An acetaldehyde-sequestering agent inhibits appetitive reinforcement and behavioral stimulation induced by ethanol in preweanling rats. *Pharmacol. Biochem. Behav.* 97, 462–469. doi: 10.1016/j.pbb.2010.10.005

Pautassi, R. M., Nizhnikov, M. E., and Spear, N. E. (2009). Assessing appetitive, aversive, and negative ethanol-mediated reinforcement through an immature rat model. *Neurosci. Biobehav. Rev.* 33, 953–974. doi: 10.1016/j.neubiorev.2009.03.008

Peana, A. T., Enrico, P., Assaretti, A. R., Pulighe, E., Muggironi, G., Nieddu, M., et al. (2008). Key role of ethanol-derived acetaldehyde in the motivational properties induced by intragastric ethanol: a conditioned place preference study in the rat. *Alcohol. Clin. Exp. Res.* 32, 249–258. doi: 10.1111/j.1530-0277.2007.00574.x

Pepino, M. Y., and Mennella, J. A. (2004). Advice given to women in Argentina about breast-feeding and the use of alcohol. *Revista Panamericada de Salud Pública* 16, 408–414. doi: 10.1590/S1020-49892004001200007

Petrov, E. S., Nizhnikov, M. E., Varlinskaya, E. I., and Spear, N. E. (2006). Dynorphin A (1-13) and responsiveness of the newborn rat to a surrogate nipple: immediate behavioral consequences and reinforcement effects in conditioning. *Behav. Brain Res.* 170, 1–14. doi: 10.1016/j.bbr.2006.03.012

Petrov, E. S., Varlinskaya, E. I., and Spear, N. E. (2003). Reinforcement from pharmacological effects of ethanol in newborn rats. *Alcohol. Clin. Exp. Res.* 27, 1583–1591. doi: 10.1097/01.ALC.0000089960.62640.58

Philpot, R. M., Badanich, K. A., and Kirstein, C. L. (2003). Place conditioning: age-related changes in the rewarding and aversive effects of alcohol. *Alcohol. Clin. Exp. Res.* 27, 593–599. doi: 10.1111/j.1530-0277.2003.tb04395.x

Quertemont, E. (2004). Genetic polymorphism in ethanol metabolism: acetaldehyde contribution to alcohol abuse and alcoholism. *Mol. Psychiatry* 9, 570–581. doi: 10.1038/sj.mp.4001497

Quertemont, E., and De Witte, P. (2001). Conditioned stimulus preference after acetaldehyde but not ethanol injections. *Pharmacol. Biochem. Behav.* 68, 449–454. doi: 10.1016/S0091-3057(00)00486-X

Quertemont, E., Tambour, S., and Tirelli, E. (2005). The role of acetaldehyde in the neurobehavioral effects of ethanol: a comprehensive review of animal studies. *Prog. Neurobiol.* 75, 247–274. doi: 10.1016/j.pneurobio.2005.03.003

Risinger, F. O., Malott, D. H., Prather, L. K., Niehus, D. R., and Cunningham, C. L. (1994). Motivational properties of ethanol in mice selectively bred for ethanol-induced locomotor differences. *Psychopharmacology (Berl.)* 116, 207–216. doi: 10.1007/BF02245064

Rodd-Henricks, Z. A., Melendez, R. I., Zaffaroni, A., Goldstein, A., Mcbride, W. J., and Li, T. K. (2002). The reinforcing effects of acetaldehyde in the posterior ventral tegmental area of alcohol-preferring rats. *Pharmacol. Biochem. Behav.* 72, 55–64. doi: 10.1016/S0091-3057(01)00733-X

Rodd, Z. A., Bell, R. L., Zhang, Y., Goldstein, A., Zaffaroni, A., Mcbride, W. J., et al. (2003). Salsolinol produces reinforcing effects in the nucleus accumbens shell of alcohol-preferring (P) rats. *Alcohol. Clin. Exp. Res.* 27, 440–449. doi: 10.1097/01.ALC.0000056612.89957.B4

Rodd, Z. A., Bell, R. L., Zhang, Y., Murphy, J. M., Goldstein, A., and Zaffaroni, A. (2005). Regional heterogeneity for intracranial self administration of ethanol and acetaldehyde within the ventral tegmental area of alcohol-preferring (P) rats: Involvement of dopamine and serotonin. *Neuropharmachology* 30, 330–338. doi: 10.1038/sj.npp.1300561

Samson, H. H., Tolliver, G. A., Pfeffer, A. O., Sadeghi, K., and Haraguchi, M. (1988). Relation of ethanol self-administration to feeding and drinking in a nonrestricted access situation in rats initiated to self-administer ethanol using the sucrose-fading technique. *Alcohol* 5, 375–385. doi: 10.1016/0741-8329(88)90024-9

Sanchez-Catalan, M. J., Hipolito, L., Zornoza, T., Polache, A., and Granero, L. (2009). Motor stimulant effects of ethanol and acetaldehyde injected into the posterior ventral tegmental area of rats: role of opioid receptors. *Psychopharmacology (Berl.)* 204, 641–653. doi: 10.1007/s00213-009-1495-6

Sanchis-Segura, C., Miquel, M., Correa, M., and Aragon, C. M. (1999). The catalase inhibitor sodium azide reduces ethanol-induced locomotor activity. *Alcohol* 19, 37–42. doi: 10.1016/S0741-8329(99)00016-6

Sanders, S., and Spear, N. E. (2007). Ethanol acceptance is high during early infancy and becomes still higher after previous ethanol ingestion. *Alcohol. Clin. Exp. Res.* 31, 1148–1158. doi: 10.1111/j.1530-0277.2007.00400.x

Serwatka, J., Molina, J. C., and Spear, N. E. (1986). Weanlings' transfer of conditioned ethanol aversion from olfaction to ingestion depends on the unconditioned stimulus. *Behav. Neural Biol.* 45, 57–70. doi: 10.1016/S0163-1047(86)80006-1

Smotherman, W. P. (1982a). In utero chemosensory experience alters taste preferences and corticosterone responsiveness. *Behav. Neural Biol.* 36, 61–68. doi: 10.1016/S0163-1047(82)90245-X

Smotherman, W. P. (1982b). Odor aversion learning by the rat fetus. *Phsysiol. Behav.* 29, 769–771. doi: 10.1016/0031-9384(82)90322-5

Smotherman, W. P., and Robinson, S. R. (1992). Kappa opioid mediation of fetal responses to milk. *Behav. Neurosci.* 106, 396–407. doi: 10.1037/0735-7044.106.2.396

Smotherman, W. P., and Robinson, S. R. (1994). Classical conditioning of opioid activity in the fetal rat. *Behav. Neurosci.* 108, 951–961. doi: 10.1037/0735-7044.108.5.951

Spear, N. E., and Molina, J. C. (2005). Fetal or infantil exposure to alcohol promotes ethanol ingestion in adolescence and adulthood: a theoretical review. *Alcohol. Clin. Exp. Res.* 25, 909–929. doi: 10.1097/01.ALC.0000171046.78556.66

Tambour, S., Closon, C., Tirelli, E., and Quertemont, E. (2007). Effects of cyanamide and acetaldehyde accumulation on the locomotor stimulant and sedative effects of ethanol in mice. *Behav. Pharmacol.* 18, 777–784. doi: 10.1097/FBP.0b013e3282f158ca

Tambour, S., Didone, V., Tirelli, E., and Quertemont, E. (2006). Locomotor effects of ethanol and acetaldehyde after peripheral and intraventricular injections in Swiss and C57BL/6J mice. *Behav. Brain Res.* 172, 145–154. doi: 10.1016/j.bbr.2006.05.010

Truxell, E., and Spear, N. E. (2004). Immediate acceptance of ethanol in infant rats: ontogenetic differences with moderate but not high ethanol concentration. *Alcohol. Clin. Exp. Res.* 28, 1200–1211. doi: 10.1097/01.ALC.0000134220.34842.18

Truxell, E. M., Molina, J. C., and Spear, N. E. (2007). Ethanol intake in the juvenile, adolescent, and adult rat: effects of age and prior exposure to ethanol. *Alcohol. Clin. Exp. Res.* 31, 755–765. doi: 10.1111/j.1530-0277.2007.00358.x

Walker, B. M., and Koob, G. F. (2008). Pharmacological evidence for a motivational role of kappa-opioid systems in ethanol dependence. *Neuropsychopharmacology* 33, 643–652. doi: 10.1038/sj.npp.1301438

Wall, T. L., Thomasson, H. R., Schuckit, M. A., and Ehlers, C. L. (1992). Subjective feelings of alcohol intoxication in Asians with genetic variations of ALDH2 alleles. *Alcohol. Clin. Exp. Res.* 16, 991–995. doi: 10.1111/j.1530-0277.1992.tb01907.x

Waller, M. B., Murphy, J. M., Mcbride, W. J., Lumeng, L., and Li, T.

K. (1986). Effect of low dose ethanol on spontaneous motor activity in alcohol-preferring and -nonpreferring lines of rats. *Pharmacol. Biochem. Behav.* 24, 617–623. doi: 10.1016/0091-3057(86)90567-8

Wee, S., and Koob, G. F. (2010). The role of the dynorphin-kappa opioid system in the reinforcing effects of drugs of abuse. *Psychopharmacology (Berl.)* 210, 121–135. doi: 10.1007/s00213-010-1825-8

West, J. R. (1994). Recent findings on the mechanisms by which alcohol damages the developing nervous system. *Alcohol Alcohol. Suppl.* 2, 395–399.

Windle, M. (2003). Alcohol use among adolescents and young adults. *Alcohol. Res. Health* 27, 79–85.

Zimatkin, S. M., and Lis, R. E. (1990). [Aldehyde dehydrogenase activity in the rat brain during ontogenesis]. *Arkh. Anat. Gistol. Embriol.* 98, 27–33.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 20 March 2013; accepted: 01 June 2013; published online: 19 June 2013.

Citation: March SM, Abate P and Molina JC (2013) Acetaldehyde involvement in ethanol's postabsortive effects during early ontogeny. Front. Behav. Neurosci. 7:70. doi: 10.3389/fnbeh.2013.00070

frontiers in
BEHAVIORAL NEUROSCIENCE

GENERAL COMMENTARY
published: 01 July 2013
doi: 10.3389/fnbeh.2013.00075

Acetaldehyde mediates the ethanol effects in developing brain

S. M. Zimatkin*

Department of Histology, Cytology and Embryology, Grodno State Medical University, Grodno, Belarus
**Correspondence: zimatkin@grsmu.by*

Edited by:
Merce Correa, Universitat Jaume I, Spain

A commentary on

Acetaldehyde involvement in ethanol postabsortive effects during early ontogeny
by March, S. M., Abate, P., and Molina, J. C. (2013). Front. Behav. Neurosci. 7:70. doi: 10.3389/fnbeh.2013.00070

The mini review of March et al. (2013) is devoted to a very interesting problem: the role of acetaldehyde in the effects of ethanol in early postnatal development. It summarizes and analyzes carefully the literature available on the problem (about 120 references in the list) and in general the review looks quite comprehensive.

In the beginning, as an introduction, the authors provide epidemiological data concerning the early initiation of alcohol consumption in children. In addition they mentioned the possibility of involuntary alcohol exposure of fetus or newborn as a result of maternal alcohol consumption during pregnancy and breastfeeding. It can induce both a dramatic disturbances in all organs, especially in the brain, and increased alcohol consumption in their future life. It may be postulated that "the earlier beginning of alcohol exposure, the higher risk of alcoholism development." It confirms the need of using the animal models to understand the mechanisms of early alcohol effects.

Then the authors describe the data available on the pharmacological, mostly behavioral, effects of ethanol in early ontogeny. They indicate a very intensive consumption and no aversion to high concentrations (doses) of ethanol in naïve infant rats, in contrast to adult ones. The neurochemical mechanisms of those specificities of alcohol related behavior is still unclear.

The next section of the review is called "The acetaldehyde hypothesis." The authors speculate there that early ethanol acceptance may be due to high acetaldehyde (AC) generation from ethanol in the brain, along with low AC generation in the periphery. Indeed, the high central AC generation can be due to the increased activity in pups brains of catalase, the main ethanol-oxidizing enzyme in the brain of adults. Unfortunately, nobody measures in pups another important enzyme, Cytochrome P450 2E1, also oxidizing ethanol in adult brains (Zimatkin et al., 2006).

Another side of the process is the removal of ethanol-derives AC by aldehyde dehydrogenase (ALDH). Therefore, it is better to talk about the accumulation of AC as a result of the balance between AC production and elimination. The accumulation of ethanol-derived AC in the brain structures in early ontogeny can be more significant then in adults due not only to its higher generation, but also its insufficient removal, because of the known lower activity of ALDH. It was found that ALDH activity (with acetaldehyde as a substrate) in the rat brain neurons of various types in fetus was very low and up to the 10th postnatal day makes 45–70% of adult level, and then increases sharply, and by the 20th day approaches the definitive level (Zimatkin and Lis, 1990). Both the higher catalase and low ALDH activity provide the conditions for higher accumulation of AC in the brain. But nobody measures the level of ethanol-derived AC in pups *in vivo*.

In addition, the induction of catalase following intragastric administration of ethanol to pups on the 5th and 6th day after birth as well as appearance of AC adducts with proteins in the brain have been found (Hamby-Mason et al., 1997). Following intragastric administration of ethanol to rats during pregnancy we found the increased activity of brain ALDH in pups. It can impact to accumulation of ethanol-derived AC in the developing brain.

Another problem is the possibilities for production and elimination of AC in the specific brain structures and types of neurons, responsible for the special brain functions, like dopaminergic neurons (with specifically high activity of catalase and low activity of ALDH) (Zimatkin and Lindros, 1996), activation of which as known mediates the reinforcing properties of ethanol.

The final section of the review is devoted to the behavioral effects of acetaldehyde during early ontogeny. The important role of AC in mediation of the motivational and motor effects of ethanol has been demonstrated. Blocking of AC formation from ethanol (by catalase inhibitors) or sequestering of central AC (by d-penicillamine) decreases the ethanol behavioral effects and stimulation of mesolimbic dopamine system directly of through the opioid system. The provided meta analysis of the literature data demonstrates a high positive correlation between brain catalase activity and ethanol consumption in developing rats, demonstrating the important role of ethanol-derived AC in alcohol craving in early ontogeny.

REFERENCES

Hamby-Mason, R., Chen, J. J., Schenker, S., Perez, A., and Henderson, G. I. (1997). Catalase mediates acetaldehyde formation from ethanol in fetal and neonatal rat brain. *Alcohol. Clin. Exp. Res.* 21, 1063–1072. doi: 10.1111/j.1530-0277.1997.tb04255.x

March, S. M., Abate, P., and Molina, J. C. (2013). Acetaldehyde involvement in ethanol postabsortive effects during early ontogeny. *Front. Behav. Neurosci.* 7:70. doi: 10.3389/fnbeh.2013.00070

Zimatkin, S. M., and Lindros, K. O. (1996). Distribution of catalase in rat brain: aminergic neurons as possible targets for ethanol effects. *Alcohol Alcohol.* 31, 167–174. doi: 10.1093/oxfordjournals.alcalc.a008128

Zimatkin, S. M., and Lis, R. E. (1990). Aldehyde dehydrogenase activity in the rat brain during ontogenesis. *Arkh. Anat. Gistol. Embriol.* 98, 27–33.

Zimatkin, S. M., Pronko, S. P., Vasiliou, V., Gonzalez, F. J., and Deitrich, R. A. (2006). Enzymatic mechanisms of ethanol oxidation in the brain. *Alcohol. Clin. Exp. Res.* 30, 1500–1505. doi: 10.1111/j.1530-0277.2006.00181.x

Received: 06 June 2013; accepted: 06 June 2013; published online: 01 July 2013.

Citation: Zimatkin SM (2013) Acetaldehyde mediates the ethanol effects in developing brain. Front. Behav. Neurosci. 7:75. doi: 10.3389/fnbeh.2013.00075

frontiers in
BEHAVIORAL NEUROSCIENCE

REVIEW ARTICLE
published: 21 June 2013
doi: 10.3389/fnbeh.2013.00071

Acetaldehyde and parkinsonism: role of CYP450 2E1

Francesca Vaglini*, Cristina Viaggi, Valentina Piro, Carla Pardini, Claudio Gerace, Marco Scarselli and Giovanni Umberto Corsini

Department of Translational Research and New Technology in Medicine, University of Pisa, Pisa, Italy

Edited by:
Merce Correa, Universitat Jaume, Spain

Reviewed by:
Merce Correa, Universitat Jaume, Spain
John D. Salamone, University of Connecticut, USA

****Correspondence:***
Francesca Vaglini, Department of Translational Research and New Technology in Medicine, University of Pisa, Via Roma 55, Pisa 56126, Italy
e-mail: francesca.vaglini@med.unipi.it

The present review update the relationship between acetaldehyde (ACE) and parkinsonism with a specific focus on the role of P450 system and CYP 2E1 isozyme particularly. We have indicated that ACE is able to enhance the parkinsonism induced in mice by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, a neurotoxin able to damage the nigrostriatal dopaminergic pathway. Similarly diethyldithiocarbamate, the main metabolite of disulfiram, a drug widely used to control alcoholism, diallylsulfide (DAS) and phenylisothiocyanate also markedly enhance the toxin-related parkinsonism. All these compounds are substrate/inhibitors of CYP450 2E1 isozyme. The presence of CYP 2E1 has been detected in the dopamine (DA) neurons of rodent Substantia Nigra (SN), but a precise function of the enzyme has not been elucidated yet. By treating CYP 2E1 knockout (KO) mice with the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, the SN induced lesion was significantly reduced when compared with the lesion observed in wild-type animals. Several *in vivo* and *in vitro* studies led to the conclusion that CYP 2E1 may enhance the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine toxicity in mice by increasing free radical production inside the dopaminergic neurons. ACE is a good substrate for CYP 2E1 enzyme as the other substrate-inhibitors and by this way may facilitate the susceptibility of dopaminergic neurons to toxic events. The literature suggests that ethanol and/or disulfiram may be responsible for toxic parkinsonism in human and it indicates that basal ganglia are the major targets of disulfiram toxicity. A very recent study reports that there are a decreased methylation of the CYP 2E1 gene and increased expression of CYP 2E1 mRNA in Parkinson's disease (PD) patient brains. This study suggests that epigenetic variants of this cytochrome contribute to the susceptibility, thus confirming multiples lines of evidence which indicate a link between environmental toxins and PD.

Keywords: CYP450 2E1 isozyme, acetaldehyde, Parkinson's disease, dopaminergic neurons, ethanol

ALCOHOL CONSUMPTION AND PARKINSON'S DISEASE

A large number of case-control studies analysed the relationship between Parkinson's disease (PD) and some environmental factors (De Lau et al., 2004). Addictive behaviors, such as cigarette smoking and coffee drinking, showed a protective effect against PD and parkinsonism. Regarding alcohol consumption, most studies report either a moderately decreased risk or no change in risk associated with alcohol intake (Benedetti et al., 2000; Paganini-Hill, 2001; Hernan et al., 2004; Wirdefeldt et al., 2011; Campdelacreu, 2012; Noyce et al., 2012; Palacios et al., 2012). In contrast with these clinical findings, experimental data in rodents showed that alcohol induces a reduction in the dopamine (DA) levels in the midbrain, even if contradictory data are present in literature and an increased oxidative stress in nigral cells (Collins, 2002; Ambhore et al., 2012) and Golgi fragmentation (Tomas et al., 2012). Likely, alcohol consumption and neurodegenerative disease (e.g., PD) induce similar effects on intracellular structures and trafficking (Ambhore et al., 2012). It's conceivable that some components of alcoholic beverages (e.g., flavonoids in red wine) could have a neuroprotective activity (Palacios et al., 2012). Beer drinkers have a lower risk of PD (Hernan et al., 2003), and this result can be explained with higher plasma urate levels that beer induces. Indeed, urate plays a protective role against PD (Xiang et al., 2008). It has been known that in parkinsonian patients addiction such as cigarette smoking, drug consumption, alcoholism and compulsive disorders such as gambling, compulsive shopping, hypersexuality are less common than into general population (De Lau et al., 2004; Xiang et al., 2008; Noyce et al., 2012). Data collected in the last decades all over the world highlight the link between dopaminergic replacement and onset of addiction behaviors and compulsive disorders in Parkinson's patients (Villa et al., 2011). However, alcohol consumption and a history of alcoholism seem to be related with higher incidence of impulse control disorders in PD patients receiving dopaminergic replacement (Evans et al., 2005; Wu et al., 2009). Some endogenous alkaloids, like salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline,SAL) and tetrahydropapaveroline (6,7-dihydroxy-1-(3′,4′-dihydroxybenzyl)-1,2,3,4-tetrahydroisoquinoline; THP), whose blood levels are increased by alcohol, have neurotoxic effects especially in the striatum and reduce DA content into basal ganglia (Young-Joon and Hyun-Jung, 2010). THP is detected at a high level in the urine of parkinsonian patients under L-DOPA therapy (Cashaw, 1993); however, there are very low levels of THP in the

urine of abstinent alcoholics. Possible implications of THP in alcohol dependence were inferred from the observation that rats which normally rejected alcohol, would drink alcohol in excessive amount following direct delivery of THP (Duncan and Deitrich, 1980). Thus, these substances might contribute to alcohol dependence (Collins, 2004; Hipólito et al., 2012; Deehan et al., 2013). These factors can be useful to identify PD patients at high risk of developing impulse control disorders during dopaminergic replacement.

ACETALDEHYDE AND PARKINSON'S DISEASE

Acetaldehyde (ACE) is the alcohol metabolite responsible for unpleasant effects such as nausea, vomiting, tachycardia and hypotension. ACE increases the toxic effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in striatum (Corsini et al., 1987; Zuddas et al., 1989a,b, 1992; Vaglini et al., 1996); this activity has been demonstrated in mice but not in rats (Fornai et al., 1997). There are no studies in humans that relate ACE to PD or parkinsonism, most likely because it's not a substance of abuse nor a drug nor a pollutant. ACE is further metabolized to acetic acid with catalysis of Aldehyde dehydrogenase (ALDH); disulfiram, a blocker of this enzyme, induces ACE accumulation and is used in treatment of alcoholism. Disulfiram can have a neurotoxic effect; namely, acute intoxication induces unilateral pallidal lesion as described by means of magnetic resonance evaluation and clinical assessment (De Mari et al., 1993) while chronic administration can induce Wernicke's encephalopathy, with progressive frontal decline and akineto-rigid parkinsonism (Charles et al., 2006). Brain MRI revealed symmetrical and reversible lesions in basal ganglia, but after discontinuation of disulfiram, clinical recovery was slow and partial. These observations suggest that basal ganglia are the major targets of disulfiram neurotoxicity (Laplane et al., 1992). Similar brain lesions are also observed in "energy deprivation syndromes," which are toxic, genetic or nutritional disorders that disrupt enzymes involved in energy production pathways (Laplane et al., 1992; Charles et al., 2006). Probably, disulfiram impairs cellular processes involved in ATP production but the exact mechanism remains unclear. It's conceivable that disulfiram toxicity is linked at least in part to the action of ACE; indeed other aldehydes, such as 3,4-dihydroxyphenilacetaldehyde (DOPALD), an oxidative metabolite of DA, have been shown to have a neurotoxic activity (Gesi et al., 2001) and higher levels of these compounds are related with neurodegenerative diseases (Marchitti et al., 2007) (**Figure 1**).

ACETALDEHYDE AND EXPERIMENTAL PARKINSONISM

Corsini et al. (1985) unexpectedly found that diethyldithiocarbamate (DDC), the main metabolite of disulfiram, markedly enhanced the MPTP-induced parkinsonism in mice. This effect was initially interpreted as due to the inhibition of superoxide dismutase leading to an increase in oxidative stress induced by the toxin. Subsequently, among numerous compounds tested, other enhancers of MPTP toxicity (ethanol and ACE) were found by the same authors (Corsini et al., 1987). After this further discovery, this group suggested that these compounds could increase the potency of the toxin via an inhibition of ALDH within the striatum. The "enhancers," at the same time, prolonged the striatal half-life of 1-methyl-4-phenylpyridinium ion (MPP^+), the toxic metabolite of MPTP, (Irwin et al., 1987; Zuddas et al., 1989b) and this was interpreted as the causative factor of this enhancement. However, in 1996 an article by Vaglini et al. demonstrated that striatal MPP^+ levels do not necessarily correlate with MPTP toxicity in the same animal species (mouse) and they further on suggested, as previously reported, that DDC-increased toxicity was probably due to an independent action on glutamate receptors (Vaglini et al., 1996). However it is likely that the prolonged storage of MPP^+ inside the DA neurons was crucial for its toxic effects. According to this interpretation, the enzymes, which may

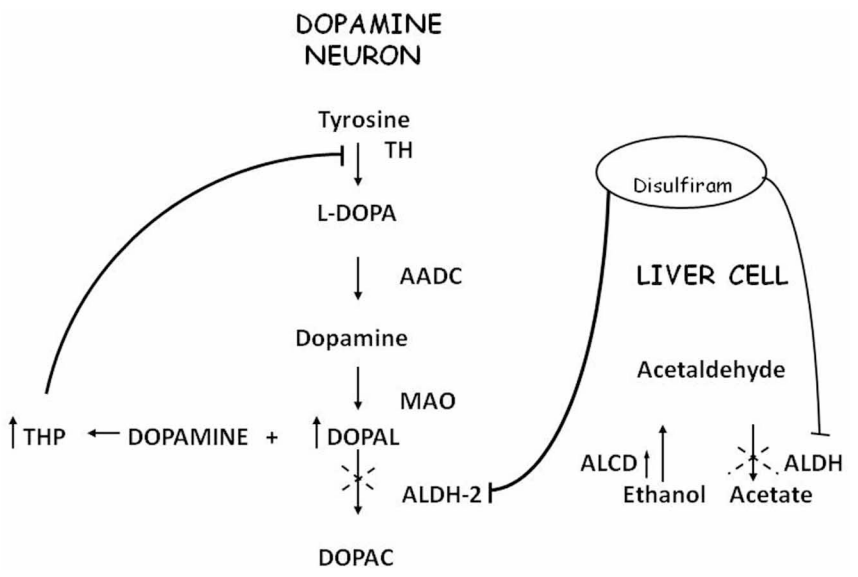


FIGURE 1 | Convergence of metabolic pathway for acetaldehyde and dopamine.

determine the disposition of MPP^+ inside the DA neurons, have a cardinal role in MPTP toxicity. CYP 2E1 and the CYP 2D family are the most widely represented isozymes within the DA neurons (Watts et al., 1998; Riedl et al., 1999) and it is likely that these two P450 enzymes are responsible for MPP^+ clearance. As a matter of fact, DDC, ethanol and ACE have been discovered to be specific substrates/inhibitors of CYP 2E1 when they are acutely administered (Stott et al., 1997) (**Figure 2**). This specific inhibition inside the DA neuron may account for the increase in MPP^+ striatal half-life, and thus toxicity.

More recently, furthermore, we demonstrated that, similar to DDC and ACE, CYP 2E1 substrates/inhibitors, such as diallylsulfide (DAS) or phenylethylisothiocyanate (PIC) markedly enhance MPTP toxicity in C57/bl brain, suggesting an involvement of CYP 2E1 in the enhancement of MPTP toxicity. However, because DAS and PIC are not true CYP 2E1 inhibitors (Nissbrandt et al., 2001), in order to provide direct evidence for CYP 2E1 involvement, CYP 2E1 knockout (KO) mice and their wild-type counterparts were challenged with the combined treatment DDC+MPTP (Vaglini et al., 2004). In that article we consistently showed that an inhibition of the enzyme, as obtained with DDC challenge, failed to enhance the toxic effect of MPTP in CYP 2E1 KO mice, whereas the effect was regularly present in wild-type animals. Focusing on this, we have studied the sensitivity of CYP 2E1 KO mice to the toxin (Viaggi et al., 2009). The lack of CYP2 E1 did not increase MPTP toxicity as expected from previous experiments with the inhibitors, and, in fact, the CYP 2E1 KO mice showed a significant resistance to DA neuronal lesions induced by the toxin in comparison with their wild-type counterparts. The reduced sensitivity to MPTP of CYP 2E1 KO mice, but not the complete insensitivity, may be due to compensatory mechanisms taking place because of the missing protein. This phenomenon was observed very clearly by Gonzalez when his team generated these mice in order to study acetaminophen-induced liver toxicity (Lee et al., 1996). This drug causes liver and kidney necrosis when it is metabolized to an alkylating intermediate by the P450 system, and more specifically by CYP 2E1 (Jollow et al., 1973; Mitchell et al., 1973; Gonzalez, 2007). CYP 2E1 KO mice were generated to strengthen the specific role of CYP 2E1 during acetaminophen toxicity (Lee et al., 1996). The CYP 2E1 KO mice were less sensitive to the hepatotoxicity of the drug but they were not completely unaffected. A compensatory isozyme of the P450, probably CYP2D6, was substituted, thus producing the same—though reduced—toxic effect. Very recently we generated mesencephalic cell cultures from CYP 2E1 KO and wild-type embryos to investigate MPP^+ toxicity and its cell distribution. In this model we demonstrated that a trace amount of MPP^+ accumulates inside the neurons from KO mesencephalic cultures in a quantity double than that from wild-type embryos, although the KO cultures are less lesioned by the toxin (manuscript in preparation). We then suggested, once inside the cell (or striatal synaptic terminal), MPP^+ entered preferentially into vesicles where its storage represented a sort of protection with respect to other toxic sites such as mitochondria.

In conclusion CYP 2E1 may facilitate the transfer of MPP^+ to mitochondria for further metabolism. Alternatively and independently from MPP^+ disposition, CYP 2E1 produces toxic reactive intermediates from endogenous or exogenous substrates which in turn impair neuronal viability.

THE P450 SYSTEM IN PD

In 1985, Barbeau et al. elegantly presented evidence for an association of a CYP 2D6 defect with PD (Barbeau et al., 1985). Indeed, they postulated that subjects with a reduced CYP 2D6 enzyme (poor metabolizers) are vulnerable for PD because of the impaired capacity of liver to detoxify those neurotoxins which are harmful for DA neurons. It is worth noting that further studies have actually indicated CYP 2D6 as the major detoxifying liver enzyme for the PD-inducing neurotoxin, MPTP (Coleman et al., 1996; Gilham et al., 1997). After this pioneering report, however, many conflicting results were obtained in phenotypic and genotypic CYP 2D6 studies, which have been outlined in a comprehensive review by Riedl et al. (1998).

Several enzymes involved in the metabolism of endogenous compounds and xenobiotics have been studied in relation to PD. However, CYP450 in particular drew attention, due to its ability to defend the body against xenobiotic aggression. In particular, six P450 enzymes have been examined with respect to PD: CYP 1A1 (Kurth and Kurth, 1993; Bennet et al., 1994; Takakubo et al., 1996), CYP 2C9 (Ferrari et al., 1990; Peeters

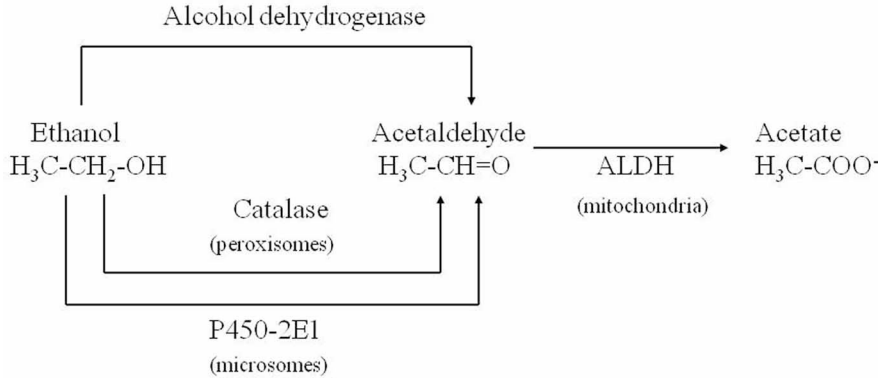


FIGURE 2 | Role of CYP 2E1 in metabolizing ethanol and acetaldehyde.

et al., 1994), CYP 2C19 (Gudjonsson et al., 1990; Tsuneoka et al., 1996), CYP 1A2, CYP 2E1 (Factor et al., 1989; Steventon et al., 1989), and CYP 2D6 (Riedl et al., 1998). Since the first enthusiastic claim, more than 50 reports have debated the role of CYP 2D6 in the pathogenesis of PD. Subsequent phenotypic studies have failed to support a link between this isozyme and PD. Similarly, the most extensive genetic studies initially confirmed this link, but a critical analysis of the recent studies from different groups again failed to draw any definitive conclusion (Riedl et al., 1998). Indeed, with respect to CYP 2D6, no laboratories have succeeded in replicating the initial report of Smith et al. (1992), according to which the frequency of poor metabolizers significantly increased in a PD population. Subsequent reports have been conflicting, although some groups have claimed differences in the allelic frequency of CYP 2D6*4 and other CYP 2D6 allelic variants in PD. Two recent meta-analyses failed to find an increased frequency of poor metabolizers among PD patients (Christensen et al., 1998; Rostami-Hodjegan et al., 1998). On the contrary, an earlier meta-analysis suggested a weak association, but this included fewer studies (McCann et al., 1997). As a result of their inability to observe any association, other authors performed sub-group analyses, thus suggesting a possible link with "young onset PD" (Agundez et al., 1995) or PD with prominent tremor (Akhmedova et al., 1995). Unfortunately, these findings have not been replicated, either (Sandy et al., 1996). Although most studies have been negative, there are some critical issues that have been addressed by Le Couteur and McCann (1998) in connection with this problem. First, it is unlikely, on the basis of current studies, to completely refute the involvement of CYP 2D6, as, in order to have a definitive study of a statistical power, one would need almost 3000 subjects to exclude a 50% increase in the frequency of poor metabolizers among PD patients. The second issue is that studies should consider only patients who have had neurotoxin exposure. If CYP 2D6 polymorphism influences vulnerability to PD by affecting the metabolism of an environmental neurotoxin, then studies should include only those subjects who have undergone this kind of neurotoxin exposure. The authors concluded that this stratification for toxin exposure is necessary in order to rule out the role of CYP 2D6 in the pathogenesis of PD.

This last concept of an environmental toxin and CYP 2D6, as its metabolizing enzyme, opens an old issue regarding the toxic hypothesis of PD, which originated from the incidental discovery of MPTP as a widespread impurity (Langston et al., 1983). Indeed, MPTP is metabolized by some P450 enzymes and by CYP 2D6 in particular (Coleman et al., 1996; Gilham et al., 1997) and has recently been discovered to be a synthetic impurity of heterocyclic drugs (Kramer et al., 1998). In this study, the authors assessed the risk of administering MPTP orally and reported that compounds containing less than 5 p.p.m. of MPTP do not involve any neurotoxicological health risk. They concluded surprisingly that it may be assumed that MPTP is also present as a yet undiscovered minor impurity in various existing drugs (Kramer et al., 1998). If this was true, MPTP or one of its analogues would represent the toxin probably responsible not for idiopathic PD, but for a specific subgroup of parkinsonism. In this case, CYP 2D6-related metabolism would be of extreme importance and phenotypic and genotypic studies should be carried out on different and selected types of subjects.

P450 IN EXPERIMENTAL PARKINSONISM

In general, MPP^+ metabolism, unlike MPTP, has been poorly investigated. Johannessen et al. (1985) postulated that MPP^+ may be transformed into free radical species, and other authors provided evidence for CYP 2D isoform involvement (Fonne-Pfister and Meyer, 1988; Jolivalt et al., 1995). It is interesting to note that CYP 2E1 is associated with the metabolism of several small planar molecules, such as nitrosoamines, benzene, alcohol and 3-hydroxypiridine (Parkinson, 1996), and is present in a functional form because its levels can be induced by prior treatment with isoniazid (Park et al., 1993). CYP 2E1 therefore may represent, in this particular case, a detoxification pathway of MPP^+, whose inhibition by DDC leads to an increased toxicity. A similar conclusion can be drawn for CYP 2D isozymes. CYP 2D6, the isoform present in humans and monkeys, metabolizes MPTP and MPP^+ probably to harmless compounds (Fonne-Pfister and Meyer, 1988; Jolivalt et al., 1995; Coleman et al., 1996; Gilham et al., 1997). Therefore, "CYP 2D6 poor metabolizers," or the drugs that inhibit this isoenzyme, may represent susceptible factors favoring the neurotoxicity induced by MPTP (Barbeau et al., 1985; Lane, 1998). It is worth noting that MPP^+ binding sites, as described by Del Zompo et al. (1986), may partly correspond in the mouse brain to the substrate recognition sites of CYP 2D isozymes. This MPP^+ binding, indeed, is displaced potently by debrisoquine and its analogues, which are good substrates for the P450 system (Del Zompo et al., 1990). MPP^+ binding has also been studied in post-mortem brain of PD patients and, among the several brain areas analyzed, only the Substantia Nigra (SN) showed a reduction in this binding in comparison with age-matched controls (Corsini et al., 1988). This reduction may be interpreted as a result of CYP 2D6 loss in the SN following DA neuron degeneration, a finding which is similar to that observed by Riedl et al. (1999) in the rat brain after 6-OHDA lesion of DA neurons. Furthermore, CYP 2D isoforms not only metabolize the neurotoxins MPTP and/or MPP^+, but also markedly participate in the metabolism of methamphetamine and its analogues (Lin et al., 1995). Actually, similar conclusions must be drawn for these toxic compounds which are widely abused by humans. The role of CYP 2D6-mediated metabolism of amphetamines must be considered not only for the hepatic enzyme, but also for the one present in DA neurons. At present, it is difficult to suggest the effective physiological role of this enzyme in the DA neuron. It is likely that it behaves like a guard against endogenous or exogenous harmful intruders (false transmitters) which may affect DA metabolism. The concept of a "false transmitter" implies that endogenous chemicals may be handled within the neurons like the natural transmitter, thereby influencing the intraneuronal disposition and release of the natural transmitter (Thoenen, 1969). Among the various false transmitters which affect DA neurons, tryptamine is one of the most widely studied (Baumgarten and Zimmermann, 1992). Tryptamine is an endogenous substrate of CYP 2D6 (Martinez et al., 1997) and its involvement in PD and in schizophrenia as well has been evaluated since the 60's

(Brune and Himwhich, 1962; Keuhl et al., 1968; Herkert and Keup, 1969; Smith and Kellow, 1969).

CYTOCHROME P450 2E1

The highest concentration of the enzyme CYP 2E1 is in the liver where it is the main P450 enzyme for ethanol metabolism (Thomas et al., 1987; de Waziers et al., 1990; Correa et al., 2009). However, it has been found in many extrahepatic organs, for example in kidney and lung. CYP 2E1 is specifically implicated in the metabolism of several compounds including toxicants and low molecular weight procarcinogens (Koop, 1992). Among the many endogenous substrates of the enzyme that have been identified (Ronis et al., 1996; Lieber, 1997; Ingelman-Sundberg, 2004), are ketones (e.g., acetone) and fatty acids such as arachidonic acid. The exogenous compounds metabolized by CYP 2E1 comprise a wide variety of xenobiotics (e.g., acetaminophen, aniline, paracetamol, N-nitrosodimetylamine, and chlorzoxazone, with the latter two often used as enzymatic probes), alcohols (e.g., ethanol, methanol), ACE, aromatic hydrocarbons such as benzene and toluene, halogenated hydrocarbons (e.g., carbon tetrachloride) and finally anesthetics including enflurane, isoflurane, and halothane. Its active site pocket is relatively small and hydrophobic, creating a suitable environment for small non-polar molecules. Due to its existence predominantly as high spin form, CYP 2E1 also is important in oxygen reduction and generation of reactive oxyradicals, potent initiators of membrane lipid peroxidation (Ekstrom and Ingelman-Sundberg, 1989; Persson et al., 1990).

Several isoenzymes of P450 have been identified in the CNS of several animal species, including man (Kalow, 1992; Warner et al., 1993). Altough the activities of the different cytocromes in the brain are very low as compared to in the liver, CYP 2E1 has been detected by immunohistochemical techniques in the DA-containing areas, the striatum and the SN (Anandatheerthavarada et al., 1993a; Gonzalez and Kimura, 1999). As in the liver, CYP 2E1 in brain is inducible by, e.g., ethanol, isoniazid or nicotine administration (Anandatheerthavarada et al., 1993b; Sohda et al., 1993; Gonzalez and Kimura, 1999). Also, induction of this enzyme during ischemic injury was shown in hippocampal and cortex astrocytes of rat and gerbil *in vivo* (Tindberg et al., 1996; Watts et al., 1998) showed that inducible CYP 2E1 existed in the same compartment as tyrosine hydroxylase in the rat SN but could not detect the enzyme in nigral glia cells. In addition, localization of the enzyme in monkey brain, as well as prenatal and adult human brain was confirmed (Brzezinski et al., 1999; Upadhya et al., 2000; Joshi and Tyndale, 2006). The active form of CYP 2E1 has been found in ER (microsomes), in the Golgi apparatus and in the plasma membrane of rat hepatocytes (Wu and Cederbaum, 1992; Loeper et al., 1993; Neve et al., 1996). It is possible that in the CNS, the active form of this enzyme is localized in the same membrane compartments as its hepatic variety.

There is evidence that interindividual variability in the expression and functional activity of this cytochrome may be considerable. Genetic polymorphisms in CYP 2E1 were identified and linked to altered susceptibility to hepatic cirrhosis induced by ethanol and esophageal and other cancers in some epidemiological studies. Therefore, it is important to evaluate how such polymorphisms affect CYP 2E1 function and whether it is possible to construct a population distribution of CYP 2E1 activity based upon the known effects of these polymorphisms and their frequency in the population (Itoga et al., 2002; Danko and Chaschin, 2005).

Recently, considering these findings on the enzymatic properties and genetic characteristics of CYP 2E1 and the fact that the enzyme is found in the SN, preliminary data demonstrated a possible association between CYP 2E1 polymorphisms and PD (Shahabi et al., 2009).

More recently Kaut et al. (2012) found decreased methylation of the cytochrome CYP 2E1 gene and increased expression of CYP 2E1 messenger RNA in PD patients' brains, suggesting that epigenetic variants of this cytochrome contribute to PD susceptibility. Alterations of gene methylation patterns may form an interface between genetic and environmental susceptibility, carrying forward long lasting changes which may have been acquired even in preceding generations (Feinberg, 2007; Suzuki and Bird, 2008; Urdinguio et al., 2009).

Summarizing the above mentioned paragraphs the use of ACE, or other CYP 2E1 substrates/inhibitors as well, revealed the role of a specific P450 enzyme in experimental parkinsonism as obtained in the MPTP mouse model. Similarly clinical studies in PD led to the conclusion that environmental factors, such as several xenobiotics, contribute to the development of the disease. Among the relevant toxic environmental chemicals, pesticides and volatile solvents are the most suspected ones which are all substrates of CYP 2E1. It is likely that the oxidative stress induced by these substrates, including ethanol and its main metabolite ACE, may trigger a chronic impairment of DA neurons leading to degeneration. CYP 2E1 epigenetic alterations may facilitate the degenerative process through the metabolism of such xenobiotics and represent the genetic susceptibility to the disease. CYP 2E1 might be just the tip of the iceberg of epigenetic alterations to be identified in apparently sporadic neurodegenerative disorders.

REFERENCES

Agundez, J. A., Jimenez-Jimenez, F. J., Luengo, A., Bernal, M. L., Molina, J. A., Ayuso, L., et al. (1995). Association between the oxidative polymorphism and early onset of Parkinson's disease. *Clin. Pharmacol. Ther.* 57, 291–298. doi: 10.1016/0009-9236(95)90154-X

Akhmedova, S. N., Pushnova, E. A., Yakimovsky, A. F., Avtonomov, V. V., and Schwartz, E. I. (1995). Frequency of a specific cytochrome P4502D6B (CYP2D6B) mutant allele in clinically differentiated groups of patients with Parkinson disease. *Biochem. Mol. Med.* 54, 88–90. doi: 10.1006/bmme.1995.1012

Ambhore, N. S., Antony, S., Mali, J. K., Kanhed, A. M., Bhalerao, A. R., and Bhojraj, S. (2012). Pharmacological and biochemical interventions of cigarette smoke, alcohol and sexual mating frequency on idiopathic rat model of Parkinson's disease. *J. Young Pharm.* 3, 177–183.

Anandatheerthavarada, H. K., Shankar, S. K., Bhamre, S., Boyd, M. R., Song, B. J., and Ravindranath, V. (1993a). Induction of brain cytochrome P-450IIE1 by chronic ethanol treatment. *Brain Res.* 601, 279–285. doi: 10.1016/0006-8993(93)91721-4

Anandatheerthavarada, H. K., Williams, J. F., and Wecker, L. (1993b). Differential effect of chronic nicotine administration on brain cytochrome P4501A1/2 and P4502E1. *Biochem. Biophys. Res. Commun.* 194, 312–318. doi: 10.1006/bbrc.1993.1821

Barbeau, A., Cloutier, T., Roy, M., Plasse, L., Paris, S., and Poirier, J. (1985). Ecogenetics of Parkinson's disease: 4-hydroxylation of debrisoquine. *Lancet* 2, 1213–1216.

Baumgarten, H. G., and Zimmermann, B. (1992). "Neurotoxic Phenylalkylamines and Indolealkylamines," in *Selective Neurotoxicity. Handbook of Experimental Pharmacology*, Vol. 102, *Ch VIII*, eds H. Herken and F. Hucho (Berlin Heidelberg: Springer-Verlag), 225–291.

Benedetti, M. D., Bower, J. H., Maraganore, D. M., McDonnell, S. K., Peterson, B. J., Ahlskog, J. E., et al. (2000). Smocking, alcohol, and coffee consumption preceding Parkinson's disease: a case-control study. *Neurology* 55, 1350–1358. doi: 10.1212/WNL.55.9.1350

Bennet, P., Ramsden, D. B., Williams, A. C., and Ho, S. L. (1994). Cytochrome P450 1A1 (CYP1A1) gene in familial and sporadic idiopathic Parkinson's disease (IPD). *Mov. Disord.* 9, 33.

Brune, G. G., and Himwhich, H. E. (1962). Indole metabolites in schizophrenic patients. *Arch. Gent. Psychiatry* 6, 324–328. doi: 10.1001/archpsyc.1962.01710220066009

Brzezinski, M. R., Boutelet-Bochan, H., Person, R. E., Fantel, A. G., and Juchau, M. R. (1999). Catalytic activity and quantitation of cytochrome P-450 2E1 in prenatal human brain. *J. Pharmacol. Exp. Ther.* 289, 1648–1653.

Campdelacreu, J. (2012). Parkinson disease and Alzheimer disease: environmental risk factors. *Neurologia*. doi: 10.1016/j.nrl.2012.04.001. [Epub ahead of print].

Cashaw, J. L. (1993). Detremination of tetrahydropapaveroline in the urine of parkinsonian patients receiving L-dopa-carbidopa (Sinemet) therapy by high performance liquid chromatography. *J. Chromatogr.* 613, 267–273.

Charles, V., Boulin, S., and Gille, M. (2006). Wernicke's encephalopathy associated with chronic disulfiram intoxication. *Rev. Neurol.* 162, 1252–1256. doi: 10.1016/S0035-3787(06)75139-6

Christensen, P. M., Gotzsche, P. C., and Brosen, K. (1998). The sparteine/debrisoquine (CYP2D6) oxidation polymorphism and the risk of Parkinson's disease: a meta-analysis. *Pharmacogenetics* 8, 473–479. doi: 10.1097/00008571-199812000-00003

Coleman, T., Ellis, S. W., Martin, I. J., Lennard, M. S., and Tucker, G. T. (1996). 1-Methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) is N-demethylated by cytochromes P450 2D6, 1A2 and 3A4-implications for susceptibility to Parkinson's disease. *J. Pharmacol. Exp. Ther.* 277, 685–690.

Collins, M. A. (2002). Alkaloids, alcohol and Parkinson's disease. *Parkinsonism Relat. Disord.* 8, 417–422. doi: 10.1016/S1353-8020(02)00024-X

Collins, M. A. (2004). Tetrahydropapaveroline in Parkinson's disease and alcoholism: a look back in honor of Merton Sandler. *Neurotoxicology* 25, 117–120. doi: 10.1016/S0161-813X(03)00145-1

Correa, M., Viaggi, C., Escrig, M. A., Pascual, M., Guerri, C., Vaglini, F., et al. (2009). Ethanol intake and ethanol-induced locomotion and locomotor sensitization in CYP2E1 knockout mice. *Pharmacogenet. Genomics* 19, 217–225. doi: 10.1097/FPC.0b013e328324e726

Corsini, G. U., Bocchetta, A., Zuddas, A., Piccardi, M. P., and Del Zompo, M. (1988). Covalent protein binding of a metabolite of 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine to mouse and monkey brain *in vivo* and *in vitro*. *Biochem. Pharmacol.* 37, 4163–4169. doi: 10.1016/0006-2952(88)90111-6

Corsini, G. U., Pintus, S., Chiueh, C. C., Weiss, J. F., and Kopin, I. J. (1985). 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxicity in mice is enhanced by pretreatment with diethyldithiocarbamate. *Eur. J. Pharmacol.* 119, 127–128.

Corsini, G. U., Zuddas, A., Bonuccelli, U., Schinelli, S., and Kopin, I. J. (1987). 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) neurotoxicity in mice is enhanced by ethanol or acetaldehyde. *Life Sci.* 40, 827–832. doi: 10.1016/0024-3205(87)90030-0

Danko, I. M., and Chaschin, N. A. (2005). Association of CYP2E1 gene polymorphism with predisposition to cancer development. *Exp. Oncol.* 27, 248–256.

Deehan, G. A., Jr, Brodie, M. S., and Rodd, Z. A. (2013). What is in that drink: the biological actions of ethanol, acetaldehyde, and salsolinol. *Curr. Top. Behav. Neurosci.* 13, 163–184. doi: 10.1007/7854_2011_198

De Lau, L. M., Giesbergen, P. C., De Rijk, M. C., Hofman, A., Koudstaal, P. J., and Breteler, M. M. (2004). Incidence of parkinsonism and Parkinson disease in a general population: the Rotterdam Study. *Neurology* 63, 1240–1244. doi: 10.1212/01.WNL.0000140706.52798.BE

De Mari, M., De Biasi, R., Lamberti, P., Carella, E., and Ferrari, E. (1993). Unilateral pallidal lesion after acute disulfirtam intoxication: a clinical and magnetic resonance study. *Mov. Disord.* 8, 247–249. doi: 10.1002/mds.870080236

de Waziers, I., Cugnenc, P. H., Yang, C. S., Leroux, J. P., and Beaune, P. H. (1990). Cytochrome P 450 isoenzymes, epoxide hydrolase and glutathione transferases in rat and human hepatic and extrahepatic tissues. *J. Pharmacol. Exp. Ther.* 253, 387–394.

Del Zompo, M., Bernardi, F., Maggio, R., Piccardi, M., Johannessen, J. N., and Corsini, G. U. (1986). High affinity binding sites for 1-methyl-4-phenyl-pyridinium ion (MPP+) are present in mouse brain. *Eur. J. Pharmacol.* 129, 87–92. doi: 10.1016/0014-2999(86)90339-0

Del Zompo, M., Ruiu, S., Maggio, R., Piccardi, M. P., and Corsini, G. U. (1990). [3H]1-methyl-4-phenyl-2, 3-dihydropyridinium ion binding sites in mouse brain: pharmacological and biological characterization. *J. Neurochem.* 54, 1905–1910. doi: 10.1111/j.1471-4159.1990.tb04889.x

Duncan, C., and Deitrich, R. A. (1980). A critical evaluation of tetrahydroisoquinoline induced ethanol preference in rats. *Pharmacol. Biochem. Behav.* 13, 265–281. doi: 10.1016/0091-3057(80)90083-0

Ekstrom, G., and Ingelman-Sundberg, M. (1989). Rat liver microsomal NADPH-supported oxidase activity and lipid peroxidation dependent on ethanol-inducible cytochrome P-450 (P-450IIE1). *Biochem. Pharmacol.* 38, 1313–1319. doi: 10.1016/0006-2952(89)90338-9

Evans, A. H., Lawrence, A. D., Potts, J., Appel, S., and Lees, A. J. (2005). Factors influencing susceptibility to compulsive dopaminergic drug use in Parkinson disease. *Neurology* 65, 1570–1574. doi: 10.1212/01.wnl.0000184487.72289.f0

Factor, S. A., Weiner, W. J., and Hefti, F. (1989). Acetaminophen metabolism by cytochrome P450 monooxygenases in Parkinson's disease. *Ann. Neurol.* 26, 286–288. doi: 10.1002/ana.410260219

Feinberg, A. P. (2007). Phenotypic plasticity and the epigenetics of human disease. *Nature* 447, 433–440. doi: 10.1038/nature05919

Ferrari, M. D., Peeters, E. A., Haan, J., Roos, R. A., Vermey, P., De Wolff, F. A., et al. (1990). Cytochrome P450 and Parkinson's disease. Poor parahydroxylation of phenytoin. *J. Neurol. Sci.* 96, 153–157. doi: 10.1016/0022-510X(90)90128-A

Fonne-Pfister, R., and Meyer, U. A. (1988). Xenobiotic and endobiotic inhibitors of cytochrome P-450dbl function, the target of the debrisoquine/sparteine type polymorphism. *Biochem. Pharmacol.* 37, 3829–3835. doi: 10.1016/0006-2952(88)90063-9

Fornai, F., Vaglini, F., Maggio, R., Bonuccelli, U., and Corsini, G. U. (1997). Species difference in the role of excitatory aminoacids in experimental parkinsonism. *Neurosci. Biobehav. Rev.* 21, 401–415. doi: 10.1016/S0149-7634(96)00042-5

Gesi, M., Santinami, A., Ruffoli, R., Conti, G., and Fornai, F. (2001). Novel aspect of dopamine oxidative metabolism (confounding outcomes take place of certainties). *Pharmacol. Toxicol.* 89, 217–224. doi: 10.1034/j.1600-0773.2001.d01-151.x

Gilham, D. E., Cairns, W., Paine, M. J., Modi, S., Poulsom, R., Roberts, G. C., et al. (1997). Metabolism of MPTP by cytochrome P4502D6 and the demonstration of 2D6 mRNA in human foetal and adult brain by *in situ* hybridization. *Xenobiotica* 27, 111–125. doi: 10.1080/004982597240802

Gonzalez, F. J. (2007). The 2006 Bernard B. Brodie Award Lecture. Cyp2e1. *Drug Metab. Dispos.* 35, 1–8. doi: 10.1124/dmd.106.012492

Gonzalez, F. J., and Kimura, S. (1999). Role of gene knockout mice in understanding the mechanisms of chemical toxicity and carcinogenesis. *Cancer Lett.* 143, 199–204. doi: 10.1016/S0304-3835(99)00125-1

Gudjonsson, O., Sanz, E., Alvan, G., Aquilonius, S. M., and Reviriego, J. (1990). Poor hydroxylator phenotypes of debrisoquine and S-mephenytoin are not over-represented in a group of patients with Parkinson's disease. *Br. J. Clin. Pharmacol.* 30, 301–302. doi: 10.1111/j.1365-2125.1990.tb03780.x

Herkert, E. E., and Keup, W. (1969). Excretion patterns of tryptamine, indoleacetic acid, and 5-hydroxyindoleacetic acid, and their correlation with mental changes in schizophrenic patients under medication with alpha-methyldopa. *Psychopharmacologia* 15, 48–59. doi: 10.1007/BF00410800

Hernan, M. A., Chen, H., Schwarzschild, M. A., and Ascherio,

A. (2003). Alcohol consumption and the incidence of Parkinson's disease. *Ann. Neurol.* 54, 170–175. doi: 10.1002/ana.10611

Hernan, M. A., Logroscino, G., and Rodriguez, L. A. (2004). A prospective study of alcoholism and the risk of Parkinson's disease. *J. Neurol.* 7, 14–17.

Hipólito, L., Sánchez-Catalán, M. J., Martí-Prats, L., Granero, L., and Polache, A. (2012). Revisiting the controversial role of salsolinol in the neurobiological effects of ethanol: old and new vistas. *Neurosci. Biobehav. Rev.* 36, 362–378. doi: 10.1016/j.neubiorev.2011.07.007

Ingelman-Sundberg, M. (2004). Human drug metabolising cytochrome P450 enzymes: properties and polymorphisms. *Naunyn Schmiedebergs Arch. Pharmacol.* 369, 89–104. doi: 10.1007/s00210-003-0819-z

Irwin, I., Wu, E. Y., DeLanney, L. E., Trevor, L., and Langston, J. W. (1987). The effect of diethyldithiocarbamate on the biodisposition of MPTP: an explanation for enhanced neurotoxicity. *Eur. J. Pharmacol.* 141, 209–217. doi: 10.1016/0014-2999(87)90265-2

Itoga, S., Nomura, F., Makino, Y., Tomonaga, T., Shimada, H., Ochiai, T., et al. (2002). Tandem repeat polymorphism of the CYP2E1 gene: an association study with esophageal cancer and lung cancer. *Alcohol. Clin. Exp. Res.* 26, 15S–19S. doi: 10.1111/j.1530-0277.2002.tb02696.x

Johannessen, J. N., Kelner, L., Hanselman, D., Shih, M. C., and Markey, S. (1985). *In vitro* oxidation of MPTP by primate neural tissue: a potential model of MPTP neurotoxicity. *Neurochem. Int.* 7, 169–176. doi: 10.1016/0197-0186(85)90022-1

Jolivalt, C., Minn, A., Vincent-Viry, M., Galteau, M. M., and Siest, G. (1995). Dextromethorphan O-demethylase activity in rat brain microsomes. *Neurosci. Lett.* 187, 65–68. doi: 10.1016/0304-3940(95)11339-X

Jollow, D. J., Mitchell, J. R., Potter, W. Z., Davis, D. C., Gillette, J. R., and Brodie, B. B. (1973). Acetaminophen-induced hepatic necrosis. II. Role of covalent binding *in vivo*. *J. Pharmacol. Exp. Ther.* 187, 195–202.

Joshi, M., and Tyndale, R. F. (2006). Induction and recovery time course of rat brain CYP2E1 after nicotine treatment. *Drug Metab. Dispos.* 34, 647–652. doi: 10.1124/dmd.105.008029

Kalow, W. (1992). Pharmacoanthropology and the genetics of drug metabolis. *Pharmacogenet. Drug Metab. Int. Encyclop. Pharmacol.* 137, 865–877.

Kaut, O., Schmitt, I., and Wüllner, U. (2012). Genome-scale methylation analysis of Parkinson's disease patients' brains reveals DNA hypomethylation and increased mRNA expression of cytochrome P450 2E1. *Neurogenetics* 13, 87–91. doi: 10.1007/s10048-011-0308-3

Keuhl, F. A., Vanden-Heuval, W. J. A., and Ormond, R. E. (1968). Urinary metabolites in Parkinson's disease. *Nature* 217, 471–490.

Koop, D. R. (1992). Oxidative and reductive metabolism by cytochrome P450 2E1. *FASEB J.* 6, 724–730.

Kramer, P. J., Caldwell, J., Hofmann, A., Tempel, P., and Weisse, G. (1998). Neurotoxicity risk assessment of MPTP (N-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine) as a synthetic impurity of drugs. *Hum. Exp. Toxicol.* 17, 283–293. doi: 10.1191/096032798678908648

Kurth, M. C., and Kurth, J. H. (1993). Variant cytochrome P450 CYP2D6 allelic frequencies in Parkinson's disease. *Am. J. Med. Genet.* 48, 166–168. doi: 10.1002/ajmg.1320480311

Lane, R. M. (1998). SSRI-induced extrapyramidal side-effects and akathisia: implications for treatment. *J. Psychopharmacol.* 12, 192–214. doi: 10.1177/0269881 19801200212

Langston, J. W., Ballard, P., Tetrud, J. W., and Irwin, I. (1983). Chronic parkinsonism in humans due to a product of meperidine-analog synthesis. *Science* 219, 979–980. doi: 10.1126/science.6823561

Laplane, D., Attal, N., Sauron, B., De Billy, A., and Dubois, B. (1992). Lesions of basal ganglia due to disulfiram neurotoxicity. *J. Neurosurg. Psychiatry* 55, 925–929. doi: 10.1136/jnnp.55.10.925

Le Couteur, D. G., and McCann, S. J. (1998). P450 enzymes and Parkinson's disease. *Mov. Disord.* 13, 851–852.

Lee, S. S., Buters, J. T., Pineau, T., Fernandez-Salguero, P., and Gonzalez, F. J. (1996). Role of CYP2E1 in the hepatotoxicity of acetaminophen. *J. Biol. Chem.* 271, 12063–12067. doi: 10.1074/jbc.271.20.12063

Lieber, C. S. (1997). Cytochrome P-4502E1: its physiological and pathological role. *Physiol. Rev.* 77, 517–544.

Lin, L. Y., Kumagai, Y., Hiratsuka, A., Narimatsu, S., Suzuki, T., Funae, Y., et al. (1995). Cytochrome P4502D isozymes catalyze the 4-hydroxylation of methamphetamine enantiomers. *Drug Metab. Dispos.* 23, 610–614.

Loeper, J., Descatoire, V., Maurice, M., Beaune, P., Belghiti, J., Houssin, D., et al. (1993). Cytochromes P-450 in human hepatocyte plasma membrane: recognition by several autoantibodies. *Gastroenterology* 104, 203–216.

Marchitti, S. A., Deitrich, R. A., and Vasiliou, V. (2007). Neurotoxicity and metabolism of the catecholamine-derived 3, 4-dihydroxyphenylacetaldehyde and 3, 4 - dihydroxyphenylglycolaldehyde: the role of aldehyde dehydrogenase. *Pharmacol. Rev.* 59, 125–150. doi: 10.1124/pr.59.2.1

Martinez, C., Agundez, J. A., Gervasini, G., Martin, R., and Benitez, J. (1997). Tryptamine: a possible endogenous substrate for CYP2D6. *Pharmacogenetics* 7, 85–93. doi: 10.1097/00008571-199704000-00001

McCann, S. J., Pond, S. M., James, K. M., and Le Couteur, D. G. (1997). The association between polymorphisms in the cytochrome P-450 2D6 gene and Parkinson's disease: a case-control study and meta-analysis. *J. Neurol. Sci.* 153, 50–53. doi: 10.1016/S0022-510X(97)00179-2

Mitchell, J. R., Jollow, D. J., Potter, W. Z., Davis, D. C., Gillette, J. R., and Brodie, B. B. (1973). Acetaminophen-induced hepatic necrosis. I. Role of drug metabolism. *J. Pharmacol. Exp. Ther.*187, 185–194.

Neve, E. P., Eliasson, E., Pronzato, M. A., Albano, E., Marinari, U., and Ingelman-Sundberg, M. (1996). Enzyme-specific transport of rat liver cytochrome P450 to the golgi apparatus. *Arch. Biochem. Biophys.* 333, 459–465. doi: 10.1006/abbi.1996.0415

Nissbrandt, H., Bergquist, F., Jonason, J., and Engberg, G. (2001). Inhibition of cytochrome P450 2E1 induces an increase in extracellular dopamine in rat substantia nigra: a new metabolic pathway? *Synapse* 40, 294–301. doi: 10.1002/syn.1052

Noyce, A. J., Bestwick, J. P., Silveira-Moriyama, L., Hawkes, C. H., Giovannoni, G., Lees, A. J., et al. (2012). Meta-analysis of early nonmotor features and risk factors for Parkinson disease *Ann. Neurol.* 72, 893–901. doi: 10.1002/ana.23687

Paganini-Hill, A. (2001). Risk factors for Parkinson's disease: the leisure world cohort study. *Neuroepidemiology* 20, 118–124. doi: 10.1159/000054770

Palacios, N., Gao, X., O'Reilly, E., Schwarzschild, M., McCullough, M. L., Mayo, T., et al. (2012). Alcohol and risk of Parkinson's disease in a large, prospective cohort of men and women. *Mov. Disord.* 27, 980–987. doi: 10.1002/mds.25050

Park, K. S., Sohn, D. H., Veech, R. L., and Song, B. J. (1993). Translational activation of ethanol-inducible cytochrome P450 (CYP2E1) by isoniazid. *Eur. J. Pharmacol.* 248, 7–14.

Parkinson, A. (1996). "Biotransformation of xenobiotics," in *Toxicology: The Basic Science of Poison*, *5th Edn*, eds M. O. Klaassen, L. J. Casarett, and J. Doull (New York, NY: McGraw-Hill; San Francisco, CA: St. Louis), 113–186.

Peeters, E. A., Bloem, B. R., Kuiper, M. A., Vermeij, P., de Wolff, F. A., Wolters, E. C., et al. (1994). Phenytoin parahydroxylation is not impaired in patients with young-onset Parkinson's disease. *Clin. Neurol. Neurosurg.* 96, 296–299. doi: 10.1016/0303-8467(94)90117-1

Persson, J. O., Terelius, Y., and Ingelman-Sundberg, M. (1990). Cytochrome P-450-dependent formation of reactive oxygen radicals: isozyme-specific inhibition of P-450-mediated reduction of oxygen and carbon tetrachloride. *Xenobiotica* 20, 887–900. doi: 10.3109/00498259009046904

Riedl, A. G., Watts, P. M., Edwards, R. J., Schulz-Utermoehl, T., Boobis, A. R., Jenner, P., et al. (1999). Growth factor-2 expression in the rat brain by the atypical antipsychotic clozapine. *Neuropharmacology* 38, 1075–1082. doi: 10.1016/S0028-3908(99)00031-3

Riedl, A. G., Watts, P. M., Jenner, P., and Marsden, C. D. (1998). P450 enzymes and Parkinson's disease: the story so far. *Mov. Disord.* 13, 212–220. doi: 10.1002/mds.870130204

Ronis, M. J. J., Lindros, K. O., and Ingelman-Sundberg, M. (1996). The CYP2 Subfamily. ed C. Ioannides (Boca Raton, FL: CRC Press), 211–239.

Rostami-Hodjegan, A., Lennard, M. S., Woods, H. F., and Tucker, G. T. (1998). Meta-analysis of studies of the CYP2D6 polymorphism in relation to lung cancer and Parkinson's disease. *Pharmacogenetics* 8, 227–238. doi: 10.1097/00008571-199806000-00005

Sandy, M. S., Armstrong, M., Tanner, C. M., Daly, A. K., Di

Monte, D. A., Langston, J. W., et al. (1996). CYP2D6 allelic frequencies in young-onset Parkinson's disease. *Neurology* 47, 225–230. doi: 10.1212/WNL.47.1.225

Shahabi, H. N., Westberg, L., Melke, J., Håkansson, A., Belin, A. C., Sydow, O., et al. (2009). Cytochrome P450 2E1 gene polymorphisms/haplotypes and Parkinson's disease in a Swedish population. *J. Neural Transm.* 116, 567–573. doi: 10.1007/s00702-009-0221-1

Smith, C. A., Gough, A. C., Leigh, P. N., Summers, B. A., Harding, A. E., Maraganore, D. M., et al. (1992). Debrisoquine hydroxylase gene polymorphism and susceptibility to Parkinson's disease. *Lancet* 339, 1375–1377. doi: 10.1016/0140-6736(92)91196-F

Smith, I., and Kellow, A. H. (1969). Aromatic amines and Parkinson's disease. *Nature* 221, 1261. doi: 10.1038/2211261a0

Sohda, T., Shimizu, M., Kamimura, S., and Okumura, M. (1993). Immunohistochemical demonstration of ethanol-inducible P450 2E1 in rat brain. *Alcohol Alcohol. Suppl.* 1B, 69–75.

Steventon, G. B., Heafield, M. T., Waring, R. H., and Williams, A. C. (1989). Xenobiotic metabolism in Parkinson's disease. *Neurology* 39, 883–887. doi: 10.1212/WNL.39.7.883

Stott, I., Murthy, A., Robinson, A., Thomas, N. W., and Fry, J. R. (1997). Low-dose diethyldithiocarbamate attenuates the hepatotoxicity of 1, 3-dichloro-2-propanol and selectively inhibits CYP2E1 activity in the rat. *Hum. Exp. Toxicol.* 16, 262–266. doi: 10.1177/096032719701600505

Suzuki, M. M., and Bird, A. (2008). DNA methylation landscapes: provocative insights from epigenomics. *Nat. Rev. Genet.* 9, 465–476. doi: 10.1038/nrg2341

Takakubo, F., Yamamoto, M., Ogawa, N., Yamashita, Y., Mizuno, Y., and Kondo, I. (1996). Genetic association between cytochrome P450IA1 gene and susceptibility to Parkinson's disease. *J. Neural Transm. Gen. Sect.* 103, 843–849. doi: 10.1007/BF01273362

Thoenen, H. (1969). Bildung und funktionelle Bedeutung adrenerger Ersatztransmitter. *Exp. Med. Pathol. Klin.* 27, 1–85.

Thomas, P. E., Bandiera, S., Reik, L. M., Maines, S. L., Ryan, D. E., and Levin, W. (1987). Polyclonal and monoclonal antibodies as probes of rat hepatic cytochrome P-450 isozymes. *Fed. Proc.* 46, 2563–2566.

Tindberg, N., Baldwin, H. A., Cross, A. J., and Ingelman-Sundberg, M. (1996). Induction of cytochrome P450 2E1 expression in rat and gerbil astrocytes by inflammatory factors and ischemic injury. *Mol. Pharmacol.* 50, 1065–1072.

Tomas, M., Marin, M. P., Martinez-Alonso, E., Esteban-Pretel, G., Diaz-Ruiz, A., Vasquez-Martinez, R., et al. (2012). Alcohol induces Golgi fragmentation in differentiated PC12 cells by deregulating Rab1-dependent ER-to-Golgi transport. *Histochem. Cell Biol.* 138, 489–501. doi: 10.1007/s00418-012-0970-z

Tsuneoka, Y., Fukushima, K., Matsuo, Y., Ichikawa, Y., and Watanabe, Y. (1996). Genotype analysis of the CYP2C19 gene in the Japanese population. *Life Sci.* 59, 1711–1715. doi: 10.1016/S0024-3205(96)00507-3

Upadhya, S. C., Tirumalai, P. S., Boyd, M. R., Mori, T., and Ravindranath, V. (2000). Cytochrome P4502E (CYP2E) in brain: constitutive expression, induction by ethanol and localization by fluorescence *in situ* hybridization. *Arch. Biochem. Biophys.* 373, 123–134. doi: 10.1006/abbi.1999.1477

Urdinguio, R. G., Sanchez-Mut, J. V., and Esteller, M. (2009). Epigenetic mechanisms in neurological diseases: genes, syndromes, and therapies. *Lancet Neurol.* 8, 1056–1072. doi: 10.1016/S1474-4422(09)70262-5

Vaglini, F., Fascetti, F., Tedeschi, D., Cavalletti, M., Fornai, F., and Corsini, G. U. (1996). Striatal MPP+ levels do not necessarily correlate with striatal dopamine levels after MPTP treatment in mice. *Neurodegeneration* 5, 129–136. doi: 10.1006/neur.1996.0019

Vaglini, F., Pardini, C., Viaggi, C., Bartoli, C., Dinucci, D., and Corsini, G. U. (2004). Involvement of cytochrome P450 2E1 in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced mouse model of Parkinson's disease. *J. Neurochem.* 2, 285–298. doi: 10.1111/j.1471-4159.2004.02720.x

Viaggi, C., Vaglini, F., Pardini, C., Caramelli, A., and Corsini, G. U. (2009). MPTP-induced model of Parkinson's disease in cytochrome P450 2E1 knockout mice. *Neuropharmacology* 56, 1075–1081. doi: 10.1016/j.neuropharm.2009.03.003

Villa, C., Pascual-Sedano, B., Pagonabarraga, J., and Kulisevsky, J. (2011). Impulse contyrol disorders and dopaminergic treatments in Parkinson's disease. *Rev. Neurol.* 167, 827–832. doi: 10.1016/j.neurol.2011.01.018

Warner, M., Strömstedt, M., Wyss, A., and Gustafsson, J. A. (1993). Regulation of cytochrome P450 in the central nervous system. *J. Steroid Biochem. Mol. Biol.* 47, 191–194. doi: 10.1016/0960-0760(93)90074-7

Watts, P. M., Riedl, A. G., Douek, D. C., Edwards, R. J., Boobis, A. R., Jenner, P., et al. (1998). Co-localization of P450 enzymes in the rat substantia nigra with tyrosine hydroxylase. *Neuroscience* 86, 511–519. doi: 10.1016/S0306-4522(97)00649-0

Wirdefeldt, K., Adami, H. O., Cole, P., Trichopoulos, D., and Mandel, J. (2011). Epidemiology and etiology of Parkinson's disease: a review of the evidence. *Eur. J. Epidemiol.* 1, 51–58. doi: 10.1007/s10654-011-9581-6

Wu, D., and Cederbaum, A. I. (1992). Presence of functionally active cytochrome P-450IIE1 in the plasma membrane of rat hepatocytes. *Hepatology* 15, 515–524. doi: 10.1002/hep.1840150326

Wu, K., Politis, M., and Piccini, P. (2009). Parkinson disease and impulse control disorders: a review of clinical features, pathophysiology and management. *Postgrad. Med. J.* 85, 590–596. doi: 10.1136/pgmj.2008.075820

Xiang, G., Honglei, C., Hyon, K. C., Curhan, G., Schwarzschild, M. A., and Ascherio, A. (2008). Diet, urate and Parkinson's disease risk in men. *Am. J. Epidemiol.* 167, 831–838. doi: 10.1093/aje/kwm385

Young-Joon, S., and Hyun-Jung, K. (2010). Neurotoxic effects of tetrahydroisoquinolines and underlying mechanisms. *Exp. Neurobiol.* 19, 63–70. doi: 10.5607/en.2010.19.2.63

Zuddas, A., Corsini, G. U., Schinelli, S., Johannessen, J. N., Di Porzio, U., and Kopin, I. J. (1989a). MPTP treatment combined with ethanol or acetaldehyde destroys dopaminergic neurons in mouse substantia nigra. *Brain Res.* 501, 1–10. doi: 10.1016/0006-8993(89)91020-2

Zuddas, A., Corsini, G. U., Schinelli, S., Barker, J. L., Kopin, I. J., and Di Porzio, U. (1989b). Acetaldehyde directly enhances MPP+ neurotoxicity and delays its elimination from the striatum *Brain Res.* 501, 11–22. doi: 10.1016/0006-8993(89)91021-4

Zuddas, A., Vaglini, F., Fornai, F., and Corsini, G. U. (1992). Selective lesion of the nigrostriatali dopaminergic pathway by MPTP and acetaldehyde or diethyldithiocarbamate. *Neurochem. Int.* 20, 287S–293S. doi: 10.1016/0197-0186(92)90254-O

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 20 March 2013; accepted: 01 June 2013; published online: 21 June 2013.

Citation: Vaglini F, Viaggi C, Piro V, Pardini C, Gerace C, Scarselli M and Corsini GU (2013) Acetaldehyde and parkinsonism: role of CYP450 2E1. Front. Behav. Neurosci. 7:71. doi: 10.3389/fnbeh.2013.00071