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Research Report

Effects of ethanol exposure and withdrawal on dendritic morphology and spine density in the nucleus accumbens core and shell

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ABSTRACT

Exposure to drugs of abuse can result in profound structural modifications on neurons in circuits involved in addiction that may contribute to drug dependence, withdrawal and related processes. Structural alterations on medium spiny neurons (MSNs) of the nucleus accumbens (NAc) have been observed following exposure to and withdrawal from a variety of drugs; however, relatively little is known about the effects of alcohol exposure and withdrawal on structural alterations of NAc MSNs. In the present study male rats were chronically exposed to vaporized ethanol for 10 days and underwent 1 or 7 days of withdrawal after which the brains were processed for Golgi–Cox staining and analysis of dendritic length, branching and spine density. MSNs of the NAc shell and core underwent different patterns of changes following ethanol exposure and withdrawal. At 1 day of withdrawal there were modest reductions in the dendritic length and branching of MSNs in both the core and the shell compared to control animals exposed only to air. At 7 days of withdrawal the length and branching of shell MSNs was reduced, whereas the length and branching of core MSNs were increased relative to the shell. The density of mature spines was increased in the core at 1 day of withdrawal, whereas the density of less mature spines was increased in both regions at 7 days of withdrawal. Collectively, these observations indicate that MSNs of the NAc core and shell undergo distinct patterns of structural modifications following ethanol exposure and withdrawal suggesting that modifications in dendritic structure in these regions may contribute differentially to ethanol withdrawal.

1. Introduction

Drugs of abuse can cause profound and persistent modifications in dendritic length, branching and spine density on neurons in circuits implicated in drug addiction and reward (Kolb et al., 2003; Rice et al., 2012; Robinson and Kolb, 1997, 1999a, 2004; Zhou et al., 2007). These types of morphological changes represent one of the primary mechanisms by which

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experience modifies the nervous system to facilitate future behavior. Importantly, these modifications can be advantageous or disadvantageous. Studies have found structural alterations in medium spiny neurons (MSNs) of the nucleus accumbens (NAc), a region implicated in drug seeking, reward learning, and reinforcement (Di Chiara, 2002; Everitt and Robbins, 2005; Ikemoto and Panksepp, 1999; Koob et al., 2014; Koob and Volkow, 2010; McFarland et al., 2003; Robinson and Berridge, 1993), following exposure to various psychoactive drugs. Modifications in dendritic morphology of NAc MSNs include increases in branching, length, spine density and/or spine head diameter following exposure to nicotine (Brown and Kolb, 2001; Gipson et al., 2013; Hamilton and Kolb, 2005), THC (Kolb et al., 2006), cocaine (Gipson et al., 2013; Kolb et al., 2003; Robinson and Kolb, 1999a), and amphetamines (Kolb et al., 2003; Robinson and Kolb, 1999a, 2004) and decreases following exposure to morphine (Robinson and Kolb, 1999b), haloperidol (Frost et al., 2010), and olanzapine (Frost et al., 2010). Ethanol exposure predominately causes reductions in MSN branching, length, and/or spine density (McMullen et al., 1984; Rice et al., 2012; Romero et al., 2013; Zhou et al., 2007); however, the direction of morphological changes following ethanol exposure varies (see, e.g. Zhou et al., 2007) perhaps owing to the diversity of exposure paradigms.

The effects of ethanol exposure and withdrawal on the morphology of NAc MSNs are not well represented in the literature, but are critically important for better understanding the neural bases and progression of ethanol addiction and withdrawal. Prenatal ethanol exposure induces long-term modifications in the nervous system associated with increased voluntary ethanol consumption in adulthood (Barbier et al., 2008, 2009) which may be partially attributed to reductions in dendritic morphology of MSNs (Rice et al., 2012) and/or elevated dopamine in the NAc (Blanchard et al., 1993). Acute analysis following prenatal and perinatal ethanol exposure, however, failed to detect effects on MSN morphology (Lawrence et al., 2012), suggesting that morphological changes in the NAc may be time dependent. In adulthood, "alcohol-preferring" (P) rats show reductions in spine density and terminal branching and increases in mushroom and multi-headed spines following chronic ethanol drinking and repeated deprivation (Zhou et al., 2007). Spiga et al. (2014) also recently reported reductions in thin spines within the nucleus accumbens early during alcohol withdrawal in young rats. The comparative lack of literature on the impact of ethanol dependence and withdrawal on dendritic morphology and spine density in the NAc of normal adult rats motivated the present study.

This study sought to characterize the effects of passive, chronic intermittent ethanol exposure and both short-term (1 day) and long-term (7 day) withdrawal on dendritic morphology on MSNs in the NAc core and shell. Examining the possibility of a dissociation between the shell and core was motivated by previous experimental data demonstrating functional dissociations between the NAc core and shell in normal rats (Di Chiara, 2002; Di Chiara and Bassareo, 2007; Horsley et al., 2007) and in relation to drug self-administration (Chaudhri et al., 2010; Gonzales et al., 2004; Meredith et al., 2008), as well as identification of the NAc core as part of a broader circuit implicated in reinstatement of drug self-administration (McFarland and Kalivas, 2001). Adult male Sprague-Dawley rats were passively exposed to vaporized ethanol (~37 mg/L; 12 h/day) for ten consecutive days; the control group received no ethanol. This exposure protocol yields blood ethanol concentrations in the 150–200 mg/dL (0.15–0.20) range, produces robust physical dependence, and increases in anxiety-like behaviors (Lack et al., 2007) that are accompanied by significant alterations in glutamatergic and GABAergic neurotransmission in the amygdala (Christian et al., 2012; Diaz et al., 2011). Further, passive vaporized ethanol exposure protocols similar to that employed here induce conspicuous signs of withdrawal (e.g., tremor) upon removal of ethanol (Macey et al., 1996; Roberts et al., 1996), anxiety (Rassnick et al., 1993; Valdez et al., 2004), increased tolerance for the hypothermic effects of ethanol (Ristuccia and Spear, 2005), reduced seizure thresholds (Ferko and Bobyock, 1977), and enhancements of subsequent ethanol seeking and operant self-administration (Buck et al., 2014; Roberts et al., 2000). Following the withdrawal period (1 day or 7 days) or control exposure the brains were extracted for Golgi–Cox staining (Gibb and Kolb, 1998) and dendritic length, branching, overall spine density, and density of specific spine types on MSNs of the NAc core and shell were quantified.

2. Results

2.1. *Length and branching*

Mean total dendritic length of shell and core MSNs for air control, 1 day and 7 day ethanol withdrawal groups are shown in Fig. 2A. An ANOVA with Group and Region (shell v. core) for overall dendritic length revealed a significant Group X Region interaction [$F(2, 22)=3.74$, $p=0.04$, $\eta_p^2=0.254$]. Neither main effect was significant [both ps > 0.066]. Analysis of simple effects of region within each group indicate that the interaction was attributable to a significant region effect for the 7 day withdrawal group [core > shell; $F(1, 8)=8.36$, $p=0.02$, $\eta_p^2=0.511$] that was not observed for either the control group [$p=0.79$, $\eta_p^2=0.01$] or 1 day withdrawal group [$p=0.67$, $\eta_p^2=0.028$].

Mean dendritic length based on distance from the soma for each combination of group and region are shown in Figs. 2B and 2C. Separate repeated measures multivariate ANOVAs (MANOVAs) with Group and Distance from soma (7 segments in 40 μm increments) as factors were conducted each region. There was a significant interaction observed for the NAc core [$\sigma=0.308$, $F(12, 34)=2.27$, $p=0.03$, $\eta_p^2=0.445$], but not the shell [$\lambda\text{ll}=0.558$, $F(12, 34)=0.96$, $p=0.50$, $\eta_p^2=0.253$]. Inspection of the group means for each segment obtained for the core indicate that the means for the 7 day withdrawal group were higher than those of the control and 1 day groups. When directly compared the 7 day withdrawal group had greater dendritic length values following Bonferroni correction than controls for segment 5 of the core [$F(1, 15)=10.96$, $p=0.005$, $\eta_p^2=0.422$]. There was a trend in the same direction for segment 6 [$F(1, 15)=3.75$, $p=0.07$, $\eta_p^2=0.20$]; however, none