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Protocadherin 10 alters γ oscillations, amino acid levels, and their coupling; baclofen partially restores these oscillatory deficits

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ABSTRACT

Approximately one in 45 children have been diagnosed with Autism Spectrum Disorder (ASD), which is characterized by social/communication impairments. Recent studies have linked a subset of familial ASD to mutations in the Protocadherin 10 (Pcdh10) gene. Additionally, Pcdh10's expression pattern, as well as its known role within protein networks, implicates the gene in ASD. Subsequently, the neurobiology of mice heterozygous for *Pcdh10* (*Pcdh10*$^{+/-}$) has been investigated as a proxy for ASD. Male *Pcdh10*$^{+/-}$ mice have demonstrated sex-specific deficits in social behavior, recapitulating the gender bias observed in ASD. Furthermore, in vitro slice preparations of these *Pcdh10*$^{+/-}$ mice demonstrate selective decreases to high frequency electrophysiological responses, mimicking clinical observations. The direct in vivo ramifications of such decreased in vitro high frequency responses are unclear. As such, *Pcdh10*$^{+/-}$ mice and their wild-type (WT) littermates underwent in vivo electrocorticography (ECoG), as well as ex vivo amino acid concentration quantification using High Performance Liquid Chromatography (HPLC). Similar to the previously observed reductions to in vitro high frequency electrophysiological responses in *Pcdh10*$^{+/-}$ mice, male *Pcdh10*$^{+/-}$ mice exhibited reduced gamma-band (30–80 Hz), but not lower frequency (10 and 20 Hz), auditory steady state responses (ASSR). In addition, male *Pcdh10*$^{+/-}$ mice exhibited decreased signal-to-noise-ratio (SNR) for high gamma-band (60–100 Hz) activity. These gamma-band perturbations for both ASSR and SNR were not observed in females. Administration of a $GABA_B$ agonist remediated these electrophysiological alterations among male *Pcdh10*$^{+/-}$ mice. *Pcdh10*$^{+/-}$ mice demonstrated increased concentrations of GABA and glutamine. Of note, a correlation of auditory gamma-band responses with underlying GABA concentrations was observed in WT mice. This correlation was not present in *Pcdh10*$^{+/-}$ mice. This study demonstrates the role of *Pcdh10* in the regulation of excitatory-inhibitory balance as a function of GABA in ASD.

1. Introduction

Autism Spectrum Disorder (ASD) is characterized by social/communication impairments (American Psychiatric Association, 2013). Estimates suggest that as many as one in 45 children between the ages of three and 17 years old have been diagnosed with ASD, of whom three quarters are male (Zablotsky et al., 2015). ASD is associated with biological alterations affecting neuronal synapses (for review see Port et al., 2014), such as the those involving cell adhesion molecules encoded by the Cadherin/Protocadherin superfamily of genes (Kim et al., 2011; Morrow et al., 2008; O'Roak et al., 2012).

Rare homozygous deletions at the Protocadherin 10 (Pcdh10, also known as OL-protocadherin) locus and its regulatory region have been

Abbreviations: AN40, auditory N40; CFC, cross-frequency coupling; ROI, region of interest; Gamma, 30–100 Hz electrophysiological activity; ASSR, auditory steady-state response; SNR, signal to noise ratio; PWC, pairwise comparison.

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linked to ASD in multiple separate cohorts (Bucan et al., 2009; Morrow et al., 2008). Further support for the relevance of Pcdh10 mutations with regards to ASD arises from the gene's murine expression pattern. *Pcdh10* is expressed during the embryonic stage of development and remains expressed even into adulthood (Hirano et al., 1999). Moreover, Pcdh10 expression follows a tightly-regulated temporal (Hirano et al., 1999) and spatial (even to the level of sub region/nuclei) (Hertel et al., 2012; Hirano et al., 1999; Kim et al., 2010, 2007) expression pattern in several brain regions, including cortex and thalamus. A similar, though not identical, spatial regulation is observed for the expression of the protein encoded by *Pcdh10*, *PCDH10* (Aoki et al., 2003). The precise nature of this expression suggests Pcdh10's importance in the development and maintenance of healthy neural function.

Additionally, *Pcdh10* is a gene target of Fragile X mental retardation protein (FMRP) and myocyte enhancer factor 2 (MEF2) (Tsai et al., 2012). FMRP binds and transports *Pcdh10* mRNA transcripts to the synapse following neural activity (Dictenberg et al., 2008; Tsai et al., 2012). Mice heterozygous for *Pcdh10* ($Pcdh10^{+/-}$) exhibit increased filamentous processes in lateral/basolateral amygdala (Schoch et al., 2017), analogous in nature to those reported for individuals with Fragile X syndrome (Irwin et al., 2001) as well as mice with *Fmr1* homozygous deletions (Comery et al., 1997). *PCDH10* functions as a PCDHδ2 activity dependent transmembrane cell-adhesion protein, encoded by the murine chromosome 3. The protein plays important roles in neuronal circuit formation and cell migration (Nakao et al., 2008), synapse elimination (Tsai et al., 2012), and is indirectly implicated in spinogenesis (Pilpel and Segal, 2005).

Recently Schoch et al. (2017) investigated $Pcdh10^{+/-}$ mice as a murine model with relevance to ASD. Male, but not female, $Pcdh10^{+/-}$ mice demonstrate impaired social behavior (Schoch et al., 2017), consistent with the gender bias in ASD. These perturbed social behaviors in male $Pcdh10^{+/-}$ mice were in addition to the aforementioned neuronal morphological alterations observed in the amygdala. It remains unknown if female $Pcdh10^{+/-}$ mice exhibit analogous morphological alterations as compared to their male counterparts, because the corresponding histological analysis was not performed/reported for female mice. As such, this murine model recapitulates several key aspects of ASD.

The $Pcdh10^{+/-}$ mice also demonstrate decreased in vitro high-frequency electrophysiological responses in the amygdala. Analogous in vivo high-frequency activity (30–100 Hz, Gamma) plays key roles in many cognitive and behavioral functions (for review see Herrmann et al., 2010). Of note, individuals with ASD exhibited reduced gamma-band responses to stimuli as well as alterations to resting-state Gamma, though the direction of change for such resting-state alterations is disputed (Rojas and Wilson, 2014). Such Gamma perturbations in ASD (Grice et al., 2001; Maxwell et al., 2013; Orekhova et al., 2007; Wilson et al., 2007) correlate to social functioning (Maxwell et al., 2013) and normalize after clinically-effective behavioral intervention (Van Hecke et al., 2013). As such, Gamma perturbations are hypothesized as biomarkers for ASD (Rojas and Wilson, 2014). Gamma reductions are also observed in first degree relatives of individuals with ASD (Rojas et al., 2008), again scaling with sociability (Rojas et al., 2011). Additionally, preliminary findings suggest the ability of Gamma metrics to predict optimal-outcome in ASD (Port et al., 2016a). Gamma perturbations are a conserved phenotype in multiple different murine models relevant to ASD (Gandal et al., 2012a, 2010; Saunders et al., 2012a). Furthermore, the normalization of preclinical Gamma perturbations are concurrent with behavioral normalization (Gandal et al., 2012b; Yizhar et al., 2011). Importantly, this in vivo murine Gamma positively correlates with underlying neurobiology (i.e. cell-specific densities (Nakamura et al., 2015) and synaptic protein levels (Gandal et al., 2010)).

The 10% delay for the 100 ms neuromagnetic response (M100) to auditory-stimuli is an additional clinical biomarker for ASD (for review see Port et al., 2015). While there has been disagreement in the literature about the exact nature of M100 latency alterations in individuals with ASD, it has been suggested that methodological differences in the stimuli presented (M100 latency delays in individuals with ASD are most apparent for 500 Hz tones), their presentation (e.g. the intensity of the presented tone can alter a M100 latency), and the subsequent analysis of neuromagnetic responses (i.e. delineating between late M50 and M100 responses) may account for such variability in observations (for more detailed discussion see Port et al., 2015). Similar magnitude delays have been observed by independent laboratories, though do not always reach significance, possibly due to smaller sample sizes (Demopoulos et al., 2017). Delayed auditory M100 latencies have been repeatedly detected in children with ASD (Edgar et al., 2015, 2014; Gage et al., 2003b; Roberts et al., 2010) and cannot be accounted for by cognitive or language abilities (Edgar et al., 2015, 2014; Roberts et al., 2010). Auditory M100 latencies are correlated with sociability (Port et al., 2016a). Additionally, the corresponding electrical response (the N1 response) demonstrates the highest correlation coefficient against ADOS severity as compared to analogous visual or multi-sensory responses (Brandwein et al., 2014). Indeed, delays to the analogous electroencephalographic middle latency response (i.e. the N40) are observed in murine models relevant to ASD (Billingslea et al., 2014; Engineer et al., 2015, 2014; Gandal et al., 2012a, 2010; Saunders et al., 2012a, 2013), with N40 latencies again associated with sociability (Billingslea et al., 2014; Saunders et al., 2013).

Gamma alterations and M100 latency delays have plausible biological bases, with perturbed synaptic transmission potentially underlying both observations (Port et al., 2015). An imbalance to excitatory and inhibitory neuronal signaling (E/I imbalance) has been posited to underlie ASD (Rubenstein and Merzenich, 2003). ASD-associated γ-aminobutyric acid (GABA) related alterations (primarily loss of function) are well documented in both *post-mortem* (Casanova et al., 2006; Fatemi et al., 2014, 2009a, 2009b, 2002) and in vivo (Gaetz et al., 2014; Harada et al., 2011; Port et al., 2016b; Rojas et al., 2014) clinical studies. Preclinical studies further support GABAergic dysfunction in ASD with alterations to GABAergic expression (Gandal et al., 2012b; Gogolla et al., 2014, 2009; Zhang et al., 2014) or function (Banerjee et al., 2013; Cellot and Cherubini, 2014; Han et al., 2012) frequently observed. Direct quantification of neural GABA concentrations in ASD-relevant rodent models are less consistent, with decreases (Bitanihirwe et al., 2010; Groves et al., 2013; Ide et al., 2005), increases (Ali and Elgoly, 2013; Gruss and Braun, 2004, 2001) and no change (Fatemi et al., 2008) reported. Such findings are confounded by concurrent, and inconsistent, alterations to glutamate (Ali and Elgoly, 2013; Bitanihirwe et al., 2010; Gruss and Braun, 2004, 2001; Ide et al., 2005).

The $GABA_B$ agonist baclofen, and its R-enantiomer arabaclofen, are proposed for ASD treatment (Berry-Kravis et al., 2012; Erickson et al., 2014). Importantly, these studies suggest that baclofen may be efficacious only in a subpopulation of individuals with ASD. Concurrently, preclinical studies have demonstrated the efficacy of baclofen in normalizing ASD-associated electrophysiological biomarkers and behavioral phenotypes (Gandal et al., 2012b; Sinclair et al., 2017), while suggesting genotype-dependent effects of baclofen (Silverman et al., 2015). Therefore, several questions remain unresolved: 1) If $Pcdh10^{+/-}$ mice demonstrate altered auditory electrophysiological responses analogous to those observed in ASD, 2) If $Pcdh10^{+/-}$ mice demonstrate altered underlying neurochemical E/I balance, 3) How do such electrophysiological and neurochemical profiles interrelate, 4) What is the role of *sex* in these aforementioned questions and 5) Are $GABA_B$ agonists effective at normalizing the electrophysiological biomarkers of ASD? To address these questions, $Pcdh10^{+/-}$ mice and their wild-type (WT; $Pcdh10^{+/+}$) littermates underwent electrocorticography (ECoG) prior to and following baclofen administration. Subsequently, High Performance Liquid Chromatography (HPLC) was utilized to quantify ex vivo amino acid concentrations for all mice. The a-priori comparison of saline condition male WT to male $Pcdh10^{+/-}$ was of specific interest for all metrics, based on the previous findings of Schoch et al. (2017).