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INTRINSIC MEMBRANE PROPERTIES AND CHOLINERGIC MODULATION OF MOUSE BASAL FOREBRAIN GLUTAMATERGIC NEURONS *IN VITRO*

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Abstract—The basal forebrain (BF) controls sleep–wake cycles, attention and reward processing. Compared to cholinergic and GABAergic neurons, BF glutamatergic neurons are less well understood, due to difficulties in identification. Here, we use vesicular glutamate transporter 2 (vGluT2)-tdTomato mice, expressing a red fluorescent protein (tdTomato) in the major group of BF glutamatergic neurons (vGluT2+) to characterize their intrinsic electrical properties and cholinergic modulation. Whole-cell, patch-clamp recordings were made from vGluT2+ neurons in coronal BF slices. Most BF vGluT2+ neurons were small/medium sized (<20 μm), exhibited moderately sized H-currents and had a maximal firing frequency of ~50 Hz. However, vGluT2+ neurons in dorsal BF (ventral pallidum) had larger H-currents and a higher maximal firing rate (83 Hz). A subset of BF vGluT2+ neurons exhibited burst/cluster firing. Most vGluT2+ neurons had low-threshold calcium spikes/currents. vGluT2+ neurons located in ventromedial regions of BF (in or adjacent to the horizontal limb of the diagonal band) were strongly hyperpolarized by the cholinergic agonist, carbachol, a finding apparently in conflict with their increased discharge during wakefulness/REM sleep and hypothesized role in wake-promotion. In contrast, most vGluT2+ neurons located in lateral BF (magnocellular preoptic area) or dorsal BF did not respond to carbachol. Our results suggest that BF glutamatergic neurons are heterogeneous and have morphological, electrical and pharmacological properties which distinguish them from BF cholinergic and GABAergic neurons. A subset of vGluT2+ neurons, possibly those neurons which project to reward-related areas such as the habenula, are hyperpolarized by cholinergic inputs, which may cause phasic inhibition during reward-related events.

Key words: vesicular glutamate transporter, sleep, cortical activation, whole-cell, Alzheimer's disease, patch-clamp.

INTRODUCTION

The basal forebrain (BF) is a subcortical brain region involved in the control of sleep–wake cycles, cortical activation, attention and reward processing (Brown et al., 2012; Zaborszky et al., 2012; Brown and McKenna, 2015; Lin et al., 2015). BF integrity and function is impaired in diverse neuropsychiatric conditions such as coma (Edlow et al., 2013), dementia (Whitehouse et al., 1982) and narcolepsy (Nishino et al., 1995; Reid et al., 1998). In particular, atrophy of BF cholinergic neurons is a prominent feature of dementia associated with Alzheimer's disease, mild cognitive impairment and Parkinson's disease (Teipel et al., 2011; Grothe et al., 2012; Muller and Bohnen, 2013). Thus, a clear understanding of the cellular properties of BF neurons and the effects of cholinergic neurons on other BF neurons is important to understand the functional consequences of loss of cholinergic neurons in these disorders.

The BF contains three, largely non-overlapping groups of neurons which release the neurotransmitters acetylcholine, GABA and glutamate (Zaborszky et al., 2012). Subsets of all three of these groups project to the neocortex (Freund and Meskenaite, 1992; Gritti et al., 2003; Henny and Jones, 2008; Kim et al., 2015; Zaborszky et al., 2015; Do et al., 2016) and have been implicated in promoting wakefulness and fast cortical rhythms (Anaclet et al., 2015; Kim et al., 2015; Xu et al., 2015; Zant et al., 2016). Other subsets project caudally to areas involved in reward processing and sleep–wake control such as the lateral hypothalamus and lateral habenula and/or locally within the BF (Zaborszky et al., 2012; Xu et al., 2015; McKenna et al., 2015a; Agostinelli

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Abbreviations: ACSF, artificial cerebrospinal fluid; AHP, afterhyperpolarization; ANOVA, analysis of variance; AP, action potential; BF, basal forebrain; Cre, Cre Recombinase; EGTA, ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid; GABA, gamma-amino-butyric-acid; HCN, hyperpolarization-activated cyclic nucleotide-gated cation channels; HDB, horizontal limb of the diagonal band; HEPES, 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid; LPO, lateral preoptic area; MCPO, magnocellular preoptic area; REM, rapid-eye-movement; RMP, resting membrane potential; TTA-P2, 3,5-dichloro-N-[1-(2,2-dimethyl-tetrahydro-pyran-4-ylmethyl)-4-fluoro-piperidin-4-ylmethyl]-benzamide; TTX, tetrodotoxin; vGluT2, vesicular glutamate transporter, subtype 2; VP, ventral pallidum; ZD7288, 4-ethylphenylamino-1,2-dimethyl-6-methylaminopyrimidinium chloride.

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et al., 2016; Brown et al., 2016; Do et al., 2016; Golden et al., 2016).

Glutamatergic neurons are the least well understood of the three main neurotransmitter phenotypes within BF. The discovery of vesicular glutamate transporters (vGluT) allowed the first definitive identification of glutamate neurons throughout the brain (Fremeau et al., 2004), including BF. In the BF, vesicular glutamate transporter 2 (vGluT2) is the predominant isoform (Hur and Zaborszky, 2005), expressed in BF glutamatergic neurons which project outside the BF to the cortex, lateral habenula, hypothalamus and other subcortical regions (Hur and Zaborszky, 2005; Henny and Jones, 2006, 2008; McKenna et al., 2015a; Agostinelli et al., 2016; Do et al., 2016). Strong optogenetic excitation of BF vGluT2+ neurons increased wakefulness (Xu et al., 2015), whereas weaker chemogenetic activation reduced cortical delta activity (Anaclet et al., 2015) suggesting that BF vGluT2+ neurons promote arousal. Furthermore, the caudal projections of BF vGluT2+ neurons to the lateral habenula and lateral hypothalamus are suggestive of a role in reward processing (Henny and Jones, 2006; McKenna et al., 2015a; Agostinelli et al., 2016; Brown et al., 2016; Do et al., 2016; Golden et al., 2016). However, the ionic and neurotransmitter mechanisms which regulate their activity remain to be elucidated.

Single-unit recordings *in vivo* from identified BF vGluT2+ neurons revealed that their discharge rate exhibits state-dependent modulation, being slightly increased during wakefulness and rapid-eye-movement (REM) sleep when compared to NREM sleep (Xu et al., 2015). However, the discharge of BF putative glutamatergic neurons is heterogeneous (Manns et al., 2003a; Hassani et al., 2009). In particular, phasic firing is a notable feature of the discharge of a subset of putative BF glutamatergic neurons (Manns et al., 2003a; Hassani et al., 2009), whereas others discharge tonically. The mechanisms underlying these state-dependent discharge patterns are unknown at present, since the intrinsic electrical properties of identified vGluT2+ neurons in regions of the BF containing neurons projecting to the neocortex have not been reported. Our results here suggest that the heterogeneity of discharge patterns of BF glutamatergic neurons observed *in vivo* (Manns et al., 2003a; Hassani et al., 2009) is matched by a diversity of intrinsic electrical properties *in vitro*. Thus, it is likely that several functionally distinct groups of BF vGluT2+ neurons exist.

Little is known about the neurotransmitter modulation of BF vGluT2+ neurons. Recent optogenetic studies *in vitro*, reported as we were conducting this study, suggested that the predominant effect of cholinergic neurons on BF vGluT2+ neurons is a hyperpolarization (Xu et al., 2015). This result is apparently in conflict with the increased discharge of vGluT2+ neurons during wakefulness/REM sleep and with a recent model of BF control of sleep and wakefulness which proposed that these neurons promote wakefulness. However, we find here that only a subset of BF vGluT2+ neurons, possibly projection neurons involved in reward processing, are inhibited by the cholinergic receptor agonist, carbachol. Other BF vGluT2+ neurons, possibly interneurons involved in maintaining the activity of BF cholinergic and GABAergic neurons during wakefulness/REM sleep, are unaffected by carbachol. Parts of these results have been presented in abstract form (Yang et al., 2015, 2016; Brown et al., 2016).

EXPERIMENTAL PROCEDURES

Animals

Mice expressing the enzyme Cre Recombinase (Cre) under the control of the vGluT2 promoter i.e. vGluT2-Cre mice (strain 016963; Jackson Laboratories, Bar Harbor, ME, USA), were crossed with a reporter strain expressing the red fluorescent marker, tdTomato in the presence of Cre (strain 007905; Jackson Laboratories, Bar Harbor, ME, USA) to generate mice which express red fluorescence in the major subset of BF glutamatergic neurons (vGluT2-tdTomato mice). Previous work has validated the selective expression of Cre in vGluT2 neurons using *in situ* hybridization (Vong et al., 2011), including in BF (Anaclet et al., 2015; Xu et al., 2015). In our own work we have found that there is virtually no overlap between tdTomato and markers of cholinergic and GABAergic neurons in BF (McKenna et al., 2015a,b). Thus, vGluT2-tdTomato mice represent a valid model to investigate glutamatergic BF neurons.

vGluT2-tdTomato mice aged 13–22 d were used for *in vitro* electrophysiological recordings, as in our previous *in vitro* studies of the intrinsic properties and cholinergic modulation of BF neurons (McKenna et al., 2013; Yang et al., 2014). Mice were housed in a temperature controlled facility with lights on at 0700 and lights off at 1900 h. Food and water were available to the animals *ad libitum*. The animal experiments described herein were approved by the IACUC committee of the VA Boston Healthcare System. All experiments were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) and were consistent with US Veterans Administration, US National Institutes of Health and Harvard University guidelines. All efforts were made to minimize the number of animals used and their suffering.

Preparation of BF slices for electrophysiological recordings

vGluT2-tdTomato mice were deeply anesthetized with isoflurane, decapitated and the brain was removed from the skull. The brain was affixed to the stage of a Vibratome 3000 or VT1200S vibrating-blade microtome (Leica Biosystems, Nussloch, Germany) and two 300 μm, coronal BF slices were cut centered on the region between 0.26 and −0.22 mm from bregma (Franklin and Paxinos, 2008). Following slicing, the brain sections were stored at room temperature in artificial cerebrospinal fluid (ACSF) with the following composition (in mM): 124 NaCl, 1.8 KCl, 25.6 $NaHCO_3$, 1.2 KH_2PO_4, 2 $CaCl_2$, 1.3 $MgSO_4$, and 10 glucose (osmolarity, 300 mOsm) and saturated with 95% O_2/5% CO_2. The brain slices were allowed to recover from the slicing pro-