Pharmacology, Biochemistry and Behavior 142 (2016) 15–22

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Localized brain differences in *Arc* expression between mice showing low vs. high propensity to ethanol sensitization

Christina N. Nona [a,b], Marcus Lam [b], José N. Nobrega [a,b,c,d,*]

[a] Department of Pharmacology and Toxicology, University of Toronto, Toronto, ON, Canada
[b] Behavioural Neurobiology Laboratory, Campbell Family Mental Health Research Institute, Centre for Addiction and Mental Health, Toronto, ON, Canada
[c] Department of Psychiatry, University of Toronto, Toronto, ON, Canada
[d] Department of Psychology, University of Toronto, Toronto, ON, Canada

ARTICLE INFO

Article history:
Received 15 June 2015
Received in revised form 11 December 2015
Accepted 13 December 2015
Available online 18 December 2015

Keywords:
Alcohol
Behavioral sensitization
Immediate early gene
Activity-regulated cytoskeleton-associated protein (Arc)
DBA/2 mice
Locomotor activity
Central amygdala

ABSTRACT

Behavioral sensitization to ethanol (EtOH) manifests as a progressive and enduring increase in locomotor activity with repeated drug exposure. However, not all mice sensitize to EtOH and the neuronal mechanisms mediating vulnerability and resistance to EtOH sensitization remain unclear. We examined regional brain expression of the immediate early gene activity-regulated cytoskeleton-associated protein (Arc) in order to identify brain areas in which neuroplastic changes may contribute to the development and expression of EtOH sensitization.

Male DBA/2J mice received 5 biweekly injections of EtOH (2.2 g/kg, i.p.) or saline (SAL). They were categorized as high- (HS) or low-sensitized (LS) on the basis of final locomotor activity scores. In both LS and HS mice sacrificed after the last sensitization injection, Arc expression was decreased throughout the brain in comparison to SAL animals. A similar pattern was seen in mice sacrificed after an EtOH challenge two weeks after the last sensitization injection. However in this cohort, Arc expression was significantly increased in the central amygdala (CeA) in LS mice and in SAL mice receiving EtOH for the first time. No significant increases in Arc expression were seen in brains of sensitized (HS) animals.

These results indicate an acute EtOH challenge results in different patterns of Arc expression in brains of LS, HS, and SAL mice. The dramatic increases in Arc expression in the CeA in LS and SAL mice showing little or no behavioral activation suggests that neural activity in this region may serve to inhibit the stimulant effects of EtOH. The observation that HS mice do not show increases in Arc expression with an EtOH challenge suggests the possibility that increased tolerance to the Arc-inducing effects of EtOH may be a factor in behavioral sensitization.

1. Introduction

A unique aspect of behavioral sensitization to ethanol (EtOH) is the variability that exists in this response, not only among strains, but also within susceptible strains. In every batch of DBA mice for example there is a group that sensitizes (high-sensitized, HS) and a group of mice that are resistant to sensitization, or that develop only a modest degree of sensitization (low-sensitized, LS) (Masur and dos Santos, 1988; Nona et al., 2013, 2014). Behavioral sensitization can be defined as a progressive and long-lasting increase in locomotor activity upon drug exposure and is common to many drugs of abuse (Stewart and Badiani, 1993). It is a form of drug-induced neurobehavioural plasticity that not only may potentially elucidate addiction processes, but also can be used to understand enduring behavioral changes in the nervous system in general (Robinson and Berridge, 2000).

The neural mechanisms underlying behavioral sensitization have been conceptualized into two temporally distinct phases, namely a development phase referring to short-term neuroadaptations induced by intermittent drug administration and an expression phase involving more persistent changes mediating supersensitivity to subsequent drug exposure (Kalivas and Stewart, 1991; Pierce and Kalivas, 1997; Vanderschuren and Kalivas, 2000; Wolf, 1998). Although the literature on the involvement of different brain areas during each phase of EtOH sensitization is limited, recent reports are beginning to corroborate the involvement of distinct neural mechanisms involved in each of these phases (Nona et al., 2014, 2015).

Abused drugs have been reported to induce the expression of immediate early genes (IEGs) (Samaha et al., 2004, 2005; Ujike et al., 2002). IEG levels are transiently increased shortly after a stimulus in the brain areas which process that stimulus (Okuno, 2011). They are considered to be markers of neuronal activity which translate extracellular

* Corresponding author at: Centre for Addiction and Mental Health, 250 College St, Toronto, ON M5T 1R8, Canada.
E-mail address: jose.nobrega@camh.ca (J.N. Nobrega).

http://dx.doi.org/10.1016/j.pbb.2015.12.006
0091-3057/

stimuli into altered patterns of neuronal gene expression and long-term changes in cellular functioning. Therefore drug-induced IEG expression can be seen as an important first step in mediating the molecular cascades underlying drug experience-dependent plasticity. Studies examining drug-induced IEG expression have primarily focused on *c-fos* and *zif268*, both of which encode transcription factors (Faria et al., 2008; Jedynak et al., 2012; Mattson et al., 2007; Valjent et al., 2006). In contrast, few studies have looked at gene expression of *activity-regulated cytoskeleton-associated protein* (*Arc*), an effector IEG that encodes a protein directly affecting neuronal plasticity. Specifically, the gene is targeted to synaptic sites undergoing strong activity, where its protein binds to actin filaments, influencing their dynamics and thus regulating spine plasticity (Bramham et al., 2008; Ujike et al., 2002). *Arc* protein plays an important role in the processes underlying the formation of long-term changes in neuron function and synaptic modifications, and is required for other forms of synaptic plasticity such as long-term potentiation (LTP), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor endocytosis, and cell migration (Bramham et al., 2008). Thus, *Arc* expression serves as an excellent marker of drug-induced neuronal activity leading to long-term changes in neuron function. The goal of the present study was to examine differences in *Arc* mRNA expression in LS, HS, and saline (SAL) mice after EtOH exposure in order to identify brain areas where neuroplastic changes may contribute to the development and expression of EtOH sensitization. We hypothesized that HS mice would show greater brain levels of *Arc* expression in comparison to LS and SAL mice.

2. Materials and methods

2.1. Subjects

Male DBA/2 mice ($n = 85$), aged 5 weeks at the beginning of the experiment, were obtained from Charles River (Quebec, Canada). Mice were housed 4 per cage polycarbonate cages (32 × 14 × 12 cm) in a room controlled for temperature, humidity (21.1 °C, 30% humidity), and photoperiod (12:12; lights on at 7 am and off at 7 pm). Food and water were provided ad libitum throughout the experiment. All procedures were approved by the Animal Care Committee at the Centre for Addiction and Mental Health and were in keeping with the guidelines and practices outlined by the Canadian Council on Animal Care.

2.2. Test apparatus

Measurements of locomotor activity (LMA) were carried out in 40 × 40 × 35 cm Plexiglas activity monitor chambers (MED Associates, St. Albans, VT) that automatically detect LMA by horizontal beam breaks. All activity testing was performed between 10 A.M. and 3 P.M. As in previous work, 15-min session was used throughout as because the stimulant effects of EtOH are observed within the first 0–15 min of drug administration, after which sedative effects dominate the behavioral profile (Crabbe et al., 1982; Nona et al., 2013, 2014).

2.3. Drugs

Anhydrous ethyl alcohol (Commercial Alcohols, Brampton, ON) was diluted with physiological saline (0.9% NaCl) to a concentration of 15%*w*/*v*. Mice received 2.2 g/kg of EtOH i.p. (15 mL/kg), or an equal volume of saline (SAL) during the development phase of the study. For the expression phase, mice were challenged with 1.8 g/kg, i.p., of EtOH. These doses were chosen because they elicit locomotor stimulant rather than sedative effects in mice and have repeatedly been shown to produce behavioral sensitization (Nona et al., 2013, 2014; Phillips et al., 1997; Quadros et al., 2002).

2.4. EtOH sensitization procedures

For the development experiment, following 7 days of acclimatization to the colony room, mice received 3 daily habituation sessions to the test chambers, where LMA was measured in the absence of drug. Following habituation sessions, mice were counterbalanced for baseline LMA and subsequently assigned to receive either EtOH ($n = 29$) or SAL ($n = 8$), for a total of 5 biweekly injections. On test days (injections 1, 3, and 5) mice were transported in their home cages to the test room and allowed to acclimatize for 30 min. They were then injected with EtOH or SAL and placed immediately in the activity boxes after injections. LMA was measured for 15 min, after which mice were returned to the colony room. On days when LMA was not measured (injections 2 and 4), mice were acclimated to the test room for 30 min, injected with EtOH or SAL, and subsequently returned to their cages. In all cases cages were left in the testing room for an additional 15 min before being returned to the colony room. Locomotor activity scores after the final injection were ranked for EtOH-treated mice in the lowest 33% of the distribution were classified as low-sensitized, whereas those in the upper 33% were classified as high-sensitized. An identical sensitization protocol was used in a separate cohort designed to test persistent sensitization effects. After the 5th injection, a 14 day drug-free period was allowed and then LS ($n = 16$), HS ($n = 16$) and SAL ($n = 16$) mice assigned to receive either an EtOH (1.8 g/kg) or a SAL challenge prior to a final LMA test and sacrifice. For both cohorts, brains were removed immediately following behavioral testing and stored at −80 °C until sectioning and processing for in situ hybridization analyses.

2.5. In situ hybridization protocol for Arc mRNA expression

Coronal brain sections, 10 μm thick, were prepared on a Leica cryostat, thaw-mounted onto Fisher Superfrost™ slides (VWR, Mississauga, ON) and then stored at −80 °C. The slides were thawed and then prehybridized at room temperature. The sections were fixed in 4% paraformaldehyde for 5 min, rinsed, treated with 0.1 M triethanolamine HCl, acetylated and rinsed in 2× SSC. Hybridization was performed using 35S-UTP labeled riboprobes complementary to the sequences of interest. RNA was extracted from the brain tissue and cDNA was prepared by reverse transcription using Superscript II enzyme (Invitrogen), primed with Oligo-dT; amplified by PCR using compound primers comprising of consensus promoter sequences for either SP6 RNA polymerase (atttaggtgacactatagaa) attached at the 5′ end of the left primer (5′-TGCCAACTAGGACTTGAGCTG-3′) complimentary to bases 1442–1462 of the mouse Arc mRNA, Genbank # NM_001276684.1, and for T7 RNA polymerase (taatacgactcactataggg) attached at the 5′ end to the right primer sequences 5′-GAGGTGCCAGGATGTCAGGT-3′, complementary to bases 1995–1976. The ^{35}S-labeled riboprobes were generated by in-vitro transcription using the Maxiscript kit (Ambion), and the PCR product as a template. Each riboprobe was diluted to a concentration of 18,000 cpm/μL in hybridization solution. Slides were incubated overnight at 60 °C. After hybridization, the sections were rinsed with agitation as follows using decreasing concentrations of SSC containing 25 g/mL sodium thiosulfate. Sections were then rinsed, air dried and then exposed to Kodak BioMax film at 4 °C for 21 days.

2.6. Image analysis

In situ hybridization signals on film were quantified using MCID Basic 7.0 image analysis software (Interfocus Imaging, Linton, UK). Standard curves obtained from calibrated radioactive standards were used to convert film optical densities to microcuries per gram of tissue. Brain regions (Table 1) were identified using the Franklin and Paxinos atlas (Franklin and Paxinos, 1997). Each region was sampled by a blinded observer under uniform background illumination conditions. Data were acquired from at least 2 sections/brain region, from 3 to 4 brain slices per mouse. Sampling was performed on images magnified