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A ROLE FOR INHIBITION IN DEAFNESS-INDUCED PLASTICITY OF THE AVIAN AUDITORY BRAINSTEM

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Abstract—To better understand the effects of deafness on the brain, these experiments examine how disrupted balance between excitatory and inhibitory neurotransmission following the loss of excitatory input from the auditory nerve alters the central auditory system. In the avian cochlear nucleus, nucleus magnocellularis (NM), deprivation of excitatory input induced by deafness triggers neuronal death. While this neuronal death was previously accredited to the loss of excitatory drive, the present experiments examine an alternative hypothesis: that inhibitory input to NM, which may also be affected by deafness, contributes to neuronal death in NM. Using an *in vitro* slice preparation in which excitatory input from the auditory nerve is absent, we pharmacologically altered GABA receptor activation in NM, and assayed an early marker of neuronal health, antigenicity for the ribosomal antibody Y10B (Y10B-ir). We found that GABA decreases Y10B-ir, and that $GABA_A$ activation is necessary for the GABA-induced effect. We further found that endogenous $GABA_A$ activation similarly decreases Y10B-ir and this decrease requires extracellular Ca^{2+}. Our results suggest that, in the absence of excitatory input, endogenous activation of ionotropic $GABA_A$ receptors is detrimental to NM neurons.

Key words: deafferentation, $GABA_A$ receptor, ribosomes, cell death, cochlear nucleus.

INTRODUCTION

Experience shapes the nervous system, not only during critical periods of development, but throughout life (Wolff and Missler, 1992). A fruitful paradigm for deciphering mechanisms underlying such neuroplasticity has been to manipulate peripheral sensory input and examine effects on the central nervous system. One common manipulation, afferent deprivation, induces significant changes to the structure and function of central circuitry as demonstrated across sensory modalities (Levi-Montalcini, 1949; Hubel and Wiesel, 1970; Van der Loos and Woolsey, 1973; Brunjes, 1994; Shao et al., 2009). Activity-dependent changes include changes in synapses (Fifková, 1970; Sanes and Kotak, 2011), changes in intrinsic physiology (Maravall et al., 2004; Leao et al., 2005), and even elimination of neurons (Born and Rubel, 1985; Frazier and Brunjes, 1988). These changes can be long-lasting, and thus implicate alterations in gene expression and protein synthesis. However, how afferent activity modulates intracellular processes to drive these alterations has not been fully elucidated.

A powerful system for studying how sensory input shapes intracellular processes is the chick auditory brainstem. This is because unilateral manipulations of auditory nerve activity, most commonly induced by unilateral cochlea removal, allows within-subject comparisons between deprived and normally-innervated cochlear nucleus neurons on opposite sides of the same brain. Removal of the cochlea initiates an array of rapid and pronounced changes in the cochlear nucleus, nucleus magnocellularis (NM). Most dramatically, 20–40% of NM neurons die within 48 h of deafferentation (Born and Rubel, 1985). Preceding neuronal death, neurons exhibit decreased immunostaining of polyribosomes with the marker Y10B (Y10B-ir) (Garden et al., 1994, 1995a; Hyson and Rubel, 1995) and other ribosomal proteins (McBride et al., 2013), an associated reduction in protein synthesis (Steward and Rubel, 1985; Hyson and Rubel, 1989), and increased intracellular concentrations of calcium ($[Ca^{2+}]_i$) (Zirpel et al., 1995; Zirpel and Rubel, 1996), all of which can be observed as early as one hour following deafness.

In searching for the mechanisms that mediate deafferentation-induced cell death in NM, research has focused on glutamatergic input from the auditory nerve. In an intact system, spontaneous and sound-evoked activity of the auditory nerve activates postsynaptic neurons by releasing glutamate from the large endbulbs of Held (Parks, 1981; Nemeth et al., 1983). So long as the cochlea is intact, the auditory nerve also drives a high rate of spontaneous activity in NM neurons even in quiet environments (Tucci et al., 1987). While driven activity in NM neurons appears dependent on iontropic glutamate receptors (iGluRs) (Nemeth et al., 1983; Hyson, 1997), there is evidence that glutamate's trophic effect is through

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Abbreviations: $[Ca^{2+}]_i$, intracellular calcium concentration; aCSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; iGluR, ionotropic glutamate receptor; IPSC, inhibitory post-synaptic potential; mGluR, metabotropic glutamate receptor; NGS, normal goat serum; NL, nucleus laminaris; NM, nucleus magnocellularis; PBS, phosphate-buffered saline; SON, superior olivary nucleus; VGCC, voltage-gated calcium channel; Y10B-ir, immunoreactivity for the Y10B antibody.

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activation of another group of receptors, metabotropic glutamate receptors (mGluRs). *In vitro* studies, designed to mimic the case of unilateral cochlea removal, have shown that unilateral auditory nerve stimulation results in greater Y10B-ir on the stimulated side of the brain slice, but this effect is eliminated by blocking mGluRs (Hyson, 1997; Nicholas and Hyson, 2004). Unilateral activation of mGluRs in the absence of auditory nerve stimulation, on the other hand, results in higher Y10B-ir on the activated side of the slice (Carzoli and Hyson, 2014). A similar pattern of results was observed when the assay for postsynaptic changes in NM was $[Ca^{2+}]_i$ (Zirpel and Rubel, 1996) or when long-term consequences of mGluR blockade were evaluated by counting the number of surviving neurons following unilateral cochlea ablation *in vivo* (Carzoli and Hyson, 2011). Because auditory nerve stimulation or application of exogenous mGluR agonists prevents these detrimental effects of deafferentation on NM neurons, mGluR activation can be considered trophic.

In contrast to this well-described role of glutamate in preventing the deafferentation-induced degradation of NM neurons, the involvement of other neurotransmitters has not been investigated. The primary inhibitory neurotransmitter in NM is GABA, which is provided primarily by the ipsilateral superior olivary nucleus (SON) (Lachica et al., 1994; Yang et al., 1999). SON activity is indirectly driven by auditory nerve activity, through glutamatergic neurons of primary and secondary auditory nuclei, (circuitry shown in Fig. 1), so its descending GABAergic projections to NM have been proposed to act as negative feedback that counters the ascending auditory input. In NM, GABA plays a prominent role in shaping physiology, improving phase locking in NM neurons (Monsivais et al., 2000; Lu and Trussell, 2001; Fukui et al., 2010). Although GABA's influence over deafferentation-induced neuronal death has not been investigated, previous publications suggest GABA has the potential to drive metabolic change in NM by influencing $[Ca^{2+}]_i$. A bath-applied agonist specific to the ionotropic receptor $GABA_A$ elicits a transient increase in $[Ca^{2+}]_i$ (Lachica et al., 1997; Wang et al., 2009a,b). Whether GABA otherwise influences metabolic activity of NM neurons, and whether it contributes to deafferentation-induced changes, such as changes in ribosomes and ultimately cell death, is unknown.

In the present set of experiments, we modulated activity at GABA receptors in deafferented NM and observed changes in Y10B-ir. Additionally, we used whole-cell patch clamp recordings to confirm spontaneous GABAergic currents are present in deafferented NM. Our results suggest that endogenous $GABA_A$ activation is involved in the reduction in Y10B-ir observed following deafness. This implies that auditory nerve input tempers the effects of descending GABA on NM neuronal metabolism, and when this excitatory input is lost, inhibition pushes NM neurons toward cell death.

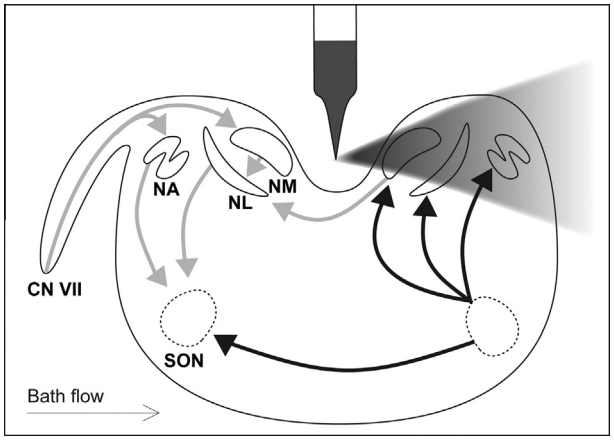

Fig. 1. Schematic of brainstem circuitry and method of drug application. *Brainstem circuitry:* Ascending input is glutamatergic (gray arrows), The auditory nerve sends glutamatergic projections to the primary auditory nuclei, angularis (NA) and magnocellularis (NM). NL in turn receives glutamatergic input from both the ipsi- and contralateral NM. Both NA and NL send glutamatergic projections to SON. Descending inhibition is primarily GABAergic (black arrows). GABAergic axons of SON project to NA, NM, and NL, and to the contralateral SON. For simplicity, connectivity is displayed unilaterally, and the more rostrally located SON (dotted lines) is displayed in the same plane. *Drug application:* A tissue section containing NM was submerged in aCSF. Puffs of vehicle or drug were delivered to the ventricle and carried across NM on one side of the tissue by aCSF flow through the bath.

EXPERIMENTAL PROCEDURES

Subjects

These experiments used 38 chicks (*Gallus gallus domesticus*) between the ages of seven and sixteen days post-hatch; three embryos between the ages E17–E18 were used for electrophysiology. Eggs were obtained from Charles River (North Franklin, CT, USA) and incubated and hatched at Florida State University facilities. All procedures conformed to the standards set by the Animal Care and Use Committee at the Florida State University, and all precautions were taken to minimize suffering.

Tissue extraction

Brain slices were prepared as described previously (Hyson and Rubel, 1989; Carzoli and Hyson, 2014). Subjects were deeply anesthetized with isoflurane and decapitated. The skull was blocked coronally, rostral to the optic tecta and through the caudal cerebellum. The blocked portion was submerged in artificial cerebrospinal fluid (aCSF) perfused with 95% O_2 and 5% CO_2. aCSF consisted of double deionized water containing (in mM): 130 NaCl, 26 $NaHCO_3$, 3 KCl, 2 $MgCl_2$-$6H_2O$, 2 $CaCl_2$-$2H_2O$, 1.23 NaH_2PO_4-H_2O, 10 dextrose. In experiments performed in a low-calcium aCSF, concentrations were altered to use 3.9 mM $MgCl_2$-$6H_2O$ and 0.1 mM $CaCl_2$-$2H_2O$. Unless otherwise stated, all chemicals used in these experiments were obtained from VWR (Radnor, PA, USA).

The blocked brain was dissected from the surrounding skull and mounted using superglue on a vibratome stage, which was again submerged in oxygenated aCSF. Using a vibrating blade tissue slicer, coronal brainstem slices