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Cell-Specific Regulation of *N*-Methyl-D-Aspartate Receptor Maturation by Mecp2 in Cortical Circuits

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ABSTRACT

BACKGROUND: Early postnatal experience shapes *N*-methyl-D-aspartate receptor (NMDAR) subunit composition and kinetics at excitatory synapses onto pyramidal cells; however, little is known about NMDAR maturation onto inhibitory interneurons.

METHODS: We combined whole-cell patch clamp recordings ($n = 440$) of NMDAR-mediated currents from layer-4-to-layer-2/3 synapses onto pyramidal and green fluorescent protein labeled parvalbumin-positive (PV) interneurons in visual cortex at three developmental ages (15, 30, and 45 postnatal days) with array tomography three-dimensional reconstructions of NMDAR subunits GluN2A- and GluN2B-positive synapses onto PV cells.

RESULTS: We show that the trajectory of the NMDAR subunit switch is slower in PV interneurons than in excitatory pyramidal cells in visual cortex. Notably, this differential time course is reversed in the absence of methyl-CpG-binding protein, MECP2, the molecular basis for cognitive decline in Rett syndrome and some cases of autism. Additional genetic reduction of GluN2A subunits, which prevents regression of vision in *Mecp2*-knockout mice, specifically rescues the accelerated NMDAR maturation in PV cells.

CONCLUSIONS: We demonstrate 1) the time course of NMDAR maturation is cell-type specific, and 2) a new cell-type specific role for Mecp2 in the development of NMDAR subunit composition. Reducing GluN2A expression in *Mecp2*-knockout mice, which prevents the decline in visual cortical function, also prevents the premature NMDAR maturation in PV cells. Thus, circuit-based therapies targeting NMDAR subunit composition on PV cells may provide novel treatments for Rett syndrome.

Keywords: Development, GluN2A, GluN2B, Parvalbumin, Rett syndrome, Visual cortex

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N-methyl-D-aspartate receptors (NMDARs) are critical for many forms of learning and memory, in part due to their activity dependence and contribution to synaptic integration and plasticity (1). The GluN2 subunit composition determines the decay kinetics of the NMDARs, which undergo an experience-dependent switch from GluN2B to GluN2A at cortical synapses during early postnatal development (2). This NMDAR subunit switch has been well studied at excitatory synapses onto pyramidal cells; however, whether a similar process underlies the development of excitatory synapses onto inhibitory interneurons in local cortical circuits remains largely unknown (3,4).

Parvalbumin positive (PV) interneurons are of particular interest, as they mature postnatally in an activity-dependent manner, directly control the firing of pyramidal cells, and trigger critical period plasticity (5,6). PV interneurons are also vulnerable across neurodevelopmental and psychiatric disorders (4,6). Both NMDARs and PV interneurons have been implicated in the pathophysiology of Rett syndrome, a childhood disorder marked by regression of cognitive and motor function after an apparently normal initial development (7,8). Most cases of Rett syndrome are caused by loss-of-function mutations in *MECP2*, a transcriptional regulator, and *Mecp2*-knockout (KO) mice also show a decline in behavioral and motor function including loss of vision (7,9). NMDAR dysfunction has also been implicated in postmortem studies of Rett syndrome (10); however, there has been disagreement among studies, primarily from tissue homogenates of *Mecp2*-KO mice, as to the nature of the dysregulation (7,11–13). Elucidating the role of NMDARs in Rett syndrome may have important clinical implications, as the visual cortical decline, along with PV circuit hyperconnectivity, found in *Mecp2*-KO mice can be prevented by an additional genetic manipulation to decrease GluN2A expression (7). The mechanism underlying such rescue is still unknown.

We utilized a circuit-based approach to investigate the NMDAR maturation in PV cells in wild-type (WT) mice and a Rett syndrome mouse model. We performed whole-cell patch clamp recordings in acute brain slices and array tomography analysis to characterize NMDAR at layer-4-to-layer-2/3 visual cortical synapses and directly compared the development of NMDAR-mediated synaptic currents from pyramidal neurons and PV interneurons. We discovered that NMDAR maturation occurs more gradually in PV than pyramidal cells and that Mecp2 regulates the NMDAR subunit switch in a cell-type specific manner. Moreover, we reveal correction of NMDAR maturation in PV cells as a possible mechanism for the rescue of vision when reducing GluN2A expression in *Mecp2*-KO mice.

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METHODS AND MATERIALS

Animals

The electrophysiology data in this study includes a total of 440 whole-cell recordings (222 pyramidal and 218 PV cells) from 157 mice (20 *Mecp2*-KO, 32 *Mecp2*-WT, 18 *Mecp2*-KO/*GluN2A*-Heterozygous [Het], 13 *Mecp2*-WT/*GluN2A*-Het, 26 *Mecp2*-KO/PV-green fluorescent protein [GFP]+, 27 *Mecp2*-WT/PV-GFP+, 11 *Mecp2*-KO/*GluN2A*-Het/PV-GFP+, 10 *Mecp2*-WT/*GluN2A*-Het/PV-GFP+) in three age groups: postnatal day (P)15 (P13–P17), P30 (P28–P31), and P45 (P45–P51); P30 and P45 for *GluN2A*-Het mice. Male *Mecp2*-KO and *Mecp2*-WT littermates were used from in-house breeding pairs of *Mecp2*-Het female mice [mouse line generated by A. Bird *et al.*, 2001, University of Edinburgh, United Kingdom (14)] crossed with C57BL/6 male mice or C57BL/6 mice that selectively expressed GFP in PV interneurons [from H. Monyer, 2002, University of Heidelberg, Germany (15)]. Double mutants for Mecp2 and GluN2A deletion were generated in-house by crossing *Mecp2*-Het female mice with *GluN2A*-KO male mice [from M. Mishina, 1995, University of Tokyo, Japan (16)]. Triple mutants for Mecp2, GluN2A, and PV-GFP were generated in-house by first crossing *GluN2A*-KO female mice with PV-GFP+ male mice and using either the *GluN2A*-KO or *GluN2A*-Het/PV-GFP+ male offspring to cross with the *Mecp2*-Het female mice. All procedures were approved by Boston Children's Hospital Institutional Animal Care and Use Committee.

Electrophysiology

Coronal visual cortical slices (300 μm thick) were prepared after decapitation under isoflurane anesthesia. Slices were incubated at room temperature in a submerged holding chamber for at least 1 hour in artificial cerebral spinal fluid containing: 119 mM sodium chloride, 2.5 mM potassium chloride, 1 mM sodium dihydrogen phosphate monohydrate, 1.3 mM magnesium chloride hexahydrate, 2.5 mM calcium chloride, 26.2 mM sodium bicarbonate, 20 mM glucose, and bubbled with carbogen gas (95% oxygen/5% carbon dioxide). All recordings were made in the presence of 5 μM bicuculline methiodide (Sigma, St. Louis, Missouri), which was sufficient to block gamma-aminobutyric acid (GABA)-ergic currents (17). To inhibit NMDA receptors containing the GluN2B subunit, slices were incubated in artificial cerebrospinal fluid with 3 μM ifenprodil (Sigma), a GluN2B subunit selective antagonist, for at least 40 minutes before and during recording (17,18). All recordings were made at room temperature.

Voltage-clamp recordings from layer 2/3 pyramidal and PV cells were made under visual guidance by infrared differential interference contrast microscopy. Patch pipettes (4–6 MΩ) were pulled from standard wall borosilicate tubing and were filled with intracellular solution containing: 140 mM cesium chloride, .2 mM ethylene glycol tetraacetic acid (EGTA), 10 mM 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), 2 mM adenosine triphosphate-magnesium, .3 mM guanosine triphosphate, 5 mM QX-314, and 5 mg/mL biocytin (pH 7.2). All chemicals were purchased from Sigma. Cells were identified under visual guidance with by their location, morphology, and in the case of GFP-expressing PV cells, by their fluorescence under ultraviolet light. In most cells, identity was also confirmed by recording a brief spike train in current clamp immediately after patching. In addition, 58 cells were histologically processed for biocytin labeling postrecording using Alexa594-conjugated streptavidin antibody for pyramidal cells and Alexa 488-conjugated streptavidin antibody for PV cells. Excitatory postsynaptic currents (EPSCs) were evoked by extracellular stimulation (.2 ms, 16–100 μA) in layer 4 of the visual cortex using a bipolar stimulating pipette pulled from standard wall borosilicate tubing and filled with artificial cerebrospinal fluid. Recordings began once the amplitude of the stimulating current and position of the electrode were adjusted to evoke a reliable monosynaptic response at a holding potential of −100 mV.

Voltage-clamp recordings were acquired at a 10 kHz sampling rate with an AxoPatch-1D (Axon Instruments, Union City, California) and an ITC-18 AD board (Instrutech, Mineola, New York). All data were acquired and analyzed using custom-made procedures in Igor-Pro Software (WaveMetrics, Lake Oswego, Oregon). The series resistance was monitored throughout the recordings. Cells were excluded in which the access resistance changed by more than 10%. Five evoked synaptic responses were recorded and used to calculate an average response at each postsynaptic holding potential from −100 mV to +60 mV. To estimate the relative contribution of NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors to the synaptic current, the current amplitude was measured at the time of the peak amplitude at −100 mV for the AMPA receptor-mediated current and at the time point after the current at −100 mV had decayed to less than <5% of its peak amplitude for the NMDA receptor-mediated current (Figure 1A), based on methods from Hestrin *et al.* (19) and Mierau *et al.* (17). NMDAR and AMPAR components were confirmed pharmacologically with 20 μM CPP and 10 μM CNQX (Supplemental Figure S1). Current-voltage plots were made with the current amplitudes for the NMDA and AMPA receptor-mediated components as a function of the holding potential (Figure 1B). The NMDAR/AMPAR ratio was calculated as the ratio of the slopes between 0 and 40 mV, where the slopes of the NMDA and AMPA receptor-mediated components were most linear. Decay time constants were estimated using a single exponential fit, which provided an adequate fit (17) (Figure 1C, D). All values are reported as mean ± SEM, unless otherwise stated. Parametric testing for normality followed by analysis of variance with post hoc *t* tests were used to compare individual groups using JMP software (SAS, Cary, North Carolina).

Array Tomography

The tissue was processed for array tomography as described in Micheva and Smith (20). Visual cortex was dissected from perfused animals and embedded in LR White resin using a bench top protocol. Ribbons with between 70 and 100 serial 70 nm ultrathin sections were prepared from P30 *Mecp2*-KO, WT, and *Mecp2*-KO/GluN2A-Het mice using an ultramicrotome (UC7, Leica Microsystems, Wetzler, Germany) and mounted side by side on subbed glass coverslips. Coverslips were immunostained using synapsin I (mouse; SYSY,