Published in final edited form as:

Biol Psychiatry. 2018 August 15; 84(4): 304–315. doi:10.1016/j.biopsych.2017.11.036.

Role of striatal direct pathway 2-arachidonoylglycerol signaling in sociability and repetitive behavior

Brian C. Shonesy^{1,*}, Walker P. Parrish¹, Hala K. Haddad¹, Jason R. Stephenson¹, Rita Báldi², Rebecca J. Bluett², Christian R. Marks¹, Samuel W. Centanni¹, Oakleigh M. Folkes², Keeley Spiess¹, Shana M. Augustin³, Ken Mackie⁴, David M. Lovinger³, Danny G. Winder¹, Sachin Patel^{1,2}, and Roger J. Colbran¹

¹Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN 37232

²Department of Psychiatry, Vanderbilt University Medical Center, Nashville, TN 37232

³Laboratory for Integrative Neuroscience, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, MD 20892

⁴The Gill Center and the Department of Psychological & Brain Sciences, Indiana University, Bloomington, IN, USA

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 (ASD). Deficits in signaling mediated by the endocannabinoid 2-arachidonoyl glycerol (2-AG) have been reported in mouse models of ASD, but a causal role for striatal 2-AG deficiency in ASD-relevant phenotypes has not been explored.

Methods—Using conditional knockout mice, we examined the electrophysiological, biochemical and behavioral effect of 2-AG deficiency by deleting its primary synthetic enzyme, diacylglycerol lipase alpha (DGLα) from D1-dopamine or A2a-adenosine 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 ventral and dorsal striatum.

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

* Address correspondence to: Brian C. Shonesy; 724 Robinson Research Building, Vanderbilt University, Nashville, TN 37232 (Tel.: 615-322-4389. Fax: 615-322-7236. brian.shonesy@vanderbilt.edu).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

FINANCIAL DISCLOSURES: The authors have no biomedical financial interests or potential conflicts of interest to report.

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

Keywords

diacylglycerol lipase; 2-arachidonoylglycerol; endocannabinoid; striatum; nucleus accumbens; autism spectrum disorders

INTRODUCTION

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 studies (5–7) implicate 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 versus iMSNs), 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/compulsive motor routines (11,12). We and others have shown that for both dMSNs and iMSNs, negative feedback-inhibition of glutamatergic release can be mediated through cannabinoid-1 receptor (CB1R) activation by 2-arachidonoyl glycerol (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 alpha (DGL α), 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 DGL α haploinsufficiency (26) as well as other alterations in DGL α gene integrity (27). Furthermore, DGL α exists in a multi-protein complex (13), with several ASD-associated proteins (28–34). These findings along with the alterations of eCBs across multiple ASD models suggest a link between 2-AG dysfunction and ASD; however, no studies have 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 hyper-glutamatergic drive onto dMSN synapses. Moreover, multiple lines of evidence in both animal (35) and human (36,37) studies support a general role of hyper-glutamatergic 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, driving circuit, and behavioral phenotypes that may contribute to ASD pathology in some individuals.

Here 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

DGLα floxed mice (DGLα^{flx/flx}) were generated as described previously (38) and were crossed with *Drd1*-Cre (D1-Cre; GENSAT Project; MGI ID: 3836633) or *Adora2a*-Cre (A2a-Cre; GENSAT Project; MGI ID: 4361654) mouse lines to generate DGLα^{flx/flx}/Cre⁻ (DGLα^{flx/flx}) and D1-Cre⁺/DGLα^{flx/flx} (DGLα^{D1-Cre+}) or A2a-Cre⁺/DGLα^{flx/flx} (DGLα^{A2A-Cre+}) mice for experiments. These lines were further crossed with *Drd1*-tdTomato (MGI ID: 4360387) mice for electrophysiology experiments. Further details are provided in Supplementary Materials. All experiments with mice were approved by the Vanderbilt University and National Institute on Alcohol Abuse and Alcoholism (NIAAA) Institutional Animal Care and Use Committee and were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Quantification of eCBs and Related Lipids

Endocannabinoid and related lipids were detected from mouse striatal punches using multiple reaction monitoring (MRM) SCIEX QTRAP 6500 in tandem with a Shimadzu Nexera X2 UHPLC. Details regarding tissue processing, LC-MS/MS analysis, and quantification are provided in Supplementary Materials.

Electrophysiology

Acute slice preparation and whole cell patch clamp recordings were performed with similar to those previously described (13,23). Voltage-clamp recordings were performed on striatal medium spiny neurons (MSNs) identified as D1-tdTomato positive (referred to as direct pathway or dMSNs throughout) and D1-tdTomato negative (indirect pathway or iMSNs throughout). Further details about patch clamp experiments including solution recipes are described in Supplementary Materials. Depolarization induced suppression of excitation (DSE) and inhibition (DSI) experiments were carried out as previously described (13). Spontaneous excitatory and inhibitory postsynaptic currents (sEPSC and sIPSC respectively) recordings were recorded at -70 mV as previously described (23,39). Further information

about recording paradigms including AMPA-NMDA ratio measurements are provided in Supplementary Materials.

Stereotaxic Injection of AAV

DGL $\alpha^{flx/flx}$ mice underwent bilateral stereotaxic surgery under isoflurane anesthesia. AAV5.CMV.HI.eGFP.Cre.WPRE.SV40 (AAV-Cre; titer 2×10^{13} GC/ml) or AAV5.CMV.PI.eGFP.WPRE.bGH (AAV-GFP; titer 7×10^{13} GC/ml) were injected bilaterally into the dorsal striatum (500 nl, AP: 0.62, ML: 1.90, DV: 3.00) or nucleus accumbens (NAc; 450 nl, AP: 1.65, ML: 0.92, DV: 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 at least 72 hours apart. DGL $\alpha^{D1-Cre+}$, DGL $\alpha^{A2A-Cre+}$ mice were run in parallel with 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. Data were initially analyzed using the Robust regression and Outlier removal (ROUT) test with $Q=5\%$, and outliers were removed. Data are presented as mean $\pm$ 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 $p < 0.05$ throughout. Further information regarding sample size selection and exclusion criteria is provided in Supplementary Materials.

RESULTS

Selective deletion of DGL α in dMSN and iMSN striatal cell populations

Floxed *Dagla* mice (DGL $\alpha^{flx/flx}$) harboring loxP sites that flank exon 8 of *Dagla* (DGL α) were generated as shown in Figure 1a and as we previously reported (38). To examine the effect of deleting DGL α from dMSNs, we crossed DGL $\alpha^{flx/flx}$ mice with *Drd1-cre* (D1-Cre) mice to generate DGL $\alpha^{D1-Cre+}$ offspring lacking DGL α in dMSNs and DGL $\alpha^{flx/flx}$ control littermates with intact DGL α . In a similar way, DGL $\alpha^{flx/flx}$ mice were crossed with *Adora2a-cre* (A2a-Cre) mice to delete DGL α from iMSNs (DGL $\alpha^{A2A-Cre+}$).

Germline deletion of DGL α results in impairment of striatal 2-AG-mediated retrograde synaptic plasticity, termed depolarization-induced suppression of excitation (DSE) (23). However, previous studies did not differentiate dMSN from iMSN to explicitly confirm that 2-AG release is DGL α -dependent in each cell-type. To address this, we crossed *Drd1-tdTomato* (D1-Tomato) reporter mice (40) onto DGL $\alpha^{D1-Cre+}$ or DGL $\alpha^{A2A-Cre+}$ mouse lines, permitting the identification of fluorescent dMSNs and non-fluorescent iMSNs during voltage-clamp recordings. DSE was significantly reduced in dMSNs from DGL $\alpha^{D1-Cre+}$ mice (Fig. 1d) and iMSNs from DGL $\alpha^{A2A-Cre+}$ mice (Fig. 1e), indicating that synaptic 2-AG retrograde signaling is mediated by DGL α in both MSN subtypes, and demonstrate successful deletion of DGL α in both D1-Cre+ and A2a-Cre+ MSNs.

To determine the contribution of each cell population to the eCB metabolome, we quantified eCB and related lipids in striatal extracts from both mouse lines via a LC/MS/MS approach (Fig. 1f-k). Deletion of DGL α from dMSNs in DGL $\alpha^{D1-Cre+}$ mice resulted in a significant reduction in striatal 2-AG levels (Fig. 1f). To determine whether these reduced 2-AG levels had any effect on striatal CB1R expression, we examined CB1R protein levels in striatal tissue punches from DGL $\alpha^{D1-Cre+}$ and DGL $\alpha^{flx/flx}$ mice and found no significant difference between the two groups (Supplementary Fig. 2). In contrast to effects seen in DGL $\alpha^{D1-Cre+}$ mice, loss of DGL α from iMSNs in DGL $\alpha^{A2A-Cre+}$ mice had no effect on total striatal 2-AG levels (Fig. 1i), even though synaptic 2-AG signaling was disrupted in iMSNs (Fig. 1e). Importantly, neither cell-type manipulation resulted in changes in the other major eCB, anandamide (AEA; Fig. 1g,j), or the primary hydrolytic metabolite of 2-AG, arachidonic acid (AA; Fig. 1h,k). Global deletion of DGL α causes increased forebrain levels of multiple arachidonate-containing diacylglycerol (DAG) species (23), indicating their utilization by DGL α as a substrate for 2-AG production. We were, however, unable to detect any significant changes in any detectable arachidonate-containing DAGs following deletion from either striatal pathway (Supplementary Table 1). We also did not detect any changes in other related lipids including Oleoylethanolamine, Palmitoylethanolamide, and 2-arachidonoyl glycerol-ether (Supplementary Table 1).

2-AG deficiency alters excitatory transmission onto dMSN synapses

To test the hypothesis that loss of 2-AG-mediated retrograde inhibition results in hyper-glutamatergic drive onto dMSNs, we recorded sEPSCs from D1-Tomato(+) MSNs in DGL $\alpha^{D1-Cre+}$ mice and their DGL $\alpha^{flx/flx}$ littermates (Fig. 2a-c). Deletion of DGL α from dMSNs led to a significantly increased frequency of sEPSCs (Fig. 2b), consistent with an enhanced probability of presynaptic release at these synapses. The sEPSC amplitude (Fig. 2c) and AMPA/NMDA ratios of evoked EPSCs (Fig. 2d) were not significantly different from controls indicating that postsynaptic excitatory function is unaffected by DGL α deletion from dMSNs. DGL α -dependent 2-AG release also mediates retrograde feedback inhibition of inhibitory transmission at GABAergic synapses (24). Thus, we measured sIPSCs in dMSNs to determine the effect of DGL α deletion on inhibitory transmission (Fig. 2e-g). In contrast to the effects on glutamatergic release, we observed no change in sIPSC frequency (Fig. 2f) or amplitude (Fig. 2g). Given the lack of an effect of DGL α deletion on basal inhibitory transmission in dMSNs, we questioned if dMSN inhibitory synapses can utilize DGL α -mediated 2-AG release, which had not been previously shown. Therefore, we tested the effect of DGL α deletion on depolarization-induced suppression of inhibition (DSI), which like DSE, is mediated by retrograde 2-AG signaling. dMSNs from DGL $\alpha^{flx/flx}$ mice displayed robust DSI, which was significantly impaired in DGL $\alpha^{D1-Cre+}$ mice (Fig. 2h). This indicates that 2-AG can be utilized by dMSN inhibitory synapses under strong depolarizing conditions. Together these data demonstrate a selective alteration at excitatory synapses in dMSNs, which suggests that impaired 2-AG signaling results in a net over-excitation of the direct pathway.

DGLα deletion from dMSNs and iMSNs does not impair locomotor function or instrumental learning

Given the canonical role of the striatum in action learning and coordination and the known ability of eCBs to affect some aspects of these behaviors, we tested the effect of dMSN or iMSN specific-deletion of DGLα on the accelerating rotarod (Supplementary Fig. 2a,b), balance beam test, (Supplementary Fig. 2c,d) and fixed ratio or progressive ratio instrumental learning paradigms (Supplementary Fig. 2e-l), but did not observe any significant differences in any of these measures.

DGLα deletion from dMSNs results in impaired sociability, repetitive behavior and decreased exploration

To examine the effect of DGLα deletion from dMSNs on social behavior, we used a 3-chamber sociability test where one outer chamber contains a non-familiar target mouse under a small wire inverted pencil cup while the other chamber contains an empty pencil cup (Fig. 3a-c). A significantly greater amount of time spent exploring the target mouse- vs. empty-chamber indicates preference for the social target (41). DGLα^{flx/flx} controls exhibited a significant preference for the social target; however, deletion of DGLα in dMSNs resulted in a loss of this preference, indicated by the loss of a significant difference between time spent in the mouse chamber vs. time in empty chamber (Fig. 3b). There were no significant differences in total distance traveled between either Cre+ groups relative to their littermate controls (Fig. 3c), suggesting that the deficit in sociability was not due to a locomotor impairment.

Another major hallmark of ASD in human patients is repetitive and stereotyped behaviors, which can manifest in mouse models of ASD as excessive grooming. Therefore, to test if deletion of DGLα in each MSN subpopulation results in the generation of repetitive behavior, we performed a standard 10-minute water-induced grooming assay. These studies revealed an increase in time spent grooming in DGLα^{D1-Cre+} mice, which lack DGLα in dMSNs (Fig. 3d), suggesting that 2-AG at dMSN synapses may be important for suppressing repetitive or compulsive behaviors. In some mouse models of repetitive behaviors, the excessive self-grooming can lead to skin lesions, which can appear after 3–6 months of age (2,42). We did not notice any increased occurrence of skin lesions on DGLα^{D1-Cre+} mice during our studies of 2–3 month old mice; however, this is anecdotal and a careful study of this was not performed. We previously reported that germline DGLα KO mice exhibit an anxiety-like phenotype (23); however, its unlikely that this contributed to the observed effects on sociability and grooming as there was no significant effect on anxiety in the light-dark box assay (Fig. 3e). In the open-field test (Fig. 3f-h), total distance traveled (Fig. 3f) and vertical exploration (rearing; Fig. 3g) were all significantly decreased in DGLα^{D1-Cre+} mice relative to their controls. These overall differences reflected an increased rate of habituation to the novel arena over time, as the decreased exploration only occurred after the first 5 minutes of the assay. Consistent with the lack of effect in the light-dark box, we did not detect any difference in open-field center time (Fig. 3h), further suggesting that loss of dMSN 2-AG signaling does not result in anxiety-like behavior. In contrast to these findings, the deletion of DGLα from iMSNs in DGLα^{A2A-Cre+} mice had no significant

effect on sociability (Fig. 3i-k), grooming (Fig. 3l), light-dark box (Fig. 3m) or open-field exploration (Fig. 3n-p).

Role of 2-AG signaling in social and repetitive behavioral domains is specific to MSNs in dorsal and ventral striatum respectively

To gain a better understanding of the striatal circuitry involved in the behavioral phenotypes observed after deletion of DGL α , we tested the effects of spatially restricted deletions of DGL α from either dorsal striatum or nucleus accumbens (NAc). To accomplish this, adeno-associated virus (AAV) encoding Cre recombinase fused to GFP (AAV-Cre) was injected into the dorsal striatum or NAc of DGL $\alpha^{flx/flx}$ mice (Fig. 4a-i). Control DGL $\alpha^{flx/flx}$ mice were injected with an AAV expressing only GFP (AAV-GFP). Immunohistochemistry studies confirmed the successful recombination of DGL α in AAV-Cre injected mice, as DGL α immunoreactivity was visibly reduced in striatal regions that were also immunoreactive for Cre and expressed GFP (Fig. 4a). In the 3-chamber social interaction assay, mice receiving control AAV-GFP into the dorsal striatum or NAc both showed a significant preference towards a novel mouse over the empty chamber (Fig. 4c,g; left). AAV-Cre mediated deletion of DGL α in the dorsal striatum impaired preference towards the social target (Fig. 4c; right), whereas deletion of DGL α in the NAc had no effect on sociability (Fig. 4g; right). There were no significant differences in distance traveled during the assay for either dorsal striatum (Fig. 4d) or NAc (Fig. 4h) injected groups; therefore, the observed social behavior was not likely influenced by a motor phenotype. Deletion of DGL α from the dorsal striatum had no effect on grooming (Fig. 4e), while expression of AAV-Cre in the NAc led to a significant repetitive grooming phenotype (Fig. 4i). We have previously reported that DGL α deletion from the NAc does not affect open-field exploratory activity (38); however, to fully understand how each sub-region could recapitulate the phenotypes observed in the DGL $\alpha^{D1-Cre+}$ mice, we tested the effect of DGL α deletion from dorsal striatum in the open-field assay. We did not detect any significant effect of DGL α deletion from dorsal striatum in open-field exploration (Supplementary Figure 3).

DISCUSSION

We utilized DGL α conditional KO mice (DGL $\alpha^{flx/flx}$) to investigate the role of the major brain eCB, 2-AG, in spatially and genetically defined MSNs of the striatum. We show that deletion of DGL α from dMSNs results in decreased striatal 2-AG levels, a loss of 2-AG dependent feedback-inhibition at both glutamatergic and GABAergic dMSN synapses, and increased basal glutamatergic release onto dMSNs. At the behavioral level, these cellular and circuit-level changes were accompanied by deficits in sociability, excessive repetitive grooming, and impairment in exploratory drive. Our data suggest that these behavioral phenotypes are derived from different striatal sub-regions, as the social and repetitive behaviors could be recapitulated by viral-mediated DGL α deletion in the dorsal and ventral striatum respectively.

We found that reduction of striatal 2-AG levels was specific to deletion of DGL α in dMSNs. Interestingly, there were no changes in the levels of AA-containing DAGs, which is in contrast to what we have previously reported in whole forebrain of germline DGL α .

knockout mice (23). This may suggest a limited availability of these 2-AG precursor lipids to DGL α , which is supported by the requirement for PLC activation in many forms of 2-AG mobilization (43). This scenario would be consistent with our previously reported finding that application of the DAG precursor through the patch pipette combined with sub-threshold depolarization, but neither alone, is sufficient to drive 2-AG-mediated synaptic depression at striatal synapses (44).

DSE was significantly impaired in both dMSNs and iMSNs following DGL α deletion. Consistent with this, previous studies have demonstrated expression of DGL α in both dMSN and iMSN striatal cell populations (14). Thus, our data demonstrate the availability of 2-AG signaling components at excitatory synapses of both dMSN and iMSN synapses, despite the selective sensitivity of tissue and behavioral effects of DGL α deletion to dMSNs. The fact that DGL α deletion in iMSNs reduces DSE while leaving total tissue levels intact may suggest that the total tissue levels of 2-AG are largely driven by 2-AG synthesis from dMSNs and not from iMSNs or other cell types. Alternatively, it is possible that iMSNs have an alternative intrinsic 2-AG synthesis pathway that is able to compensate for non-synaptic pools of 2-AG in iMSNs.

Consistent with an impairment of eCB-mediated feedback inhibition on glutamatergic drive, we identified a significant increase in sEPSC frequency upon loss of DGL α in dMSNs. To determine how this might affect net dMSN output, we further evaluated the effect of 2-AG deficiency on GABAergic synaptic function in dMSNs. Although DSI at GABAergic synapses was also impaired by deletion of DGL α , basal GABAergic transmission was unchanged in dMSNs after DGL α deletion. The lack of an effect on GABAergic transmission together with an enhancement of glutamatergic release suggest that a shift in the excitation/inhibition ratio may lead to enhanced dMSN output. Determining whether a net over-activation of dMSNs exists in mice harboring human ASD-risk genotypes will be an important line of future study to begin to develop a strategy for therapeutic intervention using the eCB signaling pathway as a target.

We previously reported that germline DGL α deletion had no effect on motor coordination or locomotion (23). However, given the opposing roles of the direct and indirect pathways on basal ganglia output, it is possible that deletion from both pathways might result in an overall lack of effect. Results from the current study indicate otherwise, as both rotarod and balance beam performance remained intact following DGL α deletion from either striatal cell-type. Although we did detect an increased rate of habituation to the open field chamber in DGL α ^{D1-Cre+} mice, locomotor activity was similar during the first 5 min of the assay, indicating that loss of striatal 2-AG signaling may affect exploratory drive but not gross motor function. This phenotype was not recapitulated by regional deletion of DGL α from dorsal striatum as we report in the present study, or by deletion of DGL α from NAc, as we have previously reported (38). Overall these findings suggest that the decreased exploratory activity we observed in the open field may derive from another D1R-expressing brain region, or that the phenotype may be masked by deletion of DGL α from dMSNs and iMSNs in one or more striatal subregions. Nevertheless, the lack of any profound effects on locomotion following any of the manipulations to DGL α in this study and our previous work (23,38) is interesting in light of the fact that activation of CB1Rs or augmentation of 2-AG levels cause

hypolocomotion (45–47). Instrumental lever-press responding in fixed- and progressive-ratio tasks was not affected by DGL α deletion in dMSNs or iMSNs. These findings are consistent with previous reports, in which disruption of eCB signaling did not affect fixed-ratio acquisition of lever-pressing (48,49). However, pharmacological inhibition or genetic deletion of CB1Rs does not seem to have a profound effect on locomotion (46,50), which together with our results suggests that the role of eCBs in regulating motor activity may be more relevant in pathological states where eCB signaling is hyperactive.

Behavioral studies revealed social interaction deficits, excessive grooming behavior, and impaired exploration due to impairment of 2-AG signaling in dMSNs. These data suggest a selective behavioral role for 2-AG signaling at dMSNs for the behaviors we analyzed. Together with reports collectively suggesting that forms of anandamide-mediated plasticity may have a lower threshold of induction at iMSN synapses (51–53), our findings suggest the possibility of selectivity in 2-AG and anandamide utilization in dMSNs and iMSNs respectively. If confirmed, this would explain many discrepancies in regards to upstream signaling mechanisms required to induce 2-AG and anandamide plasticity, and could be exploited for therapeutic benefit to allowing selective control of striatal pathway output.

The generation of repetitive grooming was specific to deletion of DGL α from the nucleus accumbens, which is interesting given the more canonical role for the dorsal striatum in learned motor sequences. These data are in agreement with another recent study, which found the generation of repetitive motor routines in neuroligin-3 KOs was due to altered function of dMSNs in the nucleus accumbens (20). Perhaps more surprising is the finding that impairment of sociability was specific to DGL α deletion from dorsal striatum. It has been postulated that since social interaction represents reward-based behavior, this aspect of ASD pathology may be derived from synaptic dysfunction in the nucleus accumbens as opposed to the dorsal striatum. However, this model does not account for the component of social behavior that requires behavioral flexibility to adapt to novel social stimuli, which is largely dependent on the dorsal striatum (54–56). Although our data do not directly address these different aspects of striatal-mediated behaviors, they do indicate a complexity in the striatal circuitry of ASD pathology that should be clarified in future investigations involving eCB-dysfunction as well as other underlying mechanisms linked to ASD-relevant behaviors.

Finally, our data suggest the novel hypothesis that disruption of 2-AG signaling in dMSN circuits could contribute to both the social and repetitive behavioral phenotypes associated with ASD. However, the regional deletions of DGL α from the dorsal striatum and NAc in this study were not specific to dMSN or iMSN cell populations. Therefore, comparing the behavioral effects of DGL α deletion from dMSNs to the effects of regional deletion should be interpreted with this important caveat in mind. In principle, this caveat may be addressed using viral-based strategies to reconstitute DGL α expression in a striatal sub-region and cell-type specific manner. However, our attempts to express DGL α in mouse brain using either lentiviral or AAV vectors have been unsuccessful, possibly due to the large size of *dagla* cDNA, which can negatively affect efficient viral packaging. Advancements in viral transduction technology will hopefully permit these studies in the future. It will also be interesting to determine the commonality of similar disruptions in 2-AG signaling or striatal circuitry across multiple mouse models of ASD. Indeed, several recent studies have reported

ASD-like behaviors in conjunction with an overactive direct pathway (20,57). However, others have reported a selective decrease in indirect pathway activity that could be corrected to rescue abnormal behavioral in the Shank3-KO model of ASD (58). In the context of our study and others reporting direct pathway hyperactivity (2,20), these findings may suggest that any imbalance between direct and indirect pathway activity may produce ASD-like behavior. Understanding how different ASD models may arrive at such an imbalance (i.e., through selective effects on excitatory or inhibitory synapses of direct or indirect pathway MSNs) will be an important future avenue of study. Furthermore, testing whether manipulation of eCB signaling may represent a useful therapeutic tool to restore these imbalances to improve ASD-like phenotypes in these models should be further explored. Such knowledge may lead to a more rational design of drug therapies that may be more effective across a broader range of ASD patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

eCB measurements were conducted at the Vanderbilt Mass Spectrometry Research Center Facility and additional equipment support from S10-OD017997, all behavioral testing was conducted at the Vanderbilt Neurobehavioral Core Facility. *Drd1*-Cre and *Adora2a*-cre mice were generated by The Gene Expression Nervous System Atlas (GENSAT) Project, NINDS Contracts N01NS02331 & HHSN271200723701C to The Rockefeller University (New York, NY). These studies were supported by NIH Grants K01-MH107765 (BCS), F31MH106192 (RJB), R01-NS078291 (RJC), NARSAD Young Investigator Grant (RB), K05 DA021696 (KM), a Vanderbilt Kennedy Center Hobbs Discovery Grant (DGW, SP, RJC), and NIAAA Division of Intramural Clinical and Biological Research, project ZIA AA000416 (SMA, DML).

References

1. American Psychiatric Association., American Psychiatric Association. DSM-5 Task Force. Diagnostic and statistical manual of mental disorders: DSM-5. 5th. Washington, DC: American Psychiatric Association; 2013.
2. Peca, Feliciano, Ting, Wang, Wells, Venkatraman, et al. Shank3 mutant mice display autistic-like behaviours and striatal dysfunction. *Nature*. 2011; 472:437–442. [PubMed: 21423165]
3. Penagarikano, Abrahams, Herman, Winden, Gdalyahu, Dong, et al. Absence of CNTNAP2 leads to epilepsy, neuronal migration abnormalities, and core autism-related deficits. *Cell*. 2011; 147:235–246. [PubMed: 21962519]
4. Gunaydin, Grosenick, Finkelstein, Kauvar, Fenno, Adhikari, et al. Natural neural projection dynamics underlying social behavior. *Cell*. 2014; 157:1535–1551. [PubMed: 24949967]
5. Di Martino, Kelly, Grzadzinski, Zuo, Mennes, Mairena, et al. Aberrant striatal functional connectivity in children with autism. *Biol Psychiatry*. 2011; 69:847–856. [PubMed: 21195388]
6. Delmonte, Gallagher, O'Hanlon, McGrath, Balsters. Functional and structural connectivity of frontostriatal circuitry in Autism Spectrum Disorder. *Front Hum Neurosci*. 2013; 7:430. [PubMed: 23964221]
7. Hollander, Anagnostou, Chaplin, Esposito, Haznedar, Licalzi, et al. Striatal volume on magnetic resonance imaging and repetitive behaviors in autism. *Biol Psychiatry*. 2005; 58:226–232. [PubMed: 15939406]
8. Wei, Lee, Cox, Karsten, Penagarikano, Geschwind, et al. Endocannabinoid signaling mediates oxytocin-driven social reward. *Proceedings of the National Academy of Sciences of the United States of America*. 2015; 112:14084–14089. [PubMed: 26504214]

9. Karhson, Hardan, Parker. Endocannabinoid signaling in social functioning: an RDoC perspective. *Translational psychiatry*. 2016; 6:e905. [PubMed: 27676446]
10. Manduca, Servadio, Damsteegt, Campolongo, Vanderschuren, Trezza. Dopaminergic Neurotransmission in the Nucleus Accumbens Modulates Social Play Behavior in Rats. *Neuropsychopharmacology*. 2016; 41:2215–2223. [PubMed: 26860202]
11. Chen, Wan, Ade, Ting, Feng, Calakos. Sapap3 deletion anomalously activates short-term endocannabinoid-mediated synaptic plasticity. *J Neurosci*. 2011; 31:9563–9573. [PubMed: 21715621]
12. Gremel, Chancey, Atwood, Luo, Neve, Ramakrishnan, et al. Endocannabinoid Modulation of Orbitofrontal Circuits Gates Habit Formation. *Neuron*. 2016; 90:1312–1324. [PubMed: 27238866]
13. Shonesy, Wang, Rose, Ramikie, Cavener, Rentz, et al. CaMKII regulates diacylglycerol lipase- α and striatal endocannabinoid signaling. *Nat Neurosci*. 2013; 16:456–463. [PubMed: 23502535]
14. Uchigashima, Narushima, Fukaya, Katona, Kano, Watanabe. Subcellular arrangement of molecules for 2-arachidonoyl-glycerol-mediated retrograde signaling and its physiological contribution to synaptic modulation in the striatum. *J Neurosci*. 2007; 27:3663–3676. [PubMed: 17409230]
15. Kano, Ohno-Shosaku, Hashimoto-dani, Uchigashima, Watanabe. Endocannabinoid-mediated control of synaptic transmission. *Physiol Rev*. 2009; 89:309–380. [PubMed: 19126760]
16. Anderson, Aoto, Tabuchi, Foldy, Covy, Yee, et al. beta-Neurexins Control Neural Circuits by Regulating Synaptic Endocannabinoid Signaling. *Cell*. 2015; 162:593–606. [PubMed: 26213384]
17. Doenni, Gray, Song, Patel, Hill, Pittman. Deficient adolescent social behavior following early-life inflammation is ameliorated by augmentation of anandamide signaling. *Brain Behav Immun*. 2016; 58:237–247. [PubMed: 27453335]
18. Foldy, Malenka, Sudhof. Autism-associated neuroligin-3 mutations commonly disrupt tonic endocannabinoid signaling. *Neuron*. 2013; 78:498–509. [PubMed: 23583622]
19. Kerr, Downey, Conboy, Finn, Roche. Alterations in the endocannabinoid system in the rat valproic acid model of autism. *Behav Brain Res*. 2013; 249:124–132. [PubMed: 23643692]
20. Rothwell, Fuccillo, Maxeiner, Hayton, Gokce, Lim, et al. Autism-associated neuroligin-3 mutations commonly impair striatal circuits to boost repetitive behaviors. *Cell*. 2014; 158:198–212. [PubMed: 24995986]
21. Speed, Masiulis, Gibson, Powell. Increased Cortical Inhibition in Autism-Linked Neuroligin-3R451C Mice Is Due in Part to Loss of Endocannabinoid Signaling. *PLoS One*. 2015; 10:e0140638. [PubMed: 26469287]
22. Maccarrone, Rossi, Bari, De Chiara, Rapino, Musella, et al. Abnormal mGlu 5 receptor/endocannabinoid coupling in mice lacking FMRP and BC1 RNA. *Neuropsychopharmacology*. 2010; 35:1500–1509. [PubMed: 20393458]
23. Shonesy, Bluett, Ramikie, Baldi, Hermanson, Kingsley, et al. Genetic disruption of 2-arachidonoylglycerol synthesis reveals a key role for endocannabinoid signaling in anxiety modulation. *Cell reports*. 2014; 9:1644–1653. [PubMed: 25466252]
24. Tanimura, Yamazaki, Hashimoto-dani, Uchigashima, Kawata, Abe, et al. The endocannabinoid 2-arachidonoylglycerol produced by diacylglycerol lipase α mediates retrograde suppression of synaptic transmission. *Neuron*. 2010; 65:320–327. [PubMed: 20159446]
25. Gao, Vasilyev, Goncalves, Howell, Hobbs, Reisenberg, et al. Loss of retrograde endocannabinoid signaling and reduced adult neurogenesis in diacylglycerol lipase knock-out mice. *J Neurosci*. 2010; 30:2017–2024. [PubMed: 20147530]
26. Prasad, Merico, Thiruvahindrapuram, Wei, Lionel, Sato, et al. A discovery resource of rare copy number variations in individuals with autism spectrum disorder. *G3*. 2012; 2:1665–1685. [PubMed: 23275889]
27. Miller, Adam, Aradhya, Biesecker, Brothman, Carter, et al. Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. *Am J Hum Genet*. 2010; 86:749–764. [PubMed: 20466091]
28. Kelleher, Geigenmuller, Hovhannisyan, Trautman, Pinard, Rathmell, et al. High-throughput sequencing of mGluR signaling pathway genes reveals enrichment of rare variants in autism. *PLoS One*. 2012; 7:e35003. [PubMed: 22558107]

29. Fatemi, Folsom, Kneeland, Yousefi, Liesch, Thuras. Impairment of fragile X mental retardation protein-metabotropic glutamate receptor 5 signaling and its downstream cognates ras-related C3 botulinum toxin substrate 1, amyloid beta A4 precursor protein, striatal-enriched protein tyrosine phosphatase, and homer 1, in autism: a postmortem study in cerebellar vermis and superior frontal cortex. *Molecular autism*. 2013; 4:21. [PubMed: 23803181]
30. Berkel, Marshall, Weiss, Howe, Roeth, Moog, et al. Mutations in the SHANK2 synaptic scaffolding gene in autism spectrum disorder and mental retardation. *Nat Genet*. 2010; 42:489–491. [PubMed: 20473310]
31. Durand, Betancur, Boeckers, Bockmann, Chaste, Fauchereau, et al. Mutations in the gene encoding the synaptic scaffolding protein SHANK3 are associated with autism spectrum disorders. *Nat Genet*. 2007; 39:25–27. [PubMed: 17173049]
32. Gauthier, Spiegelman, Piton, Lafreniere, Laurent, St-Onge, et al. Novel de novo SHANK3 mutation in autistic patients. *Am J Med Genet B Neuropsychiatr Genet*. 2009; 150B:421–424. [PubMed: 18615476]
33. Moessner, Marshall, Sutcliffe, Skaug, Pinto, Vincent, et al. Contribution of SHANK3 mutations to autism spectrum disorder. *Am J Hum Genet*. 2007; 81:1289–1297. [PubMed: 17999366]
34. Stephenson, Wang, Perfitt, Parrish, Shonesy, Marks, et al. A Novel Human CAMK2A Mutation Disrupts Dendritic Morphology and Synaptic Transmission, and Causes ASD-Related Behaviors. *J Neurosci*. 2017; 37:2216–2233. [PubMed: 28130356]
35. Ninan. Oxytocin suppresses basal glutamatergic transmission but facilitates activity-dependent synaptic potentiation in the medial prefrontal cortex. *J Neurochem*. 2011; 119:324–331. [PubMed: 21848811]
36. Ghaleiha, Asadabadi M Fau - Mohammadi, Mohammadi Mr Fau - Shahei, Shahei M Fau - Tabrizi, Tabrizi M Fau - Hajiaghaee, Hajiaghaee R Fau - Hassanzadeh, et al. Memantine as adjunctive treatment to risperidone in children with autistic disorder: a randomized, double-blind, placebo-controlled trial.
37. Bejjani, O'Neill, Kim, Frew, Yee, Ly, et al. Elevated glutamatergic compounds in pregenual anterior cingulate in pediatric autism spectrum disorder demonstrated by 1H MRS and 1H MRSI. *PLoS One*. 2012; 7:e38786. [PubMed: 22848344]
38. Bluett, Baldi, Haymer, Gaulden, Hartley, Parrish, et al. Endocannabinoid signalling modulates susceptibility to traumatic stress exposure. *Nature communications*. 2017; 8:14782.
39. Gustin, Shonesy, Robinson, Rentz, Baucum, Jalan-Sakrikar, et al. Loss of Thr286 phosphorylation disrupts synaptic CaMKIIalpha targeting, NMDAR activity and behavior in pre-adolescent mice. *Mol Cell Neurosci*. 2011; 47:286–292. [PubMed: 21627991]
40. Ade, Wan, Chen, Gloss, Calakos. An Improved BAC Transgenic Fluorescent Reporter Line for Sensitive and Specific Identification of Striatonigral Medium Spiny Neurons. *Front Syst Neurosci*. 2011; 5:32. [PubMed: 21713123]
41. Moy, Nadler, Young, Perez, Holloway, Barbaro, et al. Mouse behavioral tasks relevant to autism: phenotypes of 10 inbred strains. *Behav Brain Res*. 2007; 176:4–20. [PubMed: 16971002]
42. Welch, Lu, Rodriguiz, Trotta, Peca, Ding, et al. Cortico-striatal synaptic defects and OCD-like behaviours in Sapap3-mutant mice. *Nature*. 2007; 448:894–900. [PubMed: 17713528]
43. Hashimoto-dani, Ohno-Shosaku, Tsubokawa, Ogata, Emoto, Maejima, et al. Phospholipase Cbeta serves as a coincidence detector through its Ca2+ dependency for triggering retrograde endocannabinoid signal. *Neuron*. 2005; 45:257–268. [PubMed: 15664177]
44. Shonesy, Winder, Patel, Colbran. The initiation of synaptic 2-AG mobilization requires both an increased supply of diacylglycerol precursor and increased postsynaptic calcium. *Neuropharmacology*. 2015; 91:57–62. [PubMed: 25484252]
45. Ledent, Valverde, Cossu, Petitot, Aubert, Beslot, et al. Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knockout mice. *Science*. 1999; 283:401–404. [PubMed: 9888857]
46. Zimmer, Zimmer, Hohmann, Herkenham, Bonner. Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB1 receptor knockout mice. *Proc Natl Acad Sci U S A*. 1999; 96:5780–5785. [PubMed: 10318961]

47. Long, Li, Booker, Burston, Kinsey, Schlosburg, et al. Selective blockade of 2-arachidonoylglycerol hydrolysis produces cannabinoid behavioral effects. *Nat Chem Biol.* 2009; 5:37–44. [PubMed: 19029917]
48. Crombag, Johnson, Zimmer, Zimmer, Holland. Deficits in sensory-specific devaluation task performance following genetic deletions of cannabinoid (CB1) receptor. *Learn Mem.* 2010; 17:18–22. [PubMed: 20035014]
49. Hilario, Clouse, Yin, Costa. Endocannabinoid signaling is critical for habit formation. *Front Integr Neurosci.* 2007; 1:6. [PubMed: 18958234]
50. Marinho, Oliveira-Lima, Santos, Hollais, Baldaia, Wu-Silva, et al. Effects of rimonabant on the development of single dose-induced behavioral sensitization to ethanol, morphine and cocaine in mice. *Prog Neuropsychopharmacol Biol Psychiatry.* 2015; 58:22–31. [PubMed: 25496830]
51. Shen, Flajolet, Greengard, Surmeier. Dichotomous dopaminergic control of striatal synaptic plasticity. *Science.* 2008; 321:848–851. [PubMed: 18687967]
52. Kreitzer, Malenka. Endocannabinoid-mediated rescue of striatal LTD and motor deficits in Parkinson's disease models. *Nature.* 2007; 445:643–647. [PubMed: 17287809]
53. Lerner, Kreitzer. RGS4 is required for dopaminergic control of striatal LTD and susceptibility to Parkinsonian motor deficits. *Neuron.* 2012; 73:347–359. [PubMed: 22284188]
54. Balleine, O'Doherty. Human and rodent homologies in action control: corticostriatal determinants of goal-directed and habitual action. *Neuropsychopharmacology.* 2010; 35:48–69. [PubMed: 19776734]
55. Graybiel. Habits, rituals, and the evaluative brain. *Annu Rev Neurosci.* 2008; 31:359–387. [PubMed: 18558860]
56. Yin, Knowlton, Balleine. Inactivation of dorsolateral striatum enhances sensitivity to changes in the action-outcome contingency in instrumental conditioning. *Behav Brain Res.* 2006; 166:189–196. [PubMed: 16153716]
57. Ade, Wan, Hamann, O'Hare, Guo, Quian, et al. Increased Metabotropic Glutamate Receptor 5 Signaling Underlies Obsessive-Compulsive Disorder-like Behavioral and Striatal Circuit Abnormalities in Mice. *Biol Psychiatry.* 2016; 80:522–533. [PubMed: 27436084]
58. Wang, Li, Chen, van der Goes, Hawrot, Yao, et al. Striatopallidal dysfunction underlies repetitive behavior in Shank3-deficient model of autism. *J Clin Invest.* 2017; 127:1978–1990. [PubMed: 28414301]

Figure 1. Specific deletion of DGLα in dMSN and iMSN striatal cell populations

(a) Diagram depicting the location of the loxP sites flanking exon 9 of the endogenous *Dagla* gene. (b,c) The breeding strategy for cell-type specific deletion of DGLα from dMSNs and iMSNs is shown. (d) Depolarization-induced suppression of excitation (DSE) recorded from D1-tdTomato fluorescent striatal MSNs (dMSNs) from $DGL\alpha^{D1-Cre+}/D1$ -tdTomato(+) mice (n=6) and $DGL\alpha^{flx/flx}/D1$ -tdTomato(+) littermates (n=13). The magnitude of DSE is shown in scatter plot (right). (e) DSE recorded from D1-tdTomato negative (non- fluorescent) striatal MSNs (iMSNs) from $DGL\alpha^{A2A-Cre+}$ mice; $DGL\alpha$ -cKO; D1-tdTomato(+) mice (n=11) and their $DGL\alpha^{flx/flx}$ littermates (n=8). The magnitude of DSE is shown in scatter plot (right). (f-h) Effect of DGLα deletion from D1-Cre expressing striatal MSNs (dMSNs) on striatal levels of (f) 2-AG, (g) anandamide (AEA), and (h) arachidonic acid (AA). (i-k) Effect of DGLα deletion from A2a-Cre expressing striatal

MSNs (iMSNs) on striatal levels of (i) 2-AG, (j) AEA, and (k) AA. Statistical significance between groups was determined by unpaired two-tailed t-test, with * indicating $p < 0.05$ and ** indicating $p < 0.01$. t-statistic (t), degrees of freedom (df), and p values are shown for all significant effects. All data is presented as mean $\pm$ s.e.m. Sample sizes are shown either in parenthesis above datasets or at the base of each dataset.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 2. Increased basal synaptic transmission onto dMSNs is specific to glutamatergic synapses following deletion of $DGL\alpha$ from dMSNs

(a) Representative traces of spontaneous excitatory postsynaptic currents (sEPSCs) in D1-tdTomato fluorescent cells from $DGL\alpha^{D1-Cre+}/D1-tdTomato(+)$ mice (red; $DGL\alpha^{D1-Cre+}$) and $DGL\alpha^{flx/flx}/D1-tdTomato(+)$ controls (gray; $DGL\alpha^{flx/flx}$). (b) Effect of $DGL\alpha$ deletion from dMSNs on the cumulative distribution of sEPSC interevent intervals (left) and average sEPSC frequency (right). (c) Effect of $DGL\alpha$ deletion from dMSNs on the cumulative distribution of sEPSC amplitude (left) and average sEPSC amplitude (right). (d) Effect of $DGL\alpha$ deletion from dMSNs on AMPA/NMDA ratios. (Left) Overlaid representative traces of evoked AMPA receptor-mediated EPSC (–70 mV; downward current) and dual component evoked EPSCs (+40 mV; upward current) from $DGL\alpha^{flx/flx}$ (gray) and $DGL\alpha^{D1-Cre+}$ mice (red). (Right) Scatter plot of average AMPA/NMDA ratios. (e)

Representative traces of spontaneous inhibitory postsynaptic currents (sIPSCs) in D1-tdTomato positive cells from DGL $\alpha^{D1-Cre+}$ mice (red) and DGL $\alpha^{flx/flx}$ controls (gray). **(f)** Effect of DGL α deletion from dMSNs on the cumulative distribution of sIPSC interevent intervals (left) and average sIPSC frequency (right). **(g)** Effect of DGL α deletion from dMSNs on the cumulative distribution of sIPSC amplitude (left) and average sIPSC amplitude (right). **(h)** Depolarization-induced suppression of inhibition (DSI) recorded from D1-tdTomato fluorescent MSNs (dMSNs) from DGL $\alpha^{D1-Cre+}$ mice and DGL $\alpha^{flx/flx}$ littermates. The magnitude of DSI is shown in scatter plot (right). Statistical significance between groups was determined by unpaired two-tailed t-test. ** $p < 0.01$; **** $p < 0.0001$. t-statistic (t), degrees of freedom (df), and p values are shown for all significant effects. All data is presented as mean $\pm$ s.e.m. Sample sizes are shown either in parenthesis above datasets or at the base of each dataset.

Figure 3. dMSN deletion of DGLα causes impairment in social interaction, excessive grooming, and decreased exploratory drive

The effect of DGLα deletion in (a-h) D1- or (i-p) A2a- neurons (indicated as dMSNs and iMSNs respectively) was tested in the 3-chamber social interaction test (a-c, j-l), grooming test (d,m), light-dark box (e,n) and open field test (f-h, n-p). (a,i) Representative heat maps tracking mouse location during the social interaction test are shown for (a) dMSN and (i) iMSN DGLα-cKO groups. (b,j) Time spent in each chamber containing a novel target mouse (mouse) or empty pencil cup (empty) is shown. Preference for the mouse chamber was determined by paired two-tailed t-test comparing time in the mouse-containing chamber to the empty chamber. (c,k) Effect of DGLα deletion from (c) dMSNs and (k) iMSNs on the total distance traveled during the social interaction test. dMSN groups DGLα^{flx/flx} n=9, DGLα^{D1-Cre+} n=14; iMSN groups DGLα^{flx/flx} n=13, DGLα^{A2A-Cre+} n=14. (d,l) Effect of

DGL α deletion from **(d)** dMSNs and **(l)** iMSNs on time spent grooming during grooming test. dMSN groups DGL $\alpha^{\text{flx/flx}}$ n=21, DGL $\alpha^{\text{D1-Cre+}}$ n=22; iMSN groups DGL $\alpha^{\text{flx/flx}}$ n=18, DGL $\alpha^{\text{A2a-Cre+}}$ n=20. **(e,m)** Effect of DGL α deletion from dMSNs **(e)** or iMSNs **(m)** on the percent distance traveled in the light compartment of the light-dark box assay. dMSN groups DGL $\alpha^{\text{flx/flx}}$ n=19, DGL $\alpha^{\text{D1-Cre+}}$ n=16; iMSN groups DGL $\alpha^{\text{flx/flx}}$ n=15, DGL $\alpha^{\text{A2a-Cre+}}$ n=13. **(f,n)** Effect of DGL α deletion from dMSNs **(f)** and iMSNs **(n)** on distance traveled in open field plotted in 5 min bins across the duration of the test (left) and as a scatter plot of total distance traveled (right). **(g,o)** Effect of DGL α deletion from dMSNs **(g)** and iMSNs **(o)** on vertical exploration (rearing) counts in the open field test plotted in 5 min bins across the duration of the test (left) and as a scatter plot of total rearing counts (right). **(h,p)** Effect of DGL α deletion from dMSNs **(h)** and iMSNs **(p)** on the time spent in the center of the open field chamber. dMSN groups DGL $\alpha^{\text{flx/flx}}$ n=24, DGL $\alpha^{\text{D1-Cre+}}$ n=25; iMSN groups DGL $\alpha^{\text{flx/flx}}$ n=26, DGL $\alpha^{\text{A2a-Cre+}}$ n=24. Center time was only measured in a subset of subjects due to a technical failure in data storage during the open-field analysis of one cohort. For center time: dMSN groups DGL $\alpha^{\text{flx/flx}}$ n=15, DGL $\alpha^{\text{D1-Cre+}}$ n=17; iMSN groups DGL $\alpha^{\text{flx/flx}}$ n=14, DGL $\alpha^{\text{A2a-Cre+}}$ n=12. Statistical significance between 2 groups (scatter plots) was determined by unpaired two-tailed t-test, and the t-statistic (t), degrees of freedom (df), and p values are shown above graph for all significant effects. 2-way repeated measures (RM) ANOVA was used to compare effect of genotype over time with Holm-Sidak multiple comparisons test used after ANOVA to compare between groups at each time point. F and p values are shown above graph for all significant interactions, with significance of multiple comparisons at each time point indicated on graph. For all statistical tests, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. All data is presented as mean $\pm$ s.e.m.

Figure 4. Social deficit and excessive grooming phenotypes in DGL α -cKO mice are mediated by MSNs in the dorsal striatum and nucleus accumbens respectively

(a) Decreased DGL α immunoreactivity in dorsal striatum and nucleus accumbens from DGL $\alpha^{flx/flx}$ mice injected with AAV-Cre into the dorsal striatum and NAc.

Immunoreactivity for DGL α (red) and Cre (blue) are shown in addition to GFP fluorescence (green) from mice infected with either AAV-GFP control virus or AAV-Cre. Merged and individual signals are shown. Scale bar, 600 μ m. (b,f) Representative heat maps tracking mouse location during the social interaction test are shown for DGL $\alpha^{flx/flx}$ mice injected

with AAV-GFP or AAV-Cre into the dorsal striatum **(b)** or NAc **(f)**. **(c,g)** Time spent in each chamber containing a novel target mouse (mouse) or empty pencil cup (empty) is shown for AAV-GFP and AAV-Cre targeted to dorsal striatum (GFP n=13, Cre n=11) or NAc (GFP n=15, Cre n=15) of DGL $\alpha^{flx/flx}$ mice. Preference for the mouse chamber was determined by paired two-tailed t-test comparing time in the mouse-containing chamber to the empty chamber. **(d,h)** Effect of DGL α deletion from dorsal striatum **(d)** and NAc **(h)** on distance traveled during social interaction test. **(e,i)** Effect of DGL α deletion from dorsal striatum **(e)** and NAc **(i)** on time spent grooming during grooming test. Statistical significance between 2 groups (scatter plots) was determined by unpaired two-tailed t-test, and the t-statistic (t), degrees of freedom (df), and p values are shown above graph for all significant effects. 2-way repeated measures (RM) ANOVA was used to compare effect of genotype over time with Holm-Sidak multiple comparisons test used after ANOVA to compare between groups at each time point. F and p values are shown above graph for all significant interactions, with significance of multiple comparisons at each time point indicated on graph. For all statistical tests, *p<0.05, **p<0.01. All data is presented as mean $\pm$ s.e.m.