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DISTRIBUTION OF FRAGILE X MENTAL RETARDATION PROTEIN IN THE HUMAN AUDITORY BRAINSTEM

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Abstract—Fragile X mental retardation protein (FMRP) binds select mRNAs, functions in intracellular transport of these mRNAs and represses their translation. FMRP is highly expressed in neurons and lack of FMRP has been shown to result in dendritic dysmorphology and altered synaptic function. FMRP is known to interact with mRNAs for the Kv3.1b potassium channel which is required for neurons to fire action potentials at high rates with remarkable temporal precision. Auditory brainstem neurons are known for remarkably high spike rates and expression of Kv3.1b potassium channels. Fragile X syndrome (FXS) is a genetic disorder caused by a mutation in the fragile X mental retardation 1 gene (Fmr1) resulting in decreased expression of FMRP and subsequent intellectual disability, seizures, attention deficit and hypersensitivity to auditory and other sensory stimuli. We therefore hypothesize that the auditory difficulties in FXS result, at least in part, from dysfunction of auditory brainstem neurons. To examine this hypothesis, we have studied normal human brainstem tissue with immunohistochemical techniques and confocal microscopy. Our results demonstrate that FMRP is widely expressed in cell bodies and dendritic arbors of neurons in the human cochlear nucleus and superior olivary complex and also that coincidence detector neurons of the medial superior olive colocalization of FMRP and Kv3.1b. We interpret these observations to suggest that the lower auditory brainstem is a potential site of dysfunction in FXS.

Key words: hearing, cochlear nucleus, superior olive.

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Abbreviations: AbN, abducens nucleus; cVCN, caudal ventral cochlear nucleus; DCN, dorsal cochlear nucleus; FMR1/Fmr1, fragile X mental retardation 1 gene; Fmr1 KO, Fmr1 knockouts; FMRP, fragile X mental retardation protein; FMRP+, FMRP positive; FN, facial nucleus; FXS, fragile X syndrome; GBC, globular bushy cell; IO, principal nucleus of the inferior olive; ITDs, interaural time differences; LNTB, lateral nucleus of the trapezoid body; LSO, lateral superior olive; MNTB, medial nucleus of the trapezoid body; MSO, medial superior olive; NDS, normal donkey serum; OC, octopus cell region; PB, sodium phosphate buffer; PN, pontine nuclei; SBC, spherical bushy cell; SOC, superior olivary complex; SPON, superior paraolivary nucleus; TBS, Tris-buffered saline; VCN, ventral cochlear nucleus.

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INTRODUCTION

Fragile X mental retardation protein (FMRP) is the product of the Fmr1 gene and is widely expressed in multiple tissues from the embryonic period into adulthood (Hinds et al., 1993). FMRP is an RNA-binding protein that functions mainly in activity-dependent translational regulation of a large number of mRNAs, including Kv3.1b and slack potassium channels, and is found both pre and post-synaptically (Akins et al., 2009, 2012; Strumbos et al., 2010; Zhang et al., 2012). Although, translation-independent actions of FMRP have recently been discovered (Brown et al., 2010; Zhang et al., 2012; Deng et al., 2013). In the adult, FMRP is widely expressed in epithelia [e.g. seminiferous tubules of the testis; esophagus] and nervous tissue (Hinds et al., 1993). In the brain, FMRP is expressed in neurons and glia throughout the brainstem, forebrain and cerebellum (Hinds et al., 1993; Feng et al., 1997; Wang et al., 2004; Jacobs et al., 2012), although not ubiquitously (Devys et al., 1993).

Fragile X syndrome (FXS) is the most common inherited form of intellectual disability (Bassell and Warren, 2008) and results from a CGG triplet repeat expansion in the Fmr1 gene (Verkerk et al., 1991) and consequent repression of FMRP. FXS affects 1:3600 males and 1:8000 females (Cornish et al., 2008), is the most common genetic cause of autism (Bassell and Warren, 2008) and 15–30% of all FXS patients demonstrate autistic behaviors (Rogers et al., 2001; Hatton et al., 2006; Harris, 2011). Patients with FXS display cognitive disabilities, social deficits including language delays, seizures, autistic features, sensory hypersensitivity and hyperactivity (Eliez et al., 2001; Berry-Kravis, 2002; Hagerman et al., 2009). FXS is associated with a number of CNS dysmorphologies, including reduced volume of the cerebellar vermis, enlargement of the 4th ventricle (Mostofsky et al., 1998; Hoeft et al., 2010) and hypertrophy of the hippocampus (Kates et al., 1997) and caudate nucleus (Reiss et al., 1995; Eliez et al., 2001; Hoeft et al., 2010). Alterations in synaptic structure and function have been identified in FXS patients and animal models of FXS (e.g. Fmr1 knockouts [Fmr1 KO]; Pfeiffer and Huber, 2009) and a number of presynaptic and postsynaptic proteins have abnormal levels in Fmr1 KO (Li et al., 2002; Klemmer et al., 2011). Furthermore, in FXS and Fmr1 KO there is a high density of immature dendritic spines (human – Rudelli et al., 1985; Hinton et al., 1991; Comery et al., 1997; Irwin et al., 2000; mouse – Nimchinsky et al., 2001; Galvez et al., 2003). Cultured

hippocampal neurons from Fmr1 KO mice give rise to shorter dendrites with fewer dendritic spines compared to controls (Braun and Segal, 2000; Castren et al., 2005) and FMRP-deficient mice have abnormally arranged dendritic fields in the somatosensory cortex (Galvez et al., 2003), more primary dendrites in the olfactory bulbs (Galvez et al., 2005) and spinal motor neurons with immature dendritic arbors (Thomas et al., 2008). Fmr1 KO flies also demonstrate significant overgrowth of dendrites and axons (Zarnescu et al., 2005). These results, taken together, suggest that FMRP plays a role essential to normal maturation and function of the central nervous system.

In our previous post-mortem studies of the autistic brain, we examined the brainstem of a 32-year-old male diagnosed with autism and FXS (Kulesza and Mangunay, 2008). In this case, we observed neurons in the medial superior olive (MSO), a prominent brainstem nucleus which contains coincidence detector neurons that function in sound source localization and encoding temporal features of sound, to be significantly smaller (a nearly 50% reduction in cell body area) and significantly more round (i.e. immature) compared to an age-matched control (Kulesza and Mangunay, 2008). Moreover, there was significantly more variability in the orientation of these MSO neurons in the FXS/autism brain compared to an age-matched control. In addition, we have demonstrated that FMRP is highly expressed in brainstem coincidence detector neurons across species including the nucleus laminaris of alligator and chicken and the MSO in gerbils and human (Wang et al., 2013). Together, we interpret the dysmorphology of MSO neurons in FXS and the abundance of FMRP in the MSO to suggest that auditory-processing deficits in FXS result, at least in part, from dysfunction of brainstem centers. Further, it is believed that the cell types which express FMRP are the most severely impacted in FXS (Hinds et al., 1993) and we hypothesize that FMRP is widely expressed in neuronal cell bodies and dendritic arbors in the cochlear nuclei and superior olivary complex (SOC). Additionally, FMRP is known to play an important role in activity-dependent regulation and tonotopic expression of the Kv3.1b potassium channel in the auditory brainstem and the tonotopic gradient of Kv3.1b expression is required for accurate coding of complex sounds (Strumbos et al., 2010). To examine the distribution of FMRP in the human auditory brainstem and explore possible functional deficits in FXS, we have used immunohistochemistry and confocal microscopy to map the distribution of FMRP in control human dorsal and ventral cochlear nuclei (DCN and VCN) and SOC. Furthermore, we have examined the colocalization of FMRP and Kv3.1b in the MSO.

EXPERIMENTAL PROCEDURES

Tissue sectioning

This report is based on the examination of brainstems from seven individuals ranging in age from 57 to 96 years of age (average 78.6 ± 5.9 years; five female/two male). Table 1 shows the age, cause of death and post-mortem interval for specimens used in this study. All specimens were obtained with permission from the PA Humanities Gifts Registry. Brainstems were only included in this study if they met the following criteria: (1) the cause of death was not neurological, (2) there were no signs of degenerative disease affecting the brain on gross examination or sectioning, (3) there were no signs of pathology affecting the brainstem or posterior cranial fossa and (4) the brainstems could be preserved within 24-h of death. Brains were dissected immediately from the skull, bisected and placed in cold fixative (4% paraformaldehyde in 0.1 M sodium phosphate buffer [PB], pH 7.2) for at least 2 weeks. Before sectioning, brainstems were trimmed and placed into a solution of 30% sucrose in the same fixative until they were saturated (at least 1 week). Tissue blocks including the cochlear nucleus and superior olive were sectioned on a freezing microtome at a thickness of 40 μm and collected in 0.1 M PB. An ordered series of sections was reserved for Giemsa staining (as previously described – Kulesza, 2007, 2008) and utilized for landmarking purposes.

Antisera

FMRP. Rabbit anti-FMRP polyclonal antibody (ab17722, Abcam, Cambridge, MA) was raised against a synthetic peptide conjugated to KLH derived from within residues 550 to the C-terminus of human FMRP and is known to react with mouse, rat and human FMRP. This antibody identifies 75 and 80-kDa bands on Western blot (Abcam datasheet) and endogenous FMRP is expected to be a 71-kDa band. The difference in band size may be related to known post-translational modifications of FMRP. This antibody has been further characterized by Western blot analysis and immunohistochemistry in mouse, rat and gerbil where bands of ~80 and 70-kDa are identified (Wang et al., 2013).

Kv3.1b. Mouse anti-Kv3.1b monoclonal antibody (NeuroMab, Davis, CA) was raised against a fusion protein of amino acids 437–585 (C-terminus) of rat Kv3.1b. This antigen shares 100% identity with mouse and human Kv3.1b. This antibody identifies a band of 110-kDa on Western blot.

Immunohistochemistry

Free-floating tissue sections were rinsed in 0.1 M PB, endogenous peroxidase activity was quenched with a 10-min wash in 1.5% hydrogen peroxide in PB and tissue was permeabilized in 0.5% Triton X100 in PB. Sections were blocked in 0.1% normal donkey serum (NDS) and incubated overnight in 1% NDS and anti-FMRP antisera (1:750–1000). Tissue sections were rinsed in PB and incubated in biotinylated goat anti-rabbit secondary (1:100; Vector Labs, Inc., Burlingame, CA) for 2-h, rinsed and incubated in ABC solution (Vector Labs) for 1 h. Tissue sections were again washed in PB and then Tris-buffered saline (TBS; pH 7.7) and the final peroxidase reaction was developed in TBS with 0.05% diaminobenzidine, 0.125% nickel ammonium sulfate and 0.06% hydrogen peroxide.