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Putative Microcircuit-Level Substrates for Attention Are Disrupted in Mouse Models of Autism

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ABSTRACT

BACKGROUND: Deep layer excitatory circuits in the prefrontal cortex represent the strongest locus for genetic convergence in autism, but specific abnormalities within these circuits that mediate key features of autism, such as cognitive or attentional deficits, remain unknown. Attention normally increases the sensitivity of neural populations to incoming signals by decorrelating ongoing cortical circuit activity. Here, we investigated whether mechanisms underlying this phenomenon might be disrupted within deep layer prefrontal circuits in mouse models of autism.

METHODS: We isolated deep layer prefrontal circuits in brain slices then used single-photon GCaMP imaging to record activity from many (50 to 100) neurons simultaneously to study patterns of spontaneous activity generated by these circuits under normal conditions and in two etiologically distinct models of autism: mice exposed to valproic acid in utero and *Fmr1* knockout mice.

RESULTS: We found that modest doses of the cholinergic agonist carbachol normally decorrelate spontaneous activity generated by deep layer prefrontal networks. This effect was disrupted in both valproic acid-exposed and *Fmr1* knockout mice but intact following other manipulations that did not model autism.

CONCLUSIONS: Our results suggest that cholinergic modulation may contribute to attention by acting on local cortical microcircuits to decorrelate spontaneous activity. Furthermore, defects in this mechanism represent a microcircuit-level endophenotype that could link diverse genetic and developmental disruptions to attentional deficits in autism. Future studies could elucidate pathways leading from various etiologies to this circuit-level abnormality or use this abnormality itself as a target and identify novel therapeutic strategies that restore normal circuit function.

Keywords: Acetylcholine, Calcium imaging, Fragile X syndrome, GCaMP, Prefrontal cortex, Valproic acid

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Autism reflects disparate genetic and environmental causes, suggesting that common behavioral phenotypes may reflect convergent defects at the level of neuronal circuits controlling these behaviors. Consistent with this hypothesis, recent analyses have revealed that several genes linked to autism are co-expressed within deep layer microcircuits in the prefrontal cortex (PFC) (1,2). Identifying convergent abnormalities located within these microcircuits would yield attractive targets for future circuit-based therapeutic interventions. Until recently, a major barrier to identifying such circuit-level abnormalities has been recording from large numbers of neurons simultaneously. However, advances in imaging and electrophysiological techniques have made it possible to characterize patterns of circuit-level activity by, for example, calculating correlations between neurons (3,4). Despite these advances, cognitive processes are associated with complex neural dynamics embedded within a high dimensional state space. Thus, to reveal defects in circuit-level activity that are associated with disorders such as autism, it is valuable to first identify specific neuronal correlates or signatures for relevant cognitive processes. Identifying these signatures makes it possible to evaluate whether they are altered in the setting of diseases such as autism. Any such alterations represent putative pathophysiological mechanisms contributing to cognitive dysfunction in these disease states.

Here, we set out to identify possible microcircuit-level abnormalities associated with autism. Our approach was to look for convergent abnormalities that are 1) conserved across multiple, etiologically distinct models of autism; and 2) impact neural correlates of cognitive processes that are known to be abnormal in autism. In particular, two recent studies identified a possible neural signature of attention using multi-neuronal recording in monkeys performing a visuospatial attention task (5,6). Both studies observed the same result: during the attended portion of the task, the pairwise correlations between cortical neurons decreased. This decorrelation would reduce the overall noise of an output signal that was composed of a sum across the population—in fact, this pairwise decorrelation accounted for 80% of the total improvement in the signal-to-noise ratio (6). This phenomenon is hypothesized to reflect the decorrelation of spontaneous network activity by neuromodulation. In particular, cholinergic modulation plays a well-established role in attention and cortical decorrelation in vivo (7). Nevertheless, it remains unknown whether cholinergic

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modulation can induce such decorrelations by acting directly on cortical microcircuits and, if so, whether defects in this mechanism might be present in autism or other conditions with a high comorbidity of attentional deficits. Notably, in Fragile X syndrome (the most common known single gene cause of autism), 70% of affected children meet criteria for an attentional deficit (8,9). Overall, approximately 50% of children with autism also meet criteria for attention-deficit/hyperactivity disorder (10,11). Deficits in attention have also been observed in *Fmr1* knockout (KO) mice (12–14) and other mouse models of autism (15).

To examine how autism might affect a neural correlate of attention, we studied how cholinergic modulation affects spontaneous activity generated by deep layer prefrontal microcircuits under normal conditions and in mouse models of autism. We focused on microcircuits in deep layers of the PFC because cholinergic modulation within the PFC has been directly implicated in attention (16,17), and abnormalities associated with autism are likely to be intrinsic to deep layer prefrontal microcircuits (1). Therefore, we isolated these microcircuits in brain slices and used single-photon, wide-field GCaMP imaging (18) to measure spontaneous activity in many neurons (50 to 100) at once. As described below, we found that cholinergic modulation can indeed act directly on cortical microcircuits to decorrelate spontaneous activity, mimicking the neural signature previously linked to attention in vivo. Furthermore, this decorrelation, which represents a possible neural substrate for attention, is defective in two etiologically distinct mouse models of autism.

METHODS AND MATERIALS

All experiments were conducted in accordance with procedures established by the Administrative Panels on Laboratory Animal Care at the University of California, San Francisco.

Subjects

Postnatal day P26 to P33 mice of either sex (Charles River, Wilmington, Massachusetts) were injected unilaterally with 500 nL of AAV5/2—synapsin::GCaMP6s (University of North Carolina at Chapel Hill viral vector core) at the coordinates (in mm): 1.7 anterior-posterior, .3 mediolateral, and −2.2 dorsoventral. Experiments studying the valproic acid (VPA) model of autism used C57BL/6 mice whose pregnant mothers had been injected with a single dose of VPA (500 mg/kg, intraperitoneal) at embryonic day E10.5. For these experiments, control mice were C57BL/6 mice whose pregnant mothers had been injected with saline at E10.5. VPA solution was prepared by dissolving VPA in .9% saline to a final concentration of 150 mg/mL. Experiments studying a mouse model of Fragile X syndrome used male *Fmr1* wild-type (WT) or KO mice on a FVB background (Jackson Labs, Bar Harbor, Maine). In some cases (active artificial cerebrospinal fluid [ACSF] cohorts), these *Fmr1* WT and KO mice were littermates, while others (carbachol cohorts) were not.

For some experiments, C57BL/6 mice were treated with fluoxetine based on a previously described protocol (19). Fluoxetine was administered (5 mg kg^{-1}, intraperitoneal) once daily for 6 days before imaging with the final injection coming 24 to 48 hours before imaging. Dominant negative disrupted in schizophrenia 1 (DISC1) mutant mice were generated by crossing B6-CamKII::TtA (JAX: 00310) mice with tetO-*DISC1*dn (JAX: 008790) to yield mice expressing dominant negative DISC1 in neocortical pyramidal cells.

Slice Preparation

In all cases, 350-micron thick coronal slices were prepared from these animals 15 to 27 days after injection (6 to 8 weeks of age). Slice preparation followed our previously described protocol (20) with one deviation: immediately after brain slices were prepared, they were transferred to an N-methyl-D-glucamine (NMDG)-based recovery solution for 10 minutes before being transferred to ACSF for the remainder of their recovery (21). The NMDG-based solution was maintained at 32°C and consisted of the following (in mmol/L): 93 NMDG, 93 hydrogen chloride, 2.5 potassium chloride (KCl), 1.2 sodium dihydrogen phosphate, 30 sodium bicarbonate, 25 glucose, 20 HEPES, 5 Na-ascorbate, 5 Na-pyruvate, 2 thiourea, 10 magnesium sulfate, and .5 calcium chloride (CaCl). This NMDG preparation method was used to improve the overall health of adult slices to ensure sufficient amounts of activity for analysis. ACSF contained the following (in mmol/L): 126 sodium chloride, 26 sodium bicarbonate, 2.5 KCl, 1.25 sodium dihydrogen phosphate, 1 magnesium chloride, 2 CaCl, and 10 glucose. All recordings were at 32.5 ± 1°C. Active ACSF was identical to normal ACSF except with elevated KCl (3.5 mmol/L vs. 2 mmol/L) and reduced CaCl (1.2 mmol/L vs. 2 mmol/L).

Imaging

GCaMP imaging was performed on an Olympus BX51 upright microscope with a 20× 1.0NA water immersion lens, .5× reducer (Olympus, Tokyo, Japan), and ORCA-ER CCD Camera (Hamamatsu Photonics, Hamamatsu, Japan). Illumination was delivered using a Lambda DG4 arc lamp (Sutter Instruments, Novato, California). Light was delivered through a 472/30 excitation filter, 495 nm single-band dichroic, and 496 nm long pass emission filter (Semrock, Rochester, New York).

All movies that were analyzed consisted of 36,000 frames acquired at 10 Hz (1 hour) with 4 × 4 sensor binning yielding a final resolution of 256 × 312 pixels. Light power during imaging was 100 to 500 $\mu W/mm^2$. The Micro Manager software suite (v1.4, National Institutes of Health, Bethesda, Maryland) was used to control all camera parameters and acquire movies. Any movies that had significant drift (greater than ∼.25 soma diameters), movement, or lacked significant amounts of activity were excluded from further analysis. Significant movement could be detected during independent components analysis by the appearance of elliptical rather than circular segments.

We observed that active, GCaMP-expressing neurons were found within a discrete layer (Figure 1A) consistent with the location of layer 5 (L5) in the medial prefrontal cortex.

Signal Extraction

All analyses and signal extraction were performed using MATLAB (The Mathworks, Natick, Massachusetts). Locations of cells were automatically identified using a modified version