Endocannabinoids and disrupted synchronous oscillations in autism spectrum disorders

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Autism spectrum disorders (ASD) are highly prevalent, highly heritable neurodevelopmental disorders that occur more commonly in males, whose prominent symptom domains include deficits of social communication, narrowed interests and repetitive behaviors. Although there are diverse etiologies, impaired synaptic architecture and function as well as imbalance between central excitatory and inhibitory transmission are probable pathogenic mechanisms shared by many affected persons, which can result in disturbances of γ -synchronous oscillatory rhythms [1]. γ -synchronous oscillatory activity resulting from the influence of inhibitory GABAergic basket cells on assemblies of cortical pyramidal output neurons facilitates efficient long-range interactions between neural circuits. These oscillatory activities within and between circuits are regulated and 'fine tuned' by the endogenous cannabinoid system (ECS) [1]. Therapeutic targeting of the ECS is a promising and novel therapeutic approach to ASD.

Within the cerebral cortex and hippocampus, fast-spiking, parvalbumin

(PV)-containing GABAergic inhibitory interneurons serve as the major driver of the firing of ensembles of pyramidal cells, contributing to their synchronous oscillatory rhythmic outputs, including θ (5–10 Hz) and γ (30–80 Hz) frequencies; the power spectra of these oscillatory rhythms are associated with efficient encoding of information and higher executive functions, especially working memory [2]. Regulation of GABA and glutamate release from interneurons and recurrent pyramidal cell projections, respectively, is highly controlled and necessary for maintenance of this oscillatory activity, which is disturbed in schizophrenia and ASD, among other disorders [1,2].

The phase locking of γ -oscillations to the *in vivo* stimulation of fast-spiking, PV interneurons and their *in vivo* suppression by inhibition of these interneurons was demonstrated by using transgenic mice that selectively expressed microbial opsins in fast-spiking PV interneurons [3]. Further, using optogenetics, stimulating PV interneurons was shown to have

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“Neuroigin-3 is essential for both the structure and function of excitatory and inhibitory synapses.”

a specialized role in generating γ -oscillations in downstream pyramidal neurons in prefrontal cortical brain slices. The data strongly supported a role for the γ -oscillations generated by fast-spiking PV interneurons in facilitating information transfer within and between brain regions, processes that may be disrupted in ASD and other psychiatric disorders [3–5].

The spectral power of high frequency oscillations measured during sustained visual attention in regions least contaminated by myogenic artifacts, especially those in the gamma1 band (24.4–44.0 Hz), was increased in 40 young boys with autism (age range: 3 years and 1 month–8 years and 9 months) drawn from two sites (i.e., Gothenburg and Moscow), compared with 40 age-matched typically developing boys drawn from these same sites (age range: 3 years and 0 months–7 years and 10 months) [4]. Moreover, within the group of autistic boys, a significant correlation existed between the degree of developmental delay and gamma1 spectral power, which was not likely to be due to group differences in autonomic arousal. The data are consistent with the high comorbid occurrence of seizures in autism and hypothesized increases in the ratio of central excitation to inhibition [4].

Consistent with abnormalities of γ -oscillatory activity, 15 males with ASD (mean age = 15.1 years $\pm$ 2.9 [SD]; mean full-scale IQ = 108.6 $\pm$ 16.0 [SD]) showed a trend for reduced γ -power in right lateral electrodes, compared with 18 typically developing male controls (mean age = 14.2 years $\pm$ 2.9 [SD]; mean full-scale IQ = 110.7 $\pm$ 12.9 [SD]); digital EEG recordings were obtained from 64 electrodes while subjects were instructed ‘to relax’ with their eyes open [5]. Additionally, within the right lateral electrode group, γ -power and total score on the social responsiveness scale, a scale where higher scores reflect greater severity of social impairment, were inversely correlated across all subjects. The data suggest a relationship between γ -oscillations and social/emotional information processing [5]. Unfortunately, there is the possibility that these results may be confounded by myogenic artifacts [4].

The ECS fine tunes neural oscillations, serving as both a mechanism of pathogenesis and target for novel therapeutic interventions [2]. Specifically, 2-arachidonoylglycerol (2-AG), acting via type 1 cannabinoid receptors (CB1) located on terminals of cholecystokinin-containing GABA basket cells (CCKBC), one of 21

distinctive types of interneurons accounting for less than 15% of the GABA interneuron population, and glutamatergic terminals ‘fine tunes’ γ -oscillatory output [1,2,6,7]. CCKBC project to the dendrites and perisoma of pyramidal cells, as well as the terminals of PV GABA interneurons. The endocannabinoid (EC) 2-AG is a potent full CB1 agonist that serves as a retrograde messenger, whose on-demand synthesis occurs in an activity-dependent manner from membrane phospholipids within the excitatory postsynaptic dendritic spine, inhibiting GABA release from CCKBC and glutamate release from projections to dendritic spines, and relieves the inhibitory influence of CCKBC on PV GABA interneurons [2,7]. There are data implicating presynaptic N-type (Cav2.2) Ca^{2+} channels in the CB1-mediated inhibition of GABA release [8]. 2-AG synthesis occurs sequentially via the actions of phospholipase C- β (PLC β) and diacylglycerol lipase- α (DAGL α), leading to the on-demand local production of diacylglycerol (DAG) and 2-AG, respectively [2,6]. There are both phasic and tonic EC inhibitory influences on GABA and glutamate release. The phasic release of 2-AG results in ‘depolarization-induced suppression of inhibition’ and ‘depolarization-induced suppression of excitation’, depending on whether CB1 activation takes place on CCKBC GABAergic terminals or glutamatergic terminals [2,7]. Importantly, the action of 2-AG is constrained in a highly spatially and temporally selective manner by monoacylglycerol lipase, its catabolic enzyme that limits the lifetime and extent of 2-AG diffusion after its release [6]. Phasic release from excitatory dendritic spine synapses results from mGluR5-mediated PLC β activation via $\text{G}_{q/11}$ protein-coupled receptors, and Ca^{2+} -stimulated PLC β activation; Ca^{2+} enters the dendritic spine via voltage-gated Ca^{2+} channels whose opening occurs as a result of AMPA-mediated depolarization [2]. Although the mechanism is currently obscure, a population of inhibitory synapses are tonically silenced by the binding of 2-AG to CB1 on the terminals of CCKBC [2]. CB1-mediated tonic inhibition of a subpopulation of CCK inhibitory interneurons may represent an important switching mechanism in cortical circuits subserving processing of social–emotional information [7,9]. There are recent data implicating disruption of this tonic regulation of EC-mediated GABA release in syndromic forms of ASD due to deletion or expression of defective neuroigins (NLG), an

important class of postsynaptic adhesion molecules that bind with presynaptically located neuroligins to create the transsynaptic architecture of the inhibitory GABAergic synapse. Importantly, more than 30 mutations of the four neuroligin isoforms (abbreviated NL1–NL4) have been associated with ASD [10].

Mutations of NLG4 have been associated with ASD and NLG4 knockout (KO) mice showed alterations of inhibitory postsynaptic GABAergic synapses with reduced density of immunostained gephyrin, a scaffolding protein, and the GABA_A receptor $\gamma 2$ subunit in the perisomatic region of hippocampal CA3 stratum pyramidale neurons [11]. Peak amplitudes of spontaneous inhibitory postsynaptic currents (IPSCs) and the power spectra of induced γ -oscillations (30–80 Hz) in hippocampal slices were reduced in these mice. Thus, postsynaptic NLGs influence composition and function of inhibitory synapses and serve as more than simply the ‘glue’ aligning pre- and post-synaptic elements [11].

Neuroligin-3 (NL3) is essential for both the structure and function of excitatory and inhibitory synapses; both deletion of NL3 and a point mutation (i.e., R451C substitution) are associated with ASD [10]. Paired whole-cell recordings between presynaptic CCKBC and postsynaptic CA1 pyramidal neurons in hippocampal slices prepared from transgenic mice whose expression of NL3 was ‘KO’ or expression of the R451C substitution was ‘knocked in (KI)’ revealed that both mouse strains shared a loss of the tonic CB1-mediated inhibition of GABA release at pyramidal synapses [10]. Specifically, stimulation of CCKBC from either the KO or KI strains resulted in both increases in the amplitudes of IPSCs and the IPSC success rate. The postsynaptically located NL3 was shown to affect the function of the CCKBC presynaptic terminal (e.g., probability of presynaptic release of GABA); thus, the expected increase in IPSC amplitudes and success rates observed after bath application of AM251, a CB1 receptor antagonist and inverse agonist, to wild-type synapses was not seen after its application to hippocampal slices prepared from the NL3 KO and KI strains, consistent with loss of tonic inhibition [10]. Importantly, this loss of tonic CB1-mediated inhibition in the KO and KI strains was selective for the CCKBC nerve terminal, because in the presence of picrotoxin to inhibit GABA_A-mediated responses, bath application

of AM251 and simultaneous stimulation of Schaffer-collateral synapses while recording from CA1 pyramidal neurons did not result in increased amplitudes of EPSCs in hippocampal slices from wild-type or NL3 KO mice. Thus, loss of tonic CB1-mediated inhibition of GABA release appears selective for inhibitory pyramidal synapses in the NL3 KO and KI mouse strains [10]. Conceivably, this cell-type/synapse selectivity affords an opportunity for therapeutic targeting of at least one possible cause of impaired γ -oscillatory activity in ASD.

Unlike the cell-type selective effect of the postsynaptic NL3 adhesion molecule on CB1-mediated tonic inhibition of GABA release from presynaptic CCKBC nerve terminals, presynaptic β -neurexin cell adhesion molecules regulate CB1-mediated glutamate release at excitatory hippocampal synapses [12]. Neurexins are coded by three genes and, compared with the α -neurexins, the β -neurexins are N-terminally truncated peptides. Importantly, throughout the brain, expression of the three β -neurexins is 10- to 100-fold lower than expression of the three α -neurexins. Over 1000 distinct neurexin mRNAs result from alternative splicing of neurexin transcripts; thus, these presynaptic adhesion molecules can have highly spatially selective and specialized effects on neural circuit dynamics. Knocking out expression of all three β -neurexins resulted in both increased postsynaptic synthesis of 2-AG and tonic CB1-mediated inhibition of presynaptic glutamate release from hippocampal CA1 pyramidal neurons synapsing onto pyramidal neurons in the subiculum, the major hippocampal output pathway [12]. This CB1-mediated cell-type selective effect on excitatory synapses in the subiculum has functional relevance because mice with KO expression of β -neurexins show impaired hippocampal contextual fear conditioning. In the β -neurexin KO mouse, tonic activation of presynaptic CB1 receptors interferes with Ca²⁺ entry and its coupling to glutamate release; moreover, the absence of β -neurexins leads to an upregulation of postsynaptic PLC β -mediated synthesis of 2-AG. Conceivably, loss of the inhibitory influence of presynaptic β -neurexins on postsynaptic PLC β -mediated 2-AG synthesis leads to excessive release of this retrograde messenger and dysregulated excitatory synapses in the subiculum. The tonic activation of CB1 receptors in β -neurexin KO mice had relatively selective effects to decrease glutamate release

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in ‘burst-firing’ as opposed to ‘regular-firing’ synapses in the subiculum and to block pre-synaptic activation of long-term potentiation (LTP) in ‘burst-firing’ neurons [12]. Risk alleles of β -neurexins have been associated with ASD and their role in modulating CB1-mediated glutamate release at burst-firing synapses in the subiculum could provide opportunities for targeted endocannabinoid therapeutic interventions [12,13].

Because disturbances of the ECS in ASD are highly cell-specific and show spatial and temporal selectivity, therapeutic targeting of the ECS may be possible with both drugs and

devices (e.g., transcranial magnetic stimulation) to fine tune disturbed γ -oscillatory rhythmic activity in ASD.

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