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Archival Report

Role of Striatal Direct Pathway 2-Arachidonoylglycerol Signaling in Sociability and Repetitive Behavior

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ABSTRACT

BACKGROUND: Endocannabinoid signaling plays an important role in regulating synaptic transmission in the striatum, a brain region implicated as a central node of dysfunction in autism spectrum disorder. Deficits in signaling mediated by the endocannabinoid 2-arachidonoylglycerol (2-AG) have been reported in mouse models of autism spectrum disorder, but a causal role for striatal 2-AG deficiency in phenotypes relevant to autism spectrum disorder has not been explored.

METHODS: Using conditional knockout mice, we examined the electrophysiological, biochemical, and behavioral effects of 2-AG deficiency by deleting its primary synthetic enzyme, diacylglycerol lipase α (DGLα), from dopamine D_1 receptor–expressing or adenosine A2a receptor–expressing medium spiny neurons (MSNs) to determine the role of 2-AG signaling in striatal direct or indirect pathways, respectively. We then used viral-mediated deletion of DGLα to study the effects of 2-AG deficiency in the ventral and dorsal striatum.

RESULTS: Targeted deletion of DGLα from direct-pathway MSNs caused deficits in social interaction, excessive grooming, and decreased exploration of a novel environment. In contrast, deletion from indirect-pathway MSNs had no effect on any measure of behavior examined. Loss of 2-AG in direct-pathway MSNs also led to increased glutamatergic drive, which is consistent with a loss of retrograde feedback inhibition. Subregional DGLα deletion from the dorsal striatum produced deficits in social interaction, whereas deletion from the ventral striatum resulted in repetitive grooming.

CONCLUSIONS: These data suggest a role for 2-AG deficiency in social deficits and repetitive behavior, and they demonstrate a key role for 2-AG in regulating striatal direct-pathway MSNs.

Keywords: 2-Arachindonoylglycerol, Autism spectrum disorder, Diacylglycerol lipase, Endocannabinoid, Nucleus accumbens, Striatum

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Autism spectrum disorder (ASD) is characterized by impairments in social interaction and excessive repetitive behavior (1). Development of effective therapies has been impeded by our lack of understanding of the circuit and cellular mechanisms that underlie the core symptoms of ASD. However, converging evidence from basic (2–4) and clinical (5–7) studies implicates the striatum in ASD pathophysiology. The striatum enables behavioral action selection based on the relative activation of medium spiny neurons (MSNs) belonging to the direct or indirect pathways (dMSNs or iMSNs, respectively), which are driven by glutamatergic inputs projecting from brain regions that serve emotional, cognitive, sensory, and motor functions.

Endocannabinoid (eCB) signaling in the striatum has been implicated in the regulation of behaviors within social (8–10) and habitual and/or compulsive motor (11,12) routines. We and others have shown that for both dMSNs and iMSNs, negative feedback inhibition of glutamatergic release can be mediated through cannabinoid-1 receptor (CB_1R) activation by 2-arachidonoylglycerol (2-AG) (13,14), the most abundant eCB in the brain (15). More recently, eCB dysfunction has emerged as a common feature of multiple ASD mouse models (16–22).

2-AG is synthesized on demand in the postsynaptic neuron by diacylglycerol lipase α (DGLα), and global deletion of this enzyme results in the loss of phasic eCB-mediated retrograde suppression of neurotransmitter release in the striatum (23) and throughout the brain (24,25). Genetic studies have identified ASD patients with *DAGLA* haploinsufficiency (26) as well as other alterations in *DAGLA* gene integrity (27). Furthermore, DGLα exists in a multiprotein complex (13) with several ASD-associated proteins (28–34). These findings, along with the alterations of eCBs across multiple ASD models, suggest a link

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between 2-AG dysfunction and ASD; however, no study has directly tested the circuit mechanisms by which 2-AG regulates striatal-mediated behaviors or the effect of 2-AG deficiency on ASD-relevant behaviors.

The introduction of some ASD-associated genetic mutations in mice can result in overactivation of dMSNs (3,20), which may represent a circuit mechanism underlying the associated ASD-relevant phenotypes in these mice. One possible mechanism for this may involve hyperglutamatergic drive onto dMSN synapses. Moreover, multiple lines of evidence in both animal (35) and human (36,37) studies support a general role of hyperglutamatergic function in ASD. Given the role of 2-AG in regulating glutamate release at these synapses through feedback inhibition, we hypothesized that an impairment of 2-AG signaling in dMSNs would result in increased glutamatergic excitation, a driving circuit, and behavioral phenotypes that may contribute to ASD pathology in some individuals.

We used conditional *Dagla* knockout (KO) mice to specifically impair 2-AG signaling in genetically targeted striatal cell types and subregions to show that a loss of 2-AG signaling in dMSNs results in excessive glutamatergic drive, impaired social interaction, repetitive self-grooming, and decreased exploration of novelty. Interestingly, the effect on social behavior was recapitulated by conditional deletion of *Dagla* from the dorsal striatum, while the repetitive behavior was reproduced by deletion from the ventral striatum (nucleus accumbens [NAc]). These data indicate a crucial role for striatal dMSN 2-AG signaling in the regulation of ASD-relevant behavioral domains and reveal novel circuit- and subregion-specific mechanisms that may be relevant to ASD pathology stemming from other genetic abnormalities.

METHODS AND MATERIALS

Animals

Mice harboring DGL loxP sites flanking exon 8 of *Dagla* (DGLflx/flx) were generated as previously described (38). DGLflx/flx were crossed with *Drd1a*-Cre (D1-Cre) or *Adora2a*-Cre (A2A-Cre) mouse lines to generate DGLflx/flx/Cre– (DGLflx/flx) and D1-Cre+/DGLflx/flx (DGLD1-Cre+) or A2A-Cre+/DGLflx/flx (DGLA2A-Cre+) mice for experiments. *Drd1a*-Cre and *Adora2a*-Cre mice were generated by the Gene Expression Nervous System Atlas Project (New York, NY) (Mouse Genome Informatics database identification numbers 3836633 [*Drd1a*-Cre] and 4361654 [*Adora2a*-Cre]). These lines were further crossed with *Drd1a*-tdTomato mice for electrophysiology experiments (Mouse Genome Informatics database identification number 4360387). Further details are provided in the Supplement. All experiments with mice were approved by the Vanderbilt University and National Institute on Alcohol Abuse and Alcoholism Institutional Animal Care and Use Committee and were performed in accordance with the National Research Council's *Guide for the Care and Use of Laboratory Animals.*

Quantification of eCBs and Related Lipids

eCBs and related lipids were detected from mouse striatal punches using multiple reaction monitoring with a SCIEX QTRAP 6500 mass spectrometer in tandem with a Shimadzu Nexera X2 ultrahigh performance liquid chromatograph series system (Shimadzu, Kyoto, Japan). Details regarding tissue processing, liquid chromatography–tandem mass spectrometry analysis, and quantification are provided in the Supplement.

Electrophysiology

Acute slice preparation and whole-cell patch-clamp recordings were performed with methods similar to those previously described (13,23). Voltage-clamp recordings were performed on striatal MSNs identified as *Drd1a*-tdTomato+ (dMSNs) and *Drd1a*-tdTomato− (iMSNs). Further details about patch-clamp experiments, including solution recipes, are described in the Supplement. Depolarization-induced suppression of excitation (DSE) and depolarization-induced suppression of inhibition (DSI) experiments were performed as previously described (13). Spontaneous excitatory postsynaptic currents (sEPSCs) and spontaneous inhibitory postsynaptic currents (sIPSCs) were recorded at −70 mV as previously described (23,39). Further information about recording paradigms, including alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid:*N*-methyl-D-aspartate ratios, are provided in the Supplement.

Stereotaxic Injection of Adeno-associated Virus

$DGL\alpha^{flx/flx}$ mice underwent bilateral stereotaxic surgery under isoflurane anesthesia. Adeno-associated virus vectors (AAV) AAV5.CMV.HI.eGFP.Cre.WPRE.SV40 (AAV-Cre; titer 2 × 1013 genome copies/mL) or AAV5.CMV.PI.eGFP.WPRE.bGH (AAV-green fluorescent protein [GFP]; titer 7 × 1013 genome copies/mL) were injected bilaterally into the dorsal striatum (500 nL, anterior-posterior: 0.62, medial-lateral: 1.90, dorsal-ventral: 3.00) or into the NAc (450 nL, anterior-posterior: 1.65, medial-lateral: 0.92, dorsal-ventral: 4.80).

Behavioral Experiments

All mice were group housed with a minimum of 2 littermates per cage. All experiments were conducted during the light phase. Tests were conducted ≥72 hours apart. Tests for $DGL\alpha^{D1\text{-}Cre+}$ and $DGL\alpha^{A2A\text{-}Cre+}$ mice were run in parallel with those of their control $DGL\alpha^{flx/flx}$ littermates, with each respective line tested and analyzed separately.

Statistical Analysis

All data were analyzed using GraphPad Prism 7 (GraphPad Software, La Jolla, CA). Data were initially analyzed using the robust regression and outlier removal test with $Q = 5\%$, and outliers were removed. Data are presented as mean ± SEM throughout, with individual data points overlaid on all bar graphs. The statistical tests and parameters used are indicated in figure legends. Results were considered significant if they reached $p < .05$ throughout. Further information regarding sample size selection and exclusion criteria is provided in the Supplement.