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Increased Metabotropic Glutamate Receptor 5 Signaling Underlies Obsessive-Compulsive Disorder-like Behavioral and Striatal Circuit Abnormalities in Mice

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ABSTRACT

BACKGROUND: Development of treatments for obsessive-compulsive disorder (OCD) is hampered by a lack of mechanistic understanding about this prevalent neuropsychiatric condition. Although circuit changes such as elevated frontostriatal activity are linked to OCD, the underlying molecular signaling that drives OCD-related behaviors remains largely unknown. Here, we examine the significance of type 5 metabotropic glutamate receptors (mGluR5s) for behavioral and circuit abnormalities relevant to OCD.

METHODS: *Sapap3* knockout (KO) mice treated acutely with an mGluR5 antagonist were evaluated for OCD-relevant phenotypes of self-grooming, anxiety-like behaviors, and increased striatal activity. The role of mGluR5 in the striatal circuit abnormalities of *Sapap3* KO mice was further explored using two-photon calcium imaging to monitor striatal output from the direct and indirect pathways. A contribution of constitutive signaling to increased striatal mGluR5 activity in *Sapap3* KO mice was investigated using pharmacologic and biochemical approaches. Finally, sufficiency of mGluR5 to drive OCD-like behavior in wild-type mice was tested by potentiating mGluR5 with a positive allosteric modulator.

RESULTS: Excessive mGluR5 signaling underlies OCD-like behaviors and striatal circuit abnormalities in *Sapap3* KO mice. Accordingly, enhancing mGluR5 activity acutely recapitulates these behavioral phenotypes in wild-type mice. In *Sapap3* KO mice, elevated mGluR5 signaling is associated with constitutively active receptors and increased and imbalanced striatal output that is acutely corrected by antagonizing striatal mGluR5.

CONCLUSIONS: These findings demonstrate a causal role for increased mGluR5 signaling in driving striatal output abnormalities and behaviors with relevance to OCD and show the tractability of acute mGluR5 inhibition to remedy circuit and behavioral abnormalities.

Keywords: Circuit, Constitutive activity, mGluR5, Obsessive-compulsive disorder, Positive allosteric modulator, Striatum

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Obsessive-compulsive disorder (OCD) is a debilitating neuropsychiatric disorder affecting 1%–3% of the population worldwide (1,2). Numerous studies in both patient populations and mouse models indicate that corticostriatothalamocortical circuit dysfunction, particularly increased activity in the striatum (3–7), drives the behavioral manifestations of OCD. However, the molecular mechanisms underlying this disorder remain largely unknown.

Mice with genetic deletion of *Sapap3* (a postsynaptic scaffold protein gene, also known as DLGAP3/GKAP3) provide a relatively unique opportunity to study the molecular mechanisms underlying OCD-relevant behaviors. *Sapap3* knockout (KO) mice demonstrate several OCD-like phenotypes, including increased striatal activity (4), increased anxiety-like behaviors (8), and excessive and pathologic self-grooming that persists despite causing harmful facial lesions (8). OCD-like behaviors in *Sapap3* KO mice are treated by chronic fluoxetine (8), a first-line treatment for OCD, and several human genetic studies provide additional, although modest, support for construct validity (9–11). Finally, selective restoration of *Sapap3* expression in the striatum prevents the self-grooming and anxiety phenotypes of *Sapap3* KO mice (8), a finding that connects brain regions implicated by human studies (5–7) to the expression of OCD-like behaviors in this mouse model.

Previously, we demonstrated that a number of excitatory synaptic abnormalities in the dorsolateral striatum of *Sapap3* KO mice arise from overactive type 5 metabotropic glutamate receptor (mGluR5) signaling (12,13), leading us to hypothesize

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that excessive mGluR5 signaling drives OCD-like behavioral and circuit phenotypes. Indeed, mGluR5 antagonists are efficacious in reducing anxiety-like and repetitive behaviors in mouse models (14–16). However, the diversity of signaling pathways targeted by drugs with demonstrated efficacy [e.g., selective serotonin reuptake inhibitors (3,8), *N*-methyl-D-aspartate–type glutamate receptor antagonists (17), mGluR4 positive allosteric modulators (18), benzodiazepines and gamma-aminobutyric acid type A receptor agonists (19), as well as a number of treatments with unspecified or atypical mechanisms of action (20–22)] highlights the complexity of inferring molecular mechanism from treatment response, especially when the mechanism of the behavioral mouse model itself is unknown (14,15). In this study, we sought to establish whether increased mGluR5 signaling plays a causal role in driving OCD-relevant phenotypes.

METHODS AND MATERIALS

Animals

All animal procedures were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee of Duke University. Generation of *Sapap3* KO, *Grm5* KO, and line 6 *Drd1a*-tdTomato transgenic mice has been previously described (8,23,24).

Behavioral Experiments

Repetitive self-grooming and anxiety-like behaviors were assessed using open field (OF), light-dark emergence (LDE), and elevated zero maze (EZM) tests using methods previously described (25,26) (Supplement).

In Vivo Recordings

Mice were surgically implanted with microarray recording electrodes using previously described methods (27). Briefly, 32 electrodes were implanted bilaterally into the dorsal striatum. After a recovery period (minimum of 3 weeks), neurophysiologic recordings were performed in an empty cage similar to the home cage. Complete details are available in the Supplement.

Slice Electrophysiology

Acute coronal brain slices (300 μm) were obtained for electrophysiologic recordings as previously described (13). *Drd1a*-tdTomato transgene expression was used to distinguish between striatal projections belonging to the direct (fluorescent) and indirect (nonfluorescent) pathways (23). Whole-cell patch-clamp recordings obtained at 23°C–25°C were used to measure excitatory postsynaptic currents using methods described in the Supplement.

Calcium Imaging

Slices were prepared, and calcium imaging experiments were performed and analyzed as described in O'Hare *et al*. (28). Briefly, acute parasagittal (300 μm) slices, corresponding to tissue approximately 300–900 μm medial to the first visible lateral aspect of dorsal striatum, were bulk loaded with the calcium indicator dye, Fura-2 AM (F-1221; Thermo Fisher Scientific, Waltham, MA) (Supplement).

Slices were transferred to the microscope chamber and continuously perfused with imaging solution containing (in mmol/L) 124 NaCl, 4.5 KCl, 1.2 NaH_2PO_4, 4 $CaCl_2$, 1 $MgCl_2$, 26 $NaHCO_3$, and 10 D-glucose. Fluorescence images were acquired using a Ti:Sapphire laser (Chameleon Ultra I; Coherent) and Prairie View image acquisition software (Bruker Corp., Billerica, MA). To monitor action potentials (APs) with single-cell resolution, we performed vector line-scan imaging of the Fura-2 signal excited by 770 nm light. A concentric bipolar stimulating electrode (FHC, Inc., Bowdoin, ME) was placed on the cortical edge of the corpus callosum (Figure 2A), and slices were subjected to a series of extracellular stimuli ranging from subthreshold to suprathreshold intensities delivered in 10 single square pulses at 0.05 Hz. For additional image acquisition and analysis details, see the Supplement.

Co-immunoprecipitation and Western Blotting

Striata were dissected from wild-type (WT) and *Sapap3* KO mice and quickly frozen over dry ice. Tissue was solubilized in co-immunoprecipitation buffer (50 mmol/L Tris, pH 7.4, 120 mmol/L NaCl, 1% Triton X-100), and the soluble lysate (200 μg of protein) was tumbled overnight at 4°C with 1 μg of anti-Homer antibody (D-3 sc-17842; Santa Cruz Biotechnology, Dallas, TX), which recognizes the long but not the short Homer 1a isoform (KM Huber, Ph.D., unpublished observations, June 2011) or mouse immunoglobulin G (sc-2025; Santa Cruz Biotechnology). Protein A/G agarose bead slurry (No. 20421; Thermo Scientific) was added for 1 additional hour, and the beads were washed with co-immunoprecipitation buffer. Western blotting was performed using primary polyclonal antibodies that recognize either mGluR5 (AB5675; Millipore, Temecula, CA) or Homer (E-18 sc-8921; Santa Cruz Biotechnology).

Statistical Analysis

Two-way repeated-measures analysis of variance and unpaired *t* tests were used to determine statistical significance for behaviors in the OF, LDE, and EZM. Values outside three standard deviations were considered outliers and were excluded from analysis [one value for KO vehicle and two values for KO 3-((2-methyl-1,3-thiazol-4-yl)ethynyl)pyridine hydrochloride (MTEP) in the LDE test]. The effects of drug and vehicle on the in vivo striatal neuron firing rates were determined using one-sample and unpaired *t* tests. Unpaired Student *t* tests were used to evaluate drug effects on miniature excitatory postsynaptic currents (mEPSCs). Two-way analysis of variance was used to compare conditions for input-output experiments. Unpaired Student *t* tests were used to determine statistical significance for the Western blot experiments.

RESULTS

mGluR5 Antagonism Reduces OCD-like Phenotypes of *Sapap3* KO Mice

To determine the significance of mGluR5 signaling for the expression of OCD-relevant phenotypes, we first evaluated the effects of the short-acting mGluR5 negative allosteric modulator (NAM), MTEP, on the increased self-grooming and