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Synaptic adaptations to chronic ethanol intake in male rhesus monkey dorsal striatum depend on age of drinking onset

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ABSTRACT

One in 12 adults suffer with alcohol use disorder (AUD). Studies suggest the younger the age in which alcohol consumption begins the higher the probability of being diagnosed with AUD. Binge/excessive alcohol drinking involves a transition from flexible to inflexible behavior likely involving the dorsal striatum (caudate and putamen nuclei). A major focus of this study was to examine the effect of age of drinking onset on subsequent chronic, voluntary ethanol intake and dorsal striatal circuitry. Data from rhesus monkeys (n = 45) that started drinking as adolescents, young adults or mature adults confirms an age-related risk for heavy drinking. Striatal neuroadaptations were examined using whole-cell patch clamp electrophysiology to record AMPA receptor-mediated miniature excitatory postsynaptic currents (mEPSCs) and $GABA_A$ receptor-mediated miniature inhibitory postsynaptic currents (mIPSCs) from medium-sized spiny projection neurons located in the caudate or putamen nuclei. In controls, greater GABAergic transmission (mIPSC frequency and amplitude) was observed in the putamen compared to the caudate. With advancing age, in the absence of ethanol, an increase in mIPSC frequency concomitant with changes in mIPSC amplitude was observed in both regions. Chronic ethanol drinking decreased mIPSC frequency in the putamen regardless of age of onset. In the caudate, an ethanol drinking-induced increase in mIPSC frequency was only observed in monkeys that began drinking as young adults. Glutamatergic transmission did not differ between the dorsal striatal subregions in controls. With chronic ethanol drinking there was a decrease in the postsynaptic characteristics of rise time and area of mEPSCs in the putamen but an increase in mEPSC frequency in the caudate. Together, the observed changes in striatal physiology indicate a combined disinhibition due to youth and ethanol leading to abnormally strong activation of the putamen that could contribute to the increased risk for problem drinking in younger drinkers.

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1. Introduction

Seventeen million or 7% of individuals over the age of 12 in the US have been diagnosed with alcohol use disorder (AUD), a medical term that encompasses a range from risky patterns of drinking to alcoholism and dependence (SAMHSA, 2014). There are several environmental factors that are thought to correlate with the likelihood of being diagnosed with AUD, including the age when ethanol drinking begins. Ethanol use most often begins during late adolescence, with research showing that the average age of first use of alcohol is about 16 years (Degenhardt et al., 2008; SAMHSA, 2014). Individuals that reported their first alcoholic drink before the age of 14 were more likely to develop AUD than those that began drinking at or over the age of 21 (Grant and Dawson, 1998; Merline et al., 2004; SAMHSA, 2014). In fact, risky patterns of drinking that are associated with AUD can manifest as early as adolescence.

The fact that age of onset is a risk factor for AUD suggests a biological basis for the progression from non-harmful to harmful alcohol consumption. The dorsal striatum is implicated in the seeking and taking of drugs of abuse, and in the progression from recreational drug-use to habitual drug seeking and addiction (Gerdeman et al., 2003; Everitt and Robbins, 2005; Vollstädt-Klein et al., 2010). The anterior portion of the primate caudate nucleus,

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similar to the rodent dorsomedial striatum (DMS), receives afferent input from the association cortices and contributes directly to actions that are sensitive to outcome value (Yin et al., 2005a,b; Haber et al., 2006; Gläscher et al., 2009). The caudoventral portion of the primate putamen nucleus, or dorsolateral striatum (DLS) in rodents, receives afferent input from the sensory and motor cortices and plays a role in inflexible habit learning that leads to behavioral automatization, a process proposed to underlie the maintenance of drug use (Künzle, 1975; McGeorge and Faull, 1989; Yin et al., 2006; Tricomi et al., 2009). Alterations in circuit function and behavioral control that favor sensorimotor striatal control have also been postulated based on studies of instrumental learning and drug use (Yin et al., 2006; Corbit et al., 2012, 2014).

Recent studies indicate that the dorsal striatum of rodents is highly sensitive to the effects of ethanol (Choi et al., 2006; Fanelli et al., 2013; Corbit et al., 2014; Clarke et al., 2015; DePoy et al., 2015; Patton et al., 2015; Wang et al., 2007, 2010, 2015). Ethanol alters the function of striatal circuits in multiple ways that are thought to contribute to acute intoxication, craving, dependence, and withdrawal. In mature adult macaque monkeys (aged 7–9.5 years), we found that chronic ethanol self-administration induced a decrease in GABAergic transmission onto MSNs of the putamen, and transmission at these synapses was strongly and negatively correlated with daily alcohol intakes (Cuzon Carlson et al., 2011). We extended these findings to mice chronically consuming alcohol and observed decreased GABAergic transmission in the DLS (Wilcox et al., 2014).

The present study explored the influence of age at the onset of ethanol access on the risk for chronic heavy drinking and related neurotransmission changes in the dorsal striatum. The age range roughly corresponded to adolescence, young adulthood and mature adulthood of male rhesus macaques. A previous report on male monkeys showed that ethanol intake was greatest when drinking began as a young adult or in late adolescence (Helms et al., 2014a). Here, we tested the hypotheses that chronic ethanol will induce changes in dorsal striatal synaptic function that will: (1) differ based on the dorsal striatal subregion, (2) correlate with ethanol intake, and (3) differ with age of onset.

2. Materials and methods

2.1. Animals

A total of 45 male rhesus macaques (*Macaca mulatta*) in 4 experimental cohorts were used in this study. Animals were born and reared at the Oregon National Primate Research Center. The monkeys were individually housed indoors with temperature (20-22 °C) and humidity (65%) controlled under an 11-hour light cycle with lights on at 7:00 a.m. Each cohort was housed within the same room, allowing for continuous visual, auditory, and olfactory contact with one another. All cohorts underwent the same standard operating procedures that included a well-defined schedule-induced polydipsia (SIP) protocol as displayed in Fig. 1A, conducted according to the Guide for the Care and Use of Laboratory Animals and approved by the Oregon National Primate Research Center Institutional Animal Care and Use Committee.

The monkeys differed in the age at which they were first exposed to ethanol: adolescent (4–5 years, equivalent to ~15–18 human years; N = 8; INIA cohort 7a), young adulthood (5–6 years, ~18–24 human years; N = 13; INIA cohorts 5 and 7b) and mature adulthood (7–11 years, ~25–40 human years; N = 11; INIA cohort 4). Control monkeys were age-matched to their ethanol counterparts, but had no access to ethanol (adolescent: N = 4; young adulthood: N = 7; mature adulthood: N = 2) and the age of experimental onset (Fig. 1B; *t*-test: adolescent: $t_{10} = 0.84$, $p = .42$; young adult: $t_{18} = 0.09$, $p = .29$; mature adult: $t_{11} = 1.76$, $p = .11$) between control and ethanol-drinking monkeys was not statistically different across the groups. However, the age at necropsy was significantly different between the young adult and mature adult groups but not the adolescent group (Fig. 1B; *t*-test: adolescent: $t_{10} = 0.81$, $p = .44$; young adult: $t_{18} = 2.28$, $p = .04$; mature adult: $t_{11} = 2.66$, $p = .02$).

2.2. Chronic oral ethanol self-administration

The chronic ethanol drinking paradigm is shown schematically in Fig. 1A. One wall of the home-cage was replaced with an operant panel that was previously described (Helms et al., 2014a) and modified from previous studies (Vivian et al., 2001; Grant et al., 2008). The operant panel consisted of two drinking spouts, a row of three lights (red, white, and green) above each spout that signaled the status of the panel/session (i.e. active or inactive), a central recess containing a dowel pull, a central food cup, and a finger-poke hole with an infrared switch. Monkeys learned to obtain all fluids (water and/or ethanol) and food pellets through the panel.

Monkeys were induced to self-administer ethanol using schedule–induced polydypsia (SIP) as previously described (Vivian et al., 2001; Grant et al., 2008, Fig. 1A). Monkeys were trained to obtain all fluids (ethanol or water) and food using the operant chamber. Banana-flavored pellets were delivered on a fixed-time interval of 300-s until a volume of water or a low concentration of ethanol (4% w/v in water) in increasing volumes equivalent to 0.5, 1.0, and 1.5 g/kg per day was consumed (Fig. 1A). After a 3-hour time out, banana pellets were then delivered on a fixed-ratio of 1 until the daily ration of pellets was consumed. Every cohort had approximately 14 months of daily drinking under these "open access" conditions (Fig. 1A). Throughout the drinking protocol, monkeys complied with awake venipuncture for obtaining blood to measure blood ethanol concentration (BEC) every 5–7 days at 7 h after session start, as previously described (Grant et al., 2008).

There is strong evidence that this population of monkeys has stable, categorically separate, levels of ethanol intake derived from a Principal Component Analysis (PCA) of daily intakes from >12,000 daily sessions in the open-access condition (i.e., the 14 months of 22 h/day access to ethanol and water) (Baker et al., 2014, 2017). Very heavy drinkers are defined as having greater than or equal to 10% of open access ethanol drinking days that exceeded 4 g/kg as well as an average daily ethanol intake that exceeded 3 g/kg. Heavy drinkers are defined as those that had greater or equal to 20% of their open access ethanol drinking days with an intake that exceeded 3 g/kg. Binge drinkers are defined as having greater or equal to 55% of their open access days with an intake that exceeded 2 g/kg. Low drinkers are defined as those with intake below any of these thresholds. Cohorts of individual monkeys are a random sample of this population and contain unequal distributions of individuals in each category (www.matrr.com). This present design helped address the influence of age on this distribution.

Control monkeys were placed on the same diet, had maltose-dextrose calorically matched to ethanol intakes and had water available *ad libitum*. They were housed in the same housing rooms and had similar daily routines as that of their ethanol-drinking counterparts. Ethanol drinking data from some of the monkeys (cohorts 4, 5 and 7a) used in this study was previously reported (Helms et al., 2014a) and drinking data for all monkeys are available at www.matrr.com.

2.3. Necropsy and tissue preparation

Tissue preparation methods were previously published