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Experience-dependent and differential regulation of local and long-range excitatory neocortical circuits by postsynaptic *Mef2c*

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Abstract

Development of proper cortical circuits requires an interaction of sensory experience and genetic programs. Little is known of how experience and specific transcription factors interact to determine the development of specific neocortical circuits. Here we demonstrate that the activity-dependent transcription factor, Myocyte enhancer factor-2C (*Mef2c*), differentially regulates development of local vs. long-range excitatory synaptic inputs onto layer 2/3 neurons in the somatosensory neocortex *in vivo*. Postnatal, postsynaptic deletion of *Mef2c* in a sparse population of L2/3 neurons suppressed development of excitatory synaptic connections from all local input pathways tested. In the same cell population, *Mef2c* deletion promoted the strength of excitatory inputs originating from contralateral neocortex. Both the synapse promoting and synapse suppressing effects of *Mef2c* deletion required normal whisking experience. These results reveal a role of *Mef2c* in experience-dependent development of specific sensory neocortical circuits.

INTRODUCTION

Sensory experience and experience-driven neuronal activity are required for development of proper synaptic connectivity of neocortical circuits (Fox, 2002). Activity-regulated transcription factors and their transcripts are hypothesized to contribute to experience-dependent development and refinement of synaptic connections (West and Greenberg, 2011). In support of this idea, the activity-regulated transcription factors, NPAS4 and Myocyte Enhancer Factor-2 (MEF2) regulate inhibitory and excitatory synapse number onto developing hippocampal neurons, respectively (West and Greenberg, 2011). In response to environmental enrichment, NPAS4 differentially regulates inhibitory synapses arising from distinct input pathways onto postsynaptic hippocampal CA1 neurons (Bloodgood et al.,

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Investigation: K.E.R., K.W.L., C.F.H., and C.H. Methodology and software: K.E.R. and J.R.G. Conceptualization and writing: K.E.R., J.R.G., and K.M.H. Funding, project administration, supervision: J.R.G. and K.M.H.

2013). Whether a transcription factor regulates experience-dependent and input-specific development of excitatory circuits is unknown.

Of the family of MEF2 transcription factors (MEF2A-D), MEF2C is the most highly expressed in the neocortex, and MEF2A/D are present at lower levels (Lyons et al., 2012; Potthoff and Olson, 2007). Expression of a constitutively active MEF2C eliminates functional and structural excitatory synapses in cultured hippocampal neurons and *in vivo* (Cole et al., 2012; Flavell et al., 2006; Pfeiffer et al., 2010). Consistent with this finding, embryonic and postnatal deletion of *Mef2c* in forebrain results in increased excitatory synapse function and dendritic spines *in vivo* in granule cells of the hippocampal dentate gyrus (Adachi et al., 2015; Barbosa et al., 2008) and is necessary for synapse elimination in response to extracellular signals (Elmer et al., 2013). In contrast, embryonic brain wide deletion of *Mef2c* reduces synaptic strength onto CA1 and layer 5 neocortical neurons (Li et al., 2008). Therefore, the effects of MEF2C on excitatory synapse development may depend on the circuit examined, and/or some effects are due to indirect or non-cell autonomous effects of *Mef2c* deletion. Here we demonstrate that MEF2C in postsynaptic layer (L) 2/3 pyramidal neurons of somatosensory barrel cortex cell-autonomously and differentially regulates experience-dependent development of local and long-range excitatory input pathways. To this end, MEF2C promotes connectivity of local excitatory inputs onto L2/3 neurons but functions to suppress the strength of inputs from long-range, contralateral neocortical regions. Our results reveal new and unexpected roles for MEF2 genes in regulation of specific synaptic inputs and provide key molecular insight into experience-dependent development of neocortical circuits.

RESULTS

Postsynaptic *Mef2c* differentially regulates excitatory synaptic function from vertical and horizontal input pathways onto neocortical L2/3 pyramidal neurons

To determine the cell-autonomous role of MEF2 genes in development of neocortical circuits, we deleted the major *Mef2* genes in a sparse (1–5%) population of neocortical neurons by injecting AAV-Cre-GFP into the ventricles of mice floxed ($^{fl/fl}$) for either *Mef2c* or *Mef2a* and *Mef2d* at postnatal day 1 (P1). Because *Mef2c* is the most abundant *Mef2* gene expressed in neocortex, it was the initial focus of our study. AAV-Cre-GFP infection in dissociated neocortical cultures revealed a complete knockdown of *Mef2c* mRNA after 7 days of infection as measured with quantitative RT-PCR (% decrease in *Mef2c* mRNA after 4 days: 85%, 7 days: >99%). Acute slices containing the barrel cortex were prepared at P18–25: a period of robust neocortical synapse proliferation (Stern et al., 2001). Simultaneous whole-cell voltage clamp recordings were performed in Cre-GFP(+); $Mef2c^{fl/fl}$ or “MEF2C KO” neurons and neighboring uninfected, GFP(−); $Mef2c^{fl/fl}$ or “WT” L2/3 pyramidal neurons (Fig. 1A,B). Sparse, postnatal deletion of *Mef2c* allowed us to determine the cell-autonomous, postsynaptic role of MEF2C on synapse development while reducing confounds related to the effects *Mef2c* on migration and laminarization of neocortical neurons (Li et al., 2008).

First, we measured spontaneous or miniature (m) EPSCs in TTX to measure the total functional excitatory synaptic inputs onto WT and MEF2C KO neurons. Consistent with a

role of *Mef2c* in synapse elimination, *Mef2c* deletion robustly increased mEPSC frequency (65%) and amplitude (10%; Fig. 1C). A rank-order distribution revealed that mEPSC amplitudes in MEF2C KO neurons are increased by 1.2-fold in comparison to WT, suggesting a general scaling up of synaptic strength (Fig. S1A) (Turrigiano, 2011) in addition to the increased frequency of mEPSCs. L2/3 pyramidal neurons in the barrel cortex receive the strongest input from L4 axons within the same barrel column (Feldmeyer, 2012; Shepherd et al., 2003). To assess evoked excitatory synaptic transmission on MEF2C KO neurons, we electrically stimulated L4 within the same barrel (L4 → L2/3; Fig. 1D_1–D_2). Surprisingly, and in contrast to the observed increase in mEPSCs, evoked L4 → L2/3 EPSCs were strongly decreased (>50%) on MEF2C KO neurons, in comparison to neighboring WT neurons. Short-term plasticity of L4 → L2/3 evoked EPSCs was unaffected in MEF2C KO neurons (Fig. 1D_3), suggesting the decrease in evoked EPSCs is not due to reduced presynaptic release probability. Heterozygous deletion of *Mef2c* by expressing AAV-Cre-GFP in *Mef2c*$^{+/fl}$ mice had no effect on evoked L4 → L2/3 EPSCs, revealing that one allele of *Mef2c* is sufficient to maintain L4 → L2/3 synaptic transmission (Fig. S1B) and that AAV-Cre-GFP expression does not affect EPSCs. Inhibitory synaptic transmission, as measured by evoked and spontaneous mIPSCs, was unaffected in MEF2C KO neurons (Fig. S1C,D), indicating that *Mef2c* cell-autonomously and selectively regulates excitatory synaptic transmission. In contrast to *Mef2c*, deletion of both *Mef2a* and *Mef2d*, by injecting AAV-Cre-GFP in *MEF2a* $^{fl/fl}$/*d*$^{fl/fl}$ mice, did not affect the amplitude of evoked L4 → L2/3 EPSCs, short-term plasticity of evoked EPSCs, or mEPSC amplitude, while mEPSC frequency was slightly reduced (Fig. S2A–B). Deletion of *Mef2c* or *Mef2a/d* did not affect input resistance and minimally affected resting membrane potential (Table S1). These results indicate a primary and specific role of *Mef2c* in development of excitatory neocortical synaptic circuits.

To reconcile the opposing effects of *Mef2c* deletion on mEPSCs and evoked L4 → L2/3 responses, we hypothesized that *Mef2c* differentially regulates distinct input pathways to L2/3 neurons. To test this possibility, we evoked EPSCs onto WT and MEF2C KO neuron pairs with extracellular electrical stimulation of adjacent L2/3 through which horizontally projecting axons from both local and long-range intercortical pathways pass (Petreanu et al., 2007; Petrus et al., 2015) (Fig. 1E_1). In contrast to that observed with stimulation of vertical L4 inputs, EPSCs evoked from adjacent L2/3 were unaffected in MEF2C KO neurons in comparison to neighboring WT neurons (Fig. 1E_2–1E_3). This result revealed an input-specific effect of *Mef2c* deletion.

Mef2c promotes development of local excitatory inputs and suppresses long-range excitatory inputs onto L2/3 pyramidal neurons

Our data suggest that *Mef2c* deletion differentially regulates vertical versus horizontal excitatory inputs onto individual L2/3 pyramidal neurons. In the barrel cortex, L2/3 pyramidal neurons receive major input from local synaptic pathways within and across adjacent barrel columns (Feldmeyer, 2012; Shepherd et al., 2003). To determine if *Mef2c* deletion selectively regulates synaptic function from other neocortical input pathways, we performed laser scanning photostimulation (LSPS) to uncage glutamate and map the source and strength of monosynaptic connections from local synaptic input pathways onto

simultaneously recorded WT and MEF2C KO L2/3 neurons (Shepherd et al., 2003). LSPS maps spanned three barrel columns allowing measurement of synaptic connectivity from different layers onto WT and MEF2C KO L2/3 neurons from the same and adjacent columns. LSPS-evoked EPSCs onto both WT and MEF2C KO neurons were spatially preserved and transformed into a color map where the pixel color represents the mean synaptic strength at each stimulation location (Fig. 2A–C). Short-latency responses in which glutamate was directly uncaged onto the dendrites of the recorded neurons (i.e. direct responses) were excluded from analysis (black pixels; Fig. 2B,D). LSPS maps acquired from individual neurons were superimposed by genotype and aligned with respect to the barrels and *pia mater*. Finally, average maps representing all recorded WT and MEF2C KO L2/3 neurons were constructed (Fig. 2D). Vertical and horizontal profiles of synaptic input revealed a weakening of all local intracolumnar and transcolumnar excitatory input pathways onto MEF2C KO L2/3 neurons (Fig. 2E–F), specifically: vertical L4 → L2/3, adjacent L4 → L2/3, adjacent L2/3 → L2/3, vertical L5A → L2/3, and adjacent L5A → L2/3 (Fig. 2G–K). Therefore, *Mef2c* promotes the development and function of multiple local excitatory synaptic input pathways onto L2/3 neurons originating from different layers and adjacent columns.

LSPS mapping results revealed that all local inputs from adjacent L2/3 onto MEF2C KO neurons are weak (Fig. 2I). In contrast, inputs assessed by electrical stimulation of horizontal L2/3 projecting axons were unaffected (Fig. 1E), and mEPSCs were enhanced in MEF2C KO neurons (Fig. 1C). Therefore, other input pathways onto L2/3 neurons that are not assayed by LSPS may be either unaffected or potentiated by *Mef2c* deletion. LSPS by glutamate uncaging only probes the strength of local circuits or inputs with an intact cell body and axon in the slice (Shepherd et al., 2003). Axons from long-range input pathways are severed during slice preparation, and therefore are not activated by LSPS. Because severed axons from long-range input pathways in the slice reseal and can release glutamate (Petreanu et al., 2007), they likely contribute to mEPSCs and electrically evoked EPSCs. We hypothesized that long-range input pathways are potentiated with postsynaptic *Mef2c* deletion and may contribute to the observed enhancement of mEPSCs. To study effects of *Mef2c* deletion on long-range intercortical inputs in isolation, we expressed Channelrhodopsin-2 (ChR2) in axons projecting from the contralateral somatosensory cortex (cS1). These long-range, trans-colossal L2/3 axons project abundantly and provide reliable inputs onto L2/3 pyramidal neurons with connectivity comparable to that from local L2/3 of ipsilateral barrel cortex (Petreanu et al., 2007). At P1, the lateral ventricle of $Mef2c^{fl/fl}$ mice was injected with AAV-Cre-GFP, to sparsely delete *Mef2c*, while AAV-ChR2-mCherry was stereotaxically injected into cS1 (Fig. 2L). Slices of barrel cortex contralateral to AAV-ChR2 injection were prepared at P18–25 and expressed mCherry-positive axons, and Cre-GFP+ (MEF2C KO) cell bodies (Fig. 2M). EPSCs were evoked by stimulating trans-callosal, ChR2-expressing axons onto pairs of WT and MEF2C KO L2/3 neurons with a brief 2-ms pulse of blue light. To isolate monosynaptic EPSCs from ChR2-expressing axons, experiments were performed in TTX. Remarkably, the amplitude of EPSCs evoked from cS1 inputs was increased by 80% in MEF2C KO neurons compared to WT neurons (Fig. 2N), demonstrating potentiation of long-range inputs from cS1. Taken together with results of LSPS experiments (Fig. 2D–K), these data suggest that *Mef2c* promotes the functional

development of local inputs while suppressing long-range intercortical inputs onto individual L2/3 pyramidal neurons.

MEF2C promotes excitatory synapse number from L4 → L2/3 pyramidal neurons but suppresses synaptic strength of long-range intercortical inputs

We sought to determine the synaptic basis of MEF2C regulation at specific input pathways. *Mef2c* deletion depressed L4 → L2/3 EPSCs but did not affect short-term synaptic plasticity, suggesting this weakening is mediated by either a selective decrease in AMPA receptor (R) function or synapse number. To differentiate between these possibilities, we measured NMDA-receptor EPSCs onto WT and MEF2C KO L2/3 neurons evoked by electrical stimulation of L4. Like AMPAR-EPSCs, NMDAR-EPSCs were reduced by ~50% in MEF2C KO neurons (Fig. 3A), suggesting a decrease in L4 → L2/3 synapse number. To further test this hypothesis, we measured L4-evoked AMPAR-EPSCs onto WT and MEF2C KO L2/3 neurons in strontium (Sr^{+2}). Sr^{+2} asynchronizes glutamate release and allows the resolution of quantal synaptic events (Petrus et al., 2015). Consistent with an effect on synapse number, *Mef2c* deletion decreased the frequency (~40%), but not amplitude of L4-evoked quantal events (Fig. 3B). In normal ACSF, we measured the coefficient of variance (C.V.) and rate of synaptic failures of L4 → L2/3 EPSCs onto pairs of WT and MEF2C KO neurons. C.V. and failure rate are inversely proportional to release probability and synapse number, and both were strongly increased in MEF2C KO neurons (Fig. 3C; D) in comparison to WT neurons. Taken together, the changes in NMDAR-EPSCs, Sr^{+2} event frequency, C.V., and synaptic failures, without changes in short-term plasticity, support a role for MEF2C in promoting the number of functional connections from L4 → L2/3 and possibly all local input pathways.

In contrast to L4 → L2/3 evoked EPSCs, the C.V. of EPSCs evoked by blue light stimulation of ChR2-expressing cS1 inputs was unaffected (Fig. 3F). This result suggests that the enhanced EPSCs from cS1 inputs on MEF2C KO neurons is likely not due to a change in synapse number or release probability but may be due to an increase in the strength of individual synapses. Consistent with an increase in the strength of cS1 synaptic inputs, the amplitude of light-evoked quantal events from ChR2-expressing cS1 axons in Sr^{+2} (Fig. $3E_1$) was enhanced on MEF2C KO in comparison to WT neurons (~32%; Fig. $3E_2$), and the frequency of events was unchanged. These results, together with the lack of a change in C.V. of cS1-mediated EPSCs, indicate that individual cS1 inputs are strengthened onto MEF2C KO neurons, while connectivity or release probability is not changed (Fig. $3E_3$).

The differential regulation of distinct input pathways onto L2/3 neurons by MEF2C may be based on the dendritic compartments contacted by these inputs. Basal dendrites of L2/3 neurons receive local inputs from L4 and L2/3 within barrel columns and from adjacent columns. Proximal apical dendrites also receive local inputs from L2/3. The distal apical “tufts” receive mostly long-range intercortical input from M1, M2, and S2 (Bosman et al., 2011). To determine if *Mef2c* alters morphology or spines on specific dendritic compartments, WT or MEF2C KO L2/3 neurons were filled with biocytin and processed for imaging. MEF2C KO neurons had normal dendritic branching and length (Fig. 3G) but

displayed a reduced spine density on basal, but not distal or proximal apical dendrites (Fig. 3H–I). These findings support functional results that MEF2C KO neurons have fewer synaptic inputs from local (L4) circuits.

Sensory experience is required for MEF2C-dependent development of both local and long-range input pathways onto L2/3 neurons

Sensory deprivation by whisker trimming depresses L4 → L2/3 strength (Shepherd et al., 2003; Bender et al., 2006), mirroring what we observe with *Mef2c* deletion (Fig. 2). MEF2C and sensory experience may interact in a common signaling pathway to promote synapse development onto L2/3 neurons. If so, then sensory deprivation may occlude or prevent effects of *Mef2c* deletion on L2/3 synaptic inputs. To test this possibility, we examined the effects of sensory deprivation (whisker trimming) on both local and long-range L2/3 synaptic inputs onto WT and MEF2C KO neurons. To probe the effects of sensory deprivation on local inputs, whiskers of AAV-Cre-GFP-injected $Mef2c^{fl/fl}$ mice were unilaterally trimmed daily from P9–P18 to deprive the contralateral barrel cortex of sensory experience while the ipsilateral barrel cortex is “spared” and provides a within-animal control (Fig. 4A). LSPS maps were obtained simultaneously from WT and MEF2C KO L2/3 neuron pairs in slices from either spared or deprived barrel cortices (Fig. 4B). In spared barrel cortex, intracolumnar and transcolumnar L4 → L2/3 synaptic input strengths were reduced onto MEF2C KO, as observed in non-deprived (i.e. non-trimmed; Fig. 2D–F) mice. However, in deprived cortex, L4 synaptic inputs onto MEF2C KO and WT L2/3 neurons were similar and weak like that observed onto MEF2C KO neurons in the spared cortex (Fig. 4C–E). In other words, *Mef2c* deletion weakened synaptic inputs onto L2/3 neurons in spared, but not deprived, cortex. Thus, a functional interaction between *Mef2c* and sensory deprivation was observed for vertical L4 → L2/3 inputs ($p < 0.05$; Fig. 4E). This result was not due to strengthening of L4 → L2/3 inputs onto spared WT neurons because they were similar to L4 → L2/3 inputs onto WT neurons in non-deprived mice (Fig. S3A). Furthermore, the interaction of *Mef2c* and experience persists when comparing vertical L4 → L2/3 input onto WT and MEF2C KO neuron pairs between deprived and non-deprived (non-trimmed) barrel cortices (Fig. S3B). Similarly, local horizontal L2/3 → L2/3 and vertical L5A → L2/3 inputs (Fig. 4F,G) were reduced by *Mef2c* deletion in spared, but not deprived, cortex. These results suggest that MEF2C is required for and permits sensory experience to promote synapse function of local inputs onto neocortical L2/3 pyramidal neurons.

To examine the role of sensory experience in *Mef2c* regulation of long-range inputs from cS1, $Mef2c^{fl/fl}$ mice were injected at P1 with AAV-Cre-GFP and AAV-ChR2-mCherry in cS1 and then subjected to daily unilateral whisker trimming from P9-P18. Light-evoked EPSCs from ChR2-expressing cS1 axons were recorded in WT and MEF2C KO L2/3 neurons in the deprived cortex receiving long-range ChR2-positive inputs from spared cS1 (Fig. 4H). In contrast to non*Rajkovich* trimmed mice (Fig. 2N), cS1-evoked EPSCs in deprived WT and MEF2C KO L2/3 neurons were not different (Fig. 4I), indicating that sensory experience is required for *Mef2c* to suppress long-range cortical inputs onto L2/3 neurons.

DISCUSSION

Here we demonstrate that postsynaptic *Mef2c* bidirectionally regulates distinct excitatory synaptic input pathways onto L2/3 pyramidal neurons in the developing barrel cortex *in vivo* in an experience-dependent manner. Postsynaptic, cell-autonomous deletion of *Mef2c* decreases functional and structural synaptic connections from local L4 inputs, and likely other local input pathways from other layers within and across adjacent columns. In contrast, long-range inputs, from at least contralateral S1, are potentiated. These data suggest that, in response to experience, postsynaptic MEF2C regulates transcripts that differentially affect synaptic connectivity and strength from distinct input pathways (Fig. S4).

Transcriptional activation of MEF2 family members has been primarily implicated in elimination of excitatory synapses. Embryonic or postnatal brainwide deletion of *Mef2c* increases spine density and synaptic transmission onto dentate gyrus granule cells of the hippocampus (Adachi et al., 2015; Barbosa et al., 2008), while excitatory synaptic transmission is reduced in CA1 and onto L5 neurons (Barbosa et al., 2008). Because embryonic deletion of *Mef2c* also affects neuronal migration and gross laminarization of neocortex, the cell-autonomous and effects of *Mef2c* on synapse development and connectivity were unknown (Li et al., 2008). To address this question, we postnatally deleted *Mef2c* in a sparse population of neurons within a wildtype circuit. Based on the evidence for MEF2C in synapse elimination, the increase in mEPSCs and potentiation of distal inputs in MEF2C KO neurons may be a direct effect of *Mef2c* deletion, while the depressed local inputs may be a homeostatic response or competition for postsynaptic resources (Bian et al., 2015). Alternatively, *Mef2c* may regulate transcripts that promote development or stabilization of synapses targeted by local inputs. In support of this idea, a sumoylated transcriptional repressor form of MEF2A promotes postsynaptic maturation onto cerebellar granule neurons (Shalizi et al., 2006). In this scenario, long-range cS1 inputs may homeostatically potentiate as a consequence of weak local, excitatory drive onto MEF2C KO L2/3 neurons.

Our data suggest that sensory experience, via regulation by MEF2C, differentially modifies specific L2/3 inputs. Supporting this model, the multiple and diverse effects of sparse postnatal *Mef2c* deletion on L2/3 circuitry parallel that of sensory deprivation. Whisker trimming decreases the strength of local input pathways onto L2/3 neurons in barrel cortex (Bender et al., 2006; Shepherd et al., 2003) and prevents or occludes weakening by *Mef2c* deletion. This result suggests that *Mef2c* is necessary for experience to promote synapse development from local circuits onto L2/3 neurons. Whisker deprivation decreases connectivity between L2/3 neurons while potentiating mEPSP amplitudes, suggesting differential changes in distinct input pathways (Cheetham et al., 2007). Furthermore, visual deprivation potentiates intercortical inputs onto L2/3 while depressing vertical L4 → L2/3 inputs (Petrus et al., 2015). To our knowledge, the effects of sensory deprivation on the strength of interhemispheric excitatory inputs to L2/3 in barrel cortex or other primary sensory cortices have not been examined. MEF2C KO neurons exhibit a selective decrease in structural synapses onto basal dendrites: the primary target of vertical L4 inputs (Feldmeyer et al., 2006). Similarly, sensory experience regulates dendritic spine density onto basal but not apical L2/3 dendrites (Bian et al., 2015; Globus et al., 1973). In contrast to the

effects of *Mef2c* deletion, plucking a single whisker row results in decreased release probability of L4 → L2/3 inputs and no change in mEPSCs onto L2/3 neurons (Bender et al., 2006). The synaptic plasticity mechanisms engaged may depend on the deprivation paradigm.

Like *Mef2c* deletion, visual deprivation increases mEPSCs in L2/3 neurons which correlates with potentiation of evoked synaptic transmission from horizontal inputs, and not the depressed vertical L4 → L2/3 inputs (Petrus et al., 2015). Similarly, we show here that the potentiation of mEPSC frequency and amplitude in MEF2C KO neurons correlates with a strengthening of contralateral S1 inputs, despite a strong depression of all local inputs measured. The fact that EPSCs evoked by extracellular electrical stimulation of horizontally projecting axons in L2/3 were unchanged on MEF2C KO neurons is likely due to stimulation of a mix of axons from both local and long-range input pathways. Although L4 provides the strongest local input onto L2/3 neurons (Shepherd et al., 2003) (Fig. 2), it is estimated that L4 inputs make up only 5% of the total synapses onto neocortical L2/3 neurons (Douglas and Martin, 2007). The enhanced amplitude of quantal events evoked from ChR2-expressing cS1 axons suggests a strengthening these individual synaptic inputs in this pathway and correlates with the increased mEPSC amplitude. It is unclear why we observe an increase in mEPSC frequency onto L2/3 neurons but no change in evoked quantal frequency from cS1 axons. The mEPSC frequency changes may stem from enhanced release probability or synapse connectivity of cS1 axons that we cannot detect with the C.V. analysis. Alternatively, enhanced release probability or synaptic connections from other long-range input pathways we did not measure, such as ipsilateral S2, M1, M2 or thalamus (Feldmeyer, 2012) could underlie the increased mEPSC frequency. Furthermore, we cannot rule out the possibility that MEF2C differentially regulates evoked and spontaneous synaptic transmission from the same local or long-range inputs (Kavalali, 2015).

Transcription factors control development of axons and dendrites, as well as formation of layer-specific synaptic inputs in part via production of gradients of guidance cues (Santiago and Bashaw, 2014). Here we demonstrate that a postsynaptic transcription factor differentially regulates distinct synaptic inputs onto an individual cortical neuron without affecting dendritic morphology. The activity-regulated transcription factor, NPAS4, differentially regulates strength of inhibitory synaptic inputs impinging on different dendritic domains of CA1 pyramidal neurons in an experience-dependent manner (Bloodgood et al., 2013). Thus, experience and activity may determine the connectivity and strength of specific inhibitory and excitatory circuits, respectively, via coordinated regulation of NPAS4 and MEF2C. Dendritic spine densities were reduced on basal but not apical dendrites of MEF2C KO neurons, suggesting that MEF2C may differentially regulate inputs based on dendritic compartment. Although L4 and local L2/3 inputs synapse onto basal dendrites of L2/3 neurons, local L2/3 inputs also contact proximal apical dendrites (Feldmeyer et al., 2006), where we did not detect a change in spines. In this scenario, one would expect that inputs from cS1 to primarily contact apical dendrites of L2/3 neurons in barrel cortex, which to our knowledge, is unknown. Alternatively, the origin of the presynaptic input, local versus long-range, may determine regulation by postsynaptic MEF2C. MEF2 transcription factors regulate cell adhesion molecules, such as protocadherins and semaphorins, which may

stabilize or eliminate specific input pathways through trans-synaptic interactions (Flavell et al., 2008; Tsai et al., 2012).

Mutations in *MEF2C* are associated with intellectual disability, epilepsy, autism and schizophrenia (Rocha et al., 2016; Schizophrenia Working Group of the Psychiatric Genomics, 2014). Interestingly, imbalances in local versus long-range functional connectivity among cortical and other regions are associated with autism and perhaps schizophrenia (Cao et al., 2016; Ha et al., 2015). The differential regulation of local and long-range cortical connections by MEF2C provides a novel molecular link from the genetics of these disorders and the abnormal brain connectivity.

METHODS SUMMARY

See supplementary information for detailed methods.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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HIGHLIGHTS

- Postsynaptic MEF2C differentially regulates distinct inputs onto L2/3 neurons
- MEF2C increases the number of local excitatory inputs onto L2/3 neurons
- MEF2C suppresses the strength of long-range excitatory cortical L2/3 inputs
- Experience-dependent development of excitatory L2/3 inputs requires MEF2C

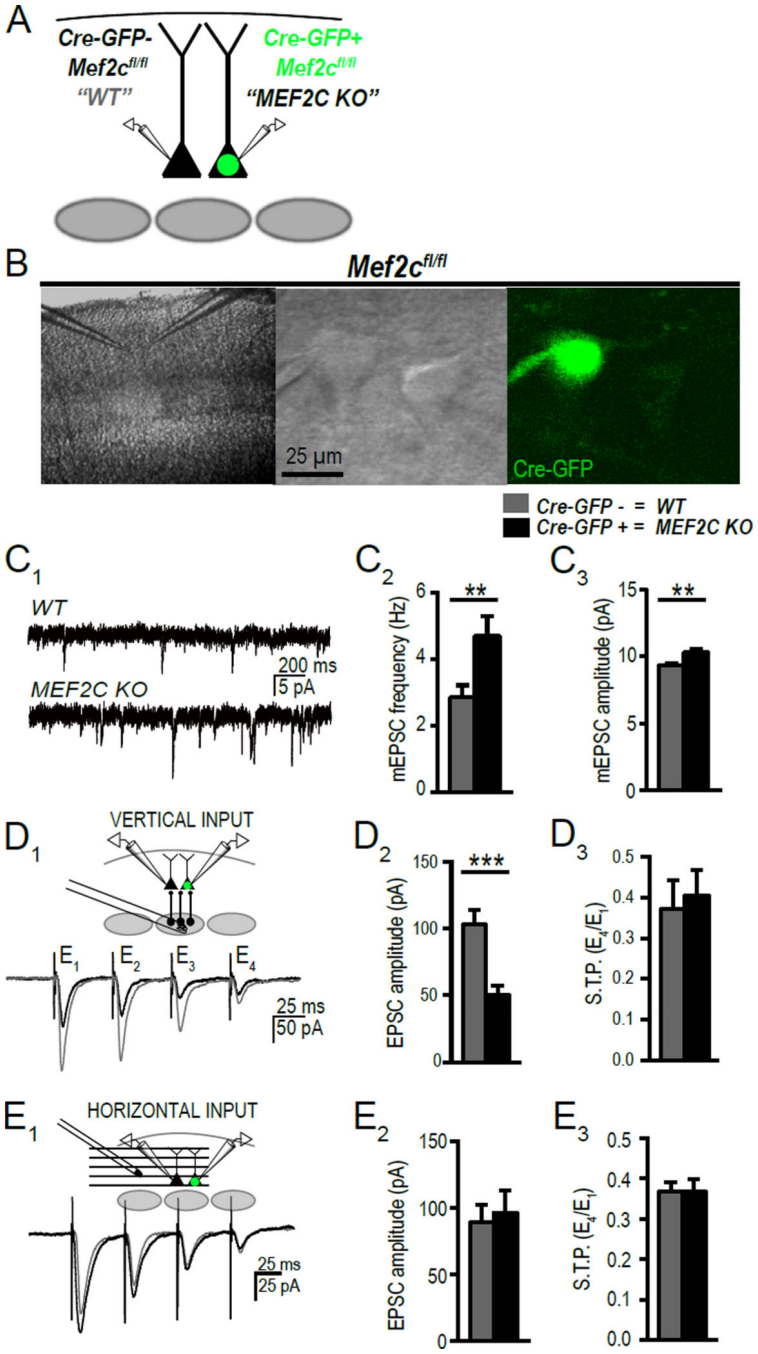

Figure 1. Postsynaptic *Mef2c* deletion differentially regulates specific L2/3 inputs

(A) Schematic of dual recordings from L2/3 neurons. L4 barrels: gray ovals. **(B)** IR-DIC and fluorescent images of recorded WT and MEF2C KO L2/3 pyramidal neurons in an acute barrel cortex slice from a *Mef2c*$^{fl/fl}$ mouse injected with AAV-Cre-GFP. **(C_1)** Example mEPSCs from WT and MEF2C KO L2/3 neurons. Average mEPSC frequency **(C_2)** and amplitude **(C_3)**. **(D_1)** Upper: Recording/stimulation configuration. Lower: Example EPSCs from WT and MEF2C KO L2/3 neurons evoked by electrical stimulation of L4. EPSC amplitude **(D_2)** and short-term plasticity (**D_3**, STP). **(E_1)** Upper: Recording/stimulation

configuration. Lower: Example EPSCs from WT and MEF2C KO L2/3 pairs evoked by stimulation of horizontal L2/3. EPSC amplitude **(E_2)** and STP **(E_3)**. For all figures, n = number of cell pairs are on bar graphs. $p < 0.05$*, 0.01**, 0.001***, 0.0001****

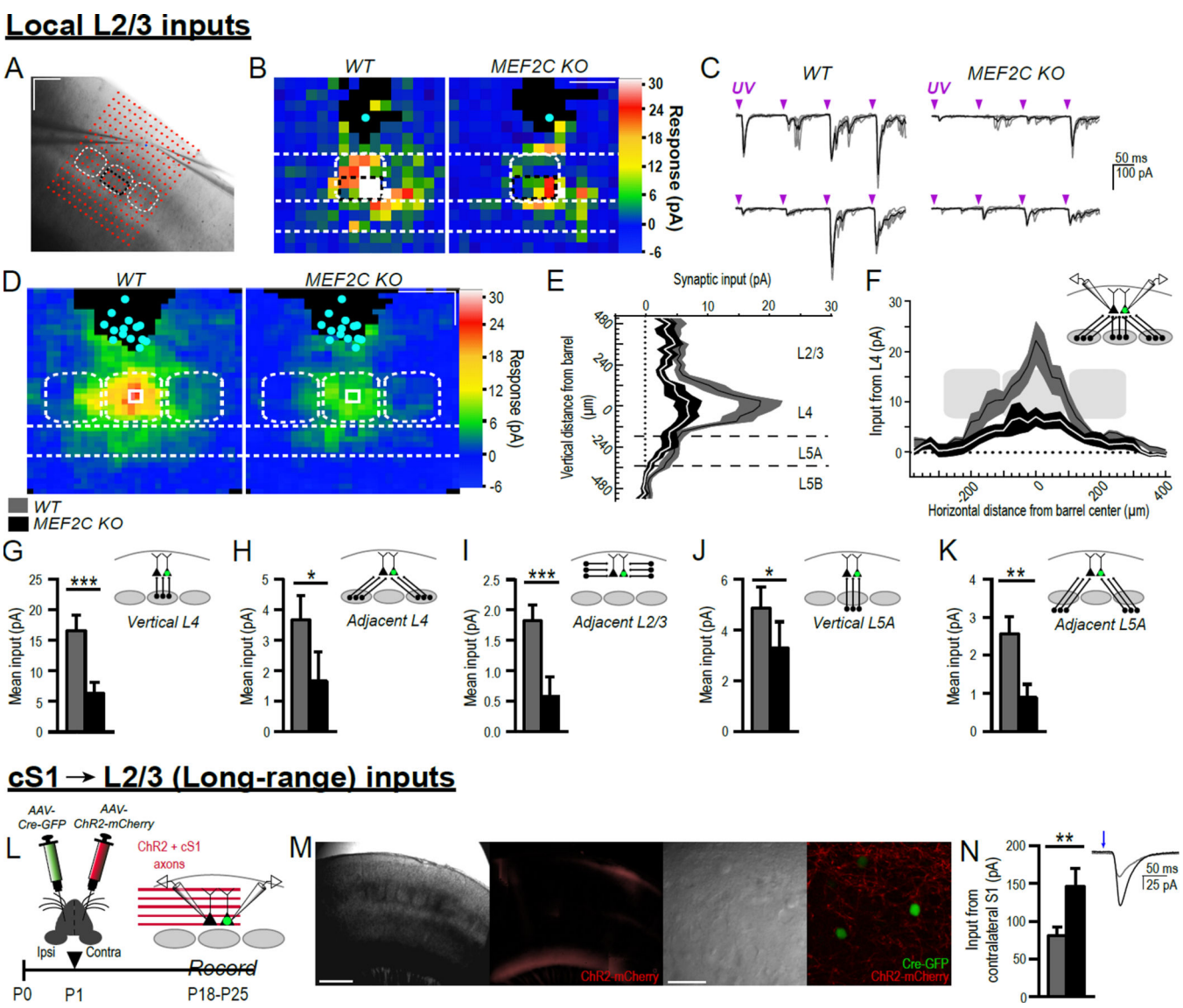

Figure 2. *Mef2c* deletion weakens excitatory synaptic inputs from local neocortical circuits, strengthens long-range inputs from contralateral cortex

(A) IR-DIC image of barrel cortex slice overlaid with LSPS stimulation grid (red dots) during recordings of WT and MEF2C KO neurons. White dotted lines outline barrel columns. **(B)** Color coded, LSPS synaptic input maps of individual WT and MEF2C KO L2/3 neurons. For all maps, cyan dots indicate soma location, direct responses are black pixels, and dashed white lines are barrels and layers. LSPS-evoked EPSC amplitudes are color coded according to z-scale. Pixels within dotted black line correspond to stimulation locations in **(A)**. **(C)** Representative LSPS-evoked EPSCs from WT and MEF2C KO neurons in **(A)** and **(B)** with preserved spatial orientation. Triangles mark UV laser pulses. Black: averaged EPSC. Gray: individual EPSCs. **(D)** Averaged LSPS maps of all WT and MEF2C KO pairs. Maps are aligned to “home” barrel center (white crosshair). **(E)** Vertical profile of mean synaptic inputs within the home barrel column of WT and MEF2C KO neurons. Shaded region represents ± S.E.M. **(F)** Horizontal profiles of mean synaptic L4 →

L2/3 input across barrel columns (gray boxes). Inset: Represented L4 → L2/3 input pathways. Mean synaptic inputs onto WT and MEF2C KO neuron pairs in the **(G)** vertical L4 → L2/3, **(H)** adjacent L4 → L2/3, **(I)** adjacent L2/3 → L2/3, **(J)** vertical L5A → L2/3, and **(K)** adjacent L5A → L2/3 input pathways from LSPS responses shown in **(D)**. **(L)** Timeline and schematic of ChR2-evoked stimulation of cS1 input. **(M)** Images of IR-DIC (i, iii); ChR2-mCherry (ii, iv) and Cre-GFP (iv) in barrel cortex. **(N)** Average amplitude of blue light-evoked EPSC of cS1 input onto WT and MEF2C KO neurons. Inset: Example blue-light-evoked EPSCs from WT and MEF2C KO neurons.

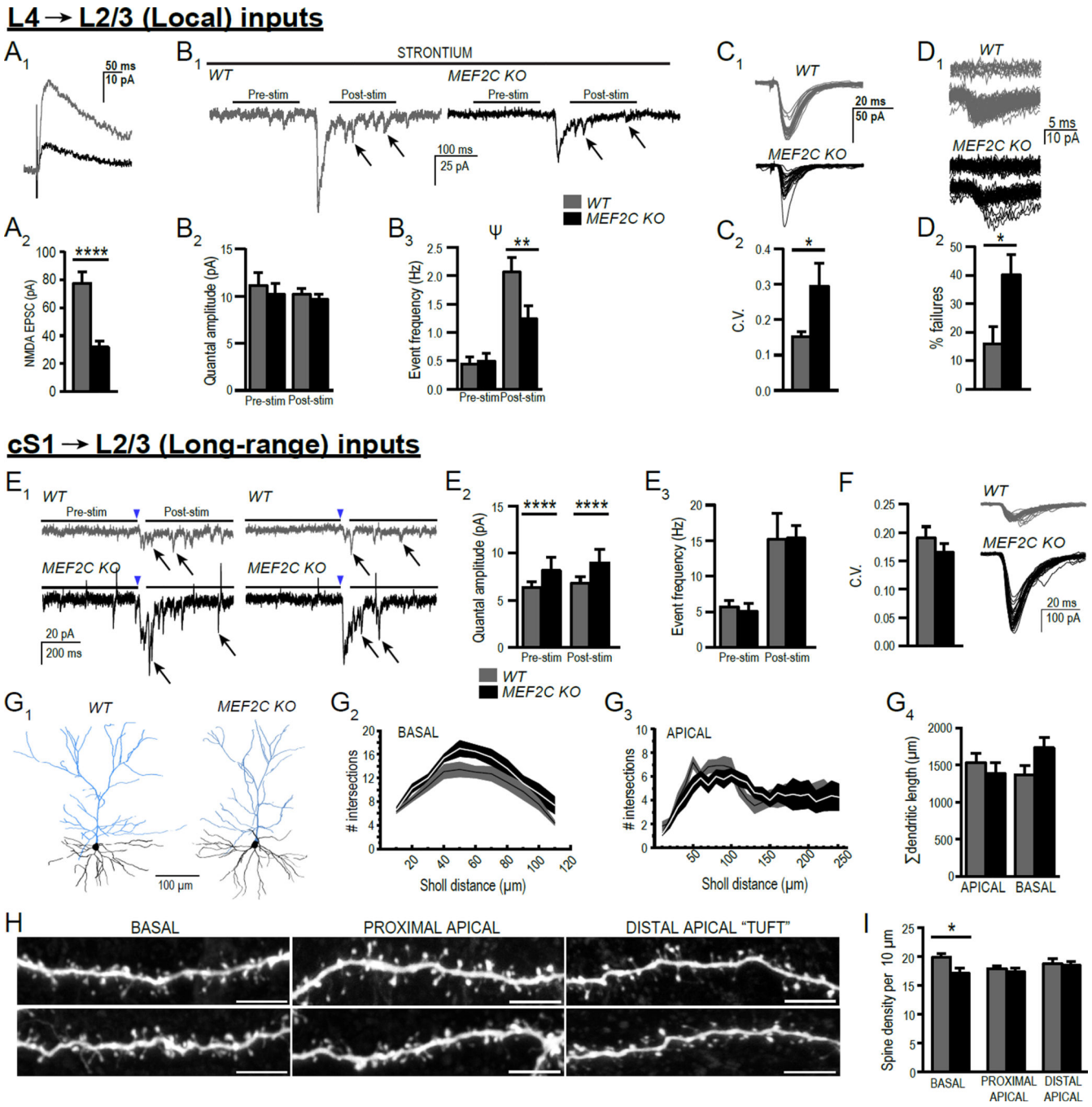

Figure 3. *Mef2c* deletion in L2/3 neurons decreases connectivity of local inputs from L4 and potentiates long-range inputs from contralateral cortex

(A_1) Example NMDAR-EPSCs from WT and MEF2C KO neurons evoked by L4 electrical stimulation. **(A_2)** Average NMDAREPSC amplitude. **(B_1)** Example evoked L4 → L2/3 EPSCs from WT and MEF2C KO neurons in Sr^{+2}. **(B_2)** Quantal event (arrows in B_1) amplitude and **(B_3)** frequency within pre- and poststimulation analysis windows (black bars in B_1). **(C_1)** Example evoked L4 → L2/3 EPSCs from WT and MEF2C KO neurons. **(C_2)** C.V. of evoked L4 → L2/3 EPSCs. **(D_1)** Example EPSCs (failures above; successes below)

evoked with minimal electrical stimulation of L4. **(D_2)** Average synaptic failure rate. **(E_1)** Example ChR2-evoked -EPSCs in Sr^{+2} from cS1 inputs. Blue arrow: blue light pulse. Quantal event (arrows in E_1) amplitude **(E_2)** and frequency **(E_3)** within pre- and post-stimulation windows (black bars in E_1). **(F)** C.V. of ChR2-evoked EPSCs from cS1 inputs. **(G_1)** Apical (blue) and basal (black) dendritic arbors of WT and MEF2C KO L2/3 neurons. Sholl analyses of **(G_2)** basal and **(G_3)** apical dendrites. **(G_4)** Summed length of apical and basal dendrites. **(H)** Representative images of spines within basal, proximal apical, and distal apical dendrites of WT and MEF2C KO L2/3 neurons. **(I)** Mean spine density in each compartment. 2-way ANOVA interaction $p < 0.05$ (Ψ).

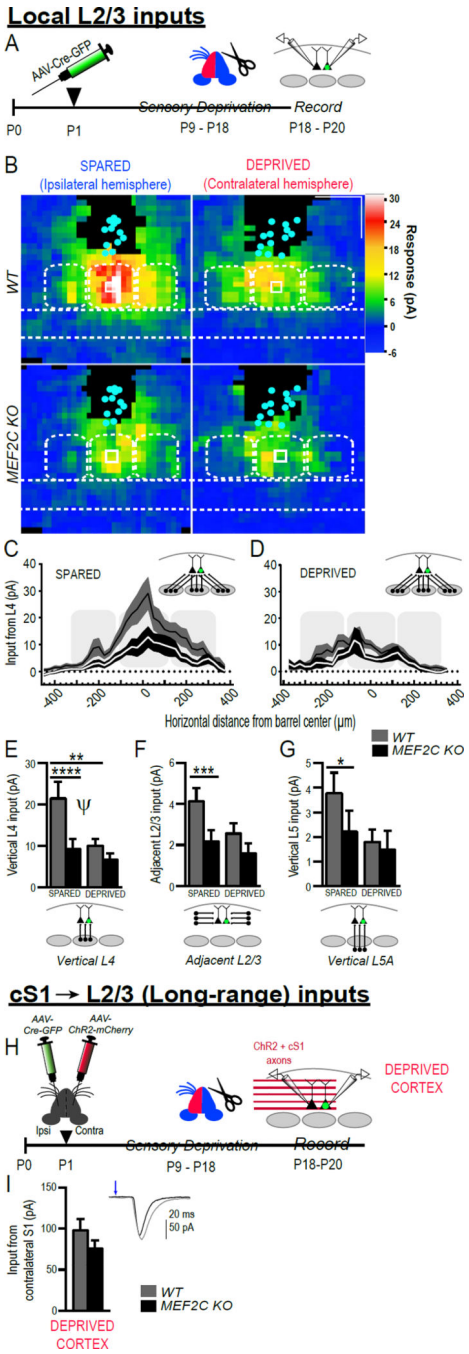

Figure 4. *Mef2c* and sensory experience interact to differentially promote excitatory synaptic local inputs and suppress long-range inputs onto L2/3 neurons

(A) Timeline of whisker trimming and recording. **(B)** Averaged LSPS maps of spared WT (B_1), deprived WT **(B_2)**, spared MEF2C KO **(B_3)**, and deprived MEF2C KO **(B_4)**. Horizontal synaptic profiles of L4 → L2/3 input onto WT and MEF2C KO L2/3 neurons in spared **(C)** and deprived **(D)** hemispheres. Average LSPS-evoked synaptic input onto WT and MEF2C KO L2/3 neurons at vertical L4 → L2/3 **(E)**, horizontal L2/3 → L2/3 **(F)**, and vertical L5A → L2/3 **(G)** pathways. **(H)** Timeline of dual neonatal AAV injections, whisker trimming, and recording of long-range cS1 inputs in WT and MEF2C KO L2/3 neurons in

deprived barrel cortex. **(I)** Average ChR2-EPSC amplitude from spared cS1 inputs onto WT and MEF2C KO neurons in deprived barrel cortex. 2-way ANOVA interaction; $p < 0.05$ (Ψ).