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A SUBSET OF VENTRAL TEGMENTAL AREA DOPAMINE NEURONS RESPONDS TO ACUTE ETHANOL

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Abstract—The mechanisms by which alcohol drinking promotes addiction in humans and self-administration in rodents remain obscure, but it is well known that alcohol can enhance dopamine (DA) neurotransmission from neurons of the ventral tegmental area (VTA) and increase DA levels within the nucleus accumbens and prefrontal cortex. We recorded from identified DA neuronal cell bodies within ventral midbrain slices prepared from transgenic mouse line (TH-GFP) mice using long-term stable extracellular recordings in a variety of locations and carefully mapped the responses to applied ethanol (EtOH). We identified a subset of DA neurons in the medial VTA located within the rostral linear and interfascicular nuclei that fired spontaneously and exhibited a concentration-dependent increase of firing frequency in response to EtOH, with some neurons responsive to as little as 20 mM EtOH. Many of these medial VTA DA neurons were also insensitive to the D2 receptor agonist quinpirole. In contrast, DA neurons in the lateral VTA (located within the parabrachial pigmented and paranigral nuclei) were either unresponsive or responded only to 100 mM EtOH. Typically, these lateral VTA DA cells had very slow firing rates, and all exhibited inhibition by quinpirole via D2 ''autoreceptors''. VTA non-DA cells did not show any significant response to low levels of EtOH. These findings are consistent with evidence for heterogeneity among midbrain DA neurons and provide an anatomical and pharmacological distinction between DA neuron sub-populations that will facilitate future mechanistic studies on the actions of EtOH in the VTA. © 2015 Published by Elsevier Ltd. on behalf of IBRO.

Key words: dopamine, VTA, tonic firing, ethanol, alcohol, addiction.

INTRODUCTION

Alcohol is certainly the most ancient, and arguably the most widely abused substance in human society. The costs associated with alcoholism and alcohol use disorders are enormous, in terms of economic performance from lost productivity and the human suffering caused by alcohol-associated disease, death and family breakdown. Ethanol (EtOH) is readily self-administered by most mammalian species and is easily studied in rodent models (Crabbe et al., 2011; Carnicella et al., 2014), in which it is highly addictive, inducing a strong conditioned place preference (Melis et al., 2007) and robust drinking in two-bottle choice paradigms. Most, if not all, drugs of abuse have been shown to activate the mesolimbic reward system to enhance dopamine (DA) transmission in the striatum (Sulzer, 2011), albeit via distinctly different mechanisms. Drugs such as cocaine and amphetamine act in part within the striatum via their effects on the DA transporter of axon terminals. Opiates and benzodiazepines target the inhibitory interneurons in ventral tegmental area (VTA) to disinhibit DA cell firing and indirectly promote DA release (Tan et al., 2010; Luscher and Malenka, 2011). In contrast to these well-characterized drugs of abuse, the manner in which alcohol alters DA physiology remains unclear and controversial (Morikawa and Morrisett, 2010).

There have been many previous studies of alcohol action in VTA *in vitro*, many of which were quite elegant in their methodology. As a result, numerous synaptic and intrinsic targets for EtOH have been suggested in recent years (Brodie et al., 1990; Okamoto et al., 2006; Nimitvilai et al., 2013). The significance of some of these findings has been difficult to interpret because of the universal observation that very high concentrations of EtOH application have been required to observe responses. Typically 80–100 mM EtOH has been used, a level of the drug that would result in unconsciousness or death in naïve animals, although tolerated in some alcoholics. The question therefore remains as to how the VTA responds to modest levels of EtOH that are typically

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Abbreviations: ACSF, artificial cerebrospinal fluid; CV, coefficient of variation; DA, dopamine; EtOH, ethanol; FF, firing frequency; GABA, gamma-aminobutyric acid; HEPES, (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; IF, interfascicular nucleus of VTA; Ih, hyperpolarization-activated non-selective cation current; ISI, interspike interval; PBP, parabrachial pigmented nucleus of VTA; RLi, rostral linear nucleus of VTA; TH, tyrosine hydroxylase; TH-GFP, transgenic mouse line; SN, substantia nigra; Vm, membrane potential; VTA, ventral tegmental area.

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associated with social intoxication. Indeed, low and moderate concentrations of EtOH (equivalent to several glasses of wine in humans) have been little studied since the early days of the field (Gessa et al., 1985), yet there is no question that these provide more accurate models of early-stage drinking.

It has been suggested by some authors that these difficulties have been amplified by other problems, such as ambiguous identification of DA neurons in VTA (Margolis et al., 2006; Ungless and Grace, 2012). Microdialysis studies in freely-moving rats have demonstrated that low doses of EtOH injection (0.5-g/kg i.p.) preferentially stimulated DA release in the nucleus accumbens (Di Chiara and Imperato, 1985). Furthermore, precise mapping of DA release sites using cyclic voltammetry *in vivo* after acute EtOH injection (0.5-g/kg injected intravenously) revealed the existence of "hot spots" of EtOH-responsive regions in nucleus accumbens core and shell, as well as clearly unresponsive regions nearby (Robinson et al., 2009). The anatomical basis for the heterogeneity of these EtOH responses is unknown, but a reasonable supposition is that this may originate in the cell bodies in the VTA.

Considerable attention has recently focused on the concept of regional heterogeneity of VTA cells, which had formerly been lumped together as a homogenous population of DA neurons bearing considerable similarity to the neighboring DA cell population in the substantia nigra (SN) *pars compacta* (Neuhoff et al., 2002; Ungless et al., 2004; Bjorklund and Dunnett, 2007; Lammel et al., 2008; Borgkvist et al., 2011; Lammel et al., 2014; Marinelli and McCutcheon, 2014). Unlike the nigral DA cells, however, the identification of DA cells in VTA by physiological criteria or pharmacology alone appears to be insufficient (Margolis et al., 2006). It is now generally accepted that verification of tyrosine hydroxylase (TH) expression is necessary to confirm DA identity (Fields et al., 2007; Ungless and Grace, 2012).

VTA cells appear to exhibit regional differences in responses to other drugs of abuse, including opioids (Ford et al., 2006; Margolis et al., 2008), nicotine (Ericson et al., 2008; Zhao-Shea et al., 2011), and cocaine (Lammel et al., 2011). Retrograde labeling studies have demonstrated that midline VTA DA cells are most sensitive to cocaine, and that the axons of these DA cells project to the medial shell of NAc and prefrontal cortex (Lammel et al., 2011). It has been suggested that responsiveness to EtOH may also exhibit regional differences (Robinson et al., 2009).

Here we undertook a simple but careful study of the alcohol responses of a large sample of 81 DA neurons in the mouse VTA, in an attempt to locate the cells that might be most sensitive to EtOH, in order to facilitate future characterization of target molecules. We selected a technologically simple, yet highly stable recording technique ("loose-patch" recording of action potentials) that obviates any problems associated with cytoplasmic dialysis during long recordings (Carta et al., 2004) and prepared midbrain slices from a transgenic mouse line (TH-GFP) expressing green fluorescent protein under the TH promoter (Sawamoto et al., 2001) in order to facilitate the identification of the DA neuron phenotype. Our results show that EtOH can accelerate DA neuron firing of a subpopulation of medial VTA DA neurons.

EXPERIMENTAL PROCEDURES

All animal procedures were performed following NIH guidelines, and were approved by the Institutional Animal Care and Use Committee at the Columbia University Medical Center. Wild-type C57BL/6J mice were obtained from the Jackson Laboratory (Bar Harbor, MA, USA). Wild-type and TH-GFP mice, in which neuronal GFP expression showed >87% co-localization with TH immunoreactivity (Sawamoto et al., 2001), were sacrificed at 3–12 weeks of age, and their brains removed for acute slice recordings.

Electrophysiological recordings in brain slice

Coronal midbrain slices (250- μm-thick) were prepared using a vibratome (Leica VT1200; Nussloch, Germany) with VTA between bregma −3.0 to −3.8 mm (primarily near bregma −3.5 mm). Brains were submerged in ice-cold cutting solution containing (in mM): 100 glucose, 75 NaCl, 26 $NaHCO_3$, 2.5 KCl, 2 $MgCl_2$-$6H_20$, 1.25 NaH_2PO_4-$6H_20$, and 0.7 $CaCl_2$. Slices were allowed to recover in the solution for 30 min at 34 °C and then transferred to a recording solution (artificial cerebrospinal fluid, ACSF) containing (in mM): 119 NaCl, 26.2 $NaHCO_3$, 2.4 $CaCl_2$, 1.8 KCl, 1.2 $MgCl_2$-$6H_20$, 1.0 NaH_2PO_4-$6H_20$, and 10 glucose. The recording chamber temperature was maintained at 32 °C (±2 °C) with an in-line heater and temperature controller (Warner Instruments, Hamden, CT, USA).

Extracellular "on-cell" recordings were obtained with pipettes (tip resistance 2–4 MΩ) pulled from borosilicate glass (G150F-4, Warner Instruments) on a P-97 Flaming-Brown micropipette puller (Sutter Instruments) and filled with ACSF solution. Seal resistances ranged from 10 MΩ to 1 GΩ, but most recordings were between 10 and −30 MΩ and were monitored in voltage-clamp mode at a command potential of 0 mV throughout the recordings. In a subset of experiments, whole-cell patch clamp recordings were performed with pipettes (tip resistance 3–4 MΩ) filled with internal solution containing (in mM): 115 K-gluconate, 20 KCl, 10 HEPES, 2 $MgCl_2$, 2 ATP-Mg, 2 ATP-Na_2, 0.3 GTP-Na, (pH = 7.3, ~290 mOsm).

DA neurons expressing GFP were visualized under a 40x water immersion objective by fluorescence and DIC optics (Olympus). Voltage-clamp and whole cell current-clamp recordings were performed with an Axopatch 200B amplifier (Molecular Devices) and digitized at 10 kHz with a Digidata 1332 (Molecular Devices). Data were acquired using Clampex 8 software (Molecular Devices). For whole-cell patch-clamp recordings, the resting membrane potential (Vm), spontaneous firing frequency (FF), and input resistance (measured by −100 pA, 100 ms duration hyperpolarizing pulses) were monitored throughout the recording. Only cells in which a baseline FF was stable for at least 5 min were

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