Experience-dependent plasticity of layer 2/3 circuits in developing somatosensory neocortex

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Abstract

Experience-dependent plasticity is the adaptability of brain circuits as a result of changes in neural activity, a phenomenon that has been proposed as the neural basis for important brain function in health and disease. The underlying mechanisms of experience-dependent plasticity can take different forms, depending on the organisms and brain areas under investigation. A better understanding of these mechanisms will help to interpret normal brain function as well as to guide therapies for neurological diseases. Mouse vibrissa system offers great experimental advantages to studying experience-dependent plasticity and the underlying molecular mechanisms at different levels.

Using sensory experience paradigms of unbalanced whisker activity, we find that sensory experience induces rapid synaptic strengthening at excitatory synapses converged onto single layer 2/3 pyramidal neurons, although the plasticity at these synapses displays remarkable input specificity. Furthermore, we discover that recently potentiated layer 4-2/3 excitatory synapses are labile and subject to activity-dependent weakening in vitro. Calcium-permeable AMPARs (CP-AMPARs) that are sometimes associated with synaptic strengthening are not essential for activity-induced synaptic weakening. Finally, we demonstrate that ongoing sensory experience triggers distinct phases of synaptic plasticity, which are tightly correlated with changes in NMDAR properties and function. Taken together, the results from this thesis show distinct manifestations and mechanisms of how sensory experience modulates synaptic properties and neuronal function that may provide insights into information processing and coding in the neocortex.

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1. Background and motivation

1.1 Barrel cortex: cytoarchitecture, sensory pathway and cortical circuitry

Primary somatosensory cortex (SI, barrel cortex) is the cortical area that corresponds to vibrissae or whiskers of the contralateral side of many mammals, including rodents. The cytoarchitecture of barrel cortex was first discovered (Woolsey and Van der Loos, 1970) by Nissl-staining as the oval-shaped multi-neuronal structures, called “barrels” in an array of representations located in layer 4 of cortex, tangentially distributed and similar to vibrissae of the mystacial pad. This interesting discovery of the alignment of the cortex with sensory periphery gave rise to the hypothesis that tactile information collected by individual whiskers is transmitted into the cortex within the column that is delineated by the barrel. Electrode-based recordings made in the barrel cortex have confirmed this hypothesis by showing that neurons in a barrel column respond preferably to the stimulus given to their principle whisker, the homologous whisker that corresponds to the barrel column where the neurons are located (Simons, 1978, 1985). Because of the anatomical and physiological correlation between the barrel columns and the vibrissae, the rodent vibrissal system has become a popular model to study cortical development, sensory processing and experience-dependent regulation of cortical pathways.

Sensory signaling from a single whisker to the cortex occurs in a highly segregated, columnar fashion. Primary sensory neurons with a diversity of nerve endings innervate whisker follicles on the snouts and respond to deflection of a specific whisker. The cell bodies of these primary sensory neurons are localized in the trigeminal ganglion and they make glutamatergic synapses with neurons in the principal trigeminal nucleus of brain stem, where these neurons form topographic cell clusters called “barrellettes” (Bates and Killackey, 1985) and cells within

each “barrellette” receive strong input from a single whisker (Veinante and Deschenes, 1999). The principal trigeminal neurons send axons to the ventral posterior medial (VPM) nucleus of the thalamus terminating in anatomical segregates termed “barreloids” and VPM neurons further project to layer 4 barrels (barrel cortex). The sensory pathway of the principle trigeminal nucleus-VPM-layer 4 barrel, is called the lemniscal pathway. Although the lemniscal pathway is thought to be the major pathway propagating and processing sensory information, other pathways exist in parallel that may process different aspects of sensory information. The paralemniscal pathway arises in the interpolaris nucleus of spinal trigeminal complex (SpVi) in the brainstem. Neurons within SpVi project to the posteriormedial thalamic nucleus (POM). Compared with a predominant single-whisker receptive field of principle neurons in “barrellettes” and “barreloids” in the lemniscal pathway, neurons in the paralemniscal pathway display multi-whisker receptive field (Diamond et al., 1992). The lemniscal and paralemiscal thalamocortical inputs have been shown to be segregated in rats as VPM and POM inputs differentially project to barrel columns (layer 4 that feeds onto layer 2/3 in the barrel column) and septal regions (layer 5A that feeds onto layer 2 in the septum-associated column) between barrel columns (Shepherd and Svoboda, 2005). Separation of these two sensory pathways are also evident in mouse barrel cortex as thalamocortical projections from VPM terminate in layer 4, 5B and 6A whereas those from POM terminate in layer 5A of barrel cortex (Bureau et al., 2006).

Electrophysiological recording and imaging of whisker-evoked neural activity have provided important information about the cortical circuitry that underlies signal propagation within barrel cortex. Dense thalamocortical inputs from VPM neurons arrive in layer 4 in the form of near-synchronous activity, attributed by strong GABAergic projections that sharpen

the firing output of VPM neurons (Brecht and Sakmann, 2002a; Bruno and Sakmann, 2006). Intracortical circuits within barrel cortex have been intensively studied anatomically and functionally using a variety of techniques. Firstly, anatomical studies have revealed that layer 4 axonal and dendritic arbors are confined within a single barrel column by reconstructing layer 4 neurons (Feldmeyer et al., 1999; Petersen and Sakmann, 2000). Two major types of excitatory neurons within layer 4, spiny stellate and pyramidal cells show distinct thalamocortical input patterns, in that spiny stellate cells receive mostly intra-columnar inputs while pyramidal cells receive both intra- and trans-columnar inputs (Schubert et al., 2003). Axons of spiny stellate cells, the predominant cell type of layer 4, terminate extensively in layer 2/3 within the same barrel column (Lubke et al., 2003). Functional analysis shows that stimulation of layer 4 results in depolarization of layer 4 (2-3 ms) followed by layer 2/3 (4-5 ms) (Armstrong-James et al., 1992). The connection between layer 4 and layer 2/3 is directional, i.e. layer 4 projects to layer 2/3, since there is very little connectivity from layer 2/3 to layer 4 (Lefort et al., 2009), a property that determines the direction of information flow within barrel cortex. Anatomical reconstructions of layer 4 spiny neurons and layer 2/3 pyramidal neurons in acute slices of barrel cortex have shown that layer 4-2/3 synapses are exclusively located on the basal dendrites of layer 2/3 pyramidal cells (Feldmeyer et al., 2002), although a relatively recent study using the method of channelrhodopsin-2 (ChR2)-assisted mapping has shown that layer 4 and layer 2/3 inputs are located in largely non-overlapping dendritic domains (Petreanu et al., 2009).

A single layer 2/3 pyramidal cell receives most of its vertical inputs from layer 4 within the same barrel column, suggesting preservation of single whisker information during the first step of signal transmission within the cortex (Petersen and Sakmann, 2001; Shepherd et al.,

2003). However, in addition to the excitatory projections from intra-columnar layer 4, layer 2/3 pyramidal neurons also receive strong excitation from other layer 2/3 neurons. Unlike those of layer 4 neurons, the axonal arbors of layer 2/3 neurons extend beyond the boundaries defined by layer 4 barrel columns (Brecht and Sakmann, 2002b; Petersen et al., 2003b). Voltage-sensitive dye (VSD) imaging in the supragranular layer has shown that deflection of a single whisker causes a spread of excitation to a broad cortical area within layer 2/3 after an initial localized activity in the home barrel column (Petersen et al., 2003a; Ferezou et al., 2006). Whole cell recordings of layer 4 stellate and layer 2/3 pyramidal cells in vivo upon deflection of a single whisker have shown that both the suprathreshold (Simons, 1978; Armstrong-James and Fox, 1987) and subthreshold (Brecht & Sakmann, 2002; Brecht et al.2003) receptive fields of layer 2/3 neurons are broader compared with those of layer 4 spiny neurons. Consistently, the rate of connectivity between local neighboring layer 2/3 pyramidal cells is similar to that between layer 4 and layer 2/3 (Lefort et al., 2009). Moreover, the innervation domains of layer 2/3 to layer 2/3 synapses are also found to be localized on the basal dendrites of layer 2/3 neurons, overlapping with those of the layer 4-2/3 excitatory synapses (Feldmeyer et al., 2006). In addition, a superficial band of layer 2 cells located between 70 and 125 μm receive excitatory inputs from layer 5A that is associated with the paralimniscal pathway (Bureau et al., 2006). Distal inputs of layer 2/3 cells arise from other cortical areas such as contralateral somatosensory cortex, motor cortex and secondary somatosensory cortex, as well as the thalamus (Petreanu et al., 2007; Petreanu et al., 2009). Conversely, layer 2/3 pyramidal neurons project to the aforementioned cortical areas and also to within-column layer 5 and 6 (Armstrong-James et al., 1992; Reyes and Sakmann, 1999). The output layer of barrel cortex, layer 5, consists of subdivisions of layer 5a and layer 5b. Layer 5a pyramidal cells send

long-range projections to the contralateral side of cortex, primary motor cortex, the second somatosensory cortex and the striatum, whereas layer 5b pyramidal cells project to a more diverse population of subcortical targets including the superior colliculus, pontine and trigeminal nuclei or spinal cord (Hattox and Nelson, 2007).

In summary, layer 2/3 pyramidal cells in barrel cortex serve as important integrators of feed-forward inputs from deep cortical layers and the sub- and supra-threshold responses of layer 2/3 cells are closely correlated with whisker-related sensory activity in the animals. These properties as well as experience-dependent plasticity of the neural circuits that layer 2/3 cells are involved in, make these cells well positioned to encode whisker-related sensory information. Indeed, optical stimulation of a sparse population (1%) of layer 2/3 excitatory neurons in awake mice that express channelrhodopsin (ChR2) has been shown to drive animal behavior in a two-way discrimination task after a period of associative learning in which photostimulation is paired with water reward in the task (Huber et al., 2008). These results indicate that layer 2/3 cells and the cortical circuits they are embedded in have the capacity to encode and drive behavior.

1.2 Experience-dependent plasticity in barrel cortex

Reorganization of cortical presentation of the sensory periphery has been shown to occur in response to changes in the pattern of sensory stimuli, which is termed experience-dependent map plasticity. Experience-dependent map plasticity in primary sensory cortices is important for normal brain function such as storage of new sensory information and perception, as well as neuronal repair and functional rehabilitation (Fox, 2009). Although experience-dependent receptive field plasticity was first described in visual cortex (Wiesel and

Hubel, 1965), a substantial amount of studies have been carried out in barrel cortex because of its clear one-to-one correspondence between whiskers and cortical map and the accessibility of sensory manipulation. Different paradigms of sensory manipulation have been used to study experience-dependent plasticity, including all whisker deprivation (Glazewski et al., 1998), single whisker(s) deprivation (Glazewski et al., 1998; Allen et al., 2003), single whisker sparing (Fox, 1992; Bender et al., 2006a; Clem and Barth, 2006; Benedetti et al., 2009), double whisker sparing (Diamond et al., 1994) and chessboard deprivation (Wallace and Fox, 1999b, a). Thus barrel cortex has become a popular model system to study the cellular and synaptic mechanisms of experience-dependent plasticity.

The initial topographic map of barrel cortex is determined by genetic programs. Genetic knockout of certain genes in mice, including NMDA receptors, phospholipase C-beta 1, metabotropic glutamate receptors, adenylyl cyclase 1 and monoamine oxidase A, has resulted in the loss or undefined appearance of barrel cortex (Petersen, 2007). Despite the instrumental role of genetic programs in the initial stage of map establishment, the developmental refinement of cortical map requires sensory experience. In the neonates, whisker de-afferentation disrupts the formation of corresponding barrels (Van der Loos and Woolsey, 1973). This experience-dependent change in large-scale anatomical organization of barrel cortex is followed by a fine-scale functional change in the cortical circuitry that persists through adulthood. Trimming or plucking of all but one whisker, a manipulation that continues for weeks during the first four postnatal days has resulted in an increase of cortical responsiveness of neurons in layer 4 and other cortical layers to the deflection of the spared whisker (Fox, 1992). This early phase of plasticity is primarily mediated by thalamocortical synapses onto layer 4 neurons. During this period, thalamocortical synapses display a form of

NMDAR-dependent long-term potentiation (LTP), which coincides with the early sensitive period for experience-dependent plasticity in layer 4 (Crair and Malenka, 1995). This form of LTP involves conversion of "silent" synapses which do not express AMPARs to active synapses where AMPARs are inserted into the synapses (Isaac et al., 1997). These findings suggest NMDAR-dependent, AMPAR-mediated synaptic strengthening serves as a putative synaptic mechanism for early plasticity within layer 4. After P4, experience-dependent plasticity in layer 4 rapidly disappears while plasticity in layer 2/3 is maintained at comparable levels and persists into adulthood, which suggests that in older animals intracortical circuits are the major sites of plasticity. In animals with all whiskers intact, layer 2/3 neurons preferentially respond to the homologous whisker input, but to a much less extent to the inputs from whiskers surrounding the homologous whisker (i.e. surround whiskers). It has been shown that sensory deprivation-induced cortical plasticity in layer 2/3 neurons of adolescent rats (1-2 months) involves two separate components, one being response depression to the deprived input from the homologous whisker and the other being response potentiation to the inputs from the spared surround whiskers. Plucking of all but a single whisker leads to an initial depression of layer 2/3 firing in the deprived barrel columns to the principle deprived whisker inputs (after they are regrown), followed by a later increase of responses to the spared surround whisker inputs (Glazewski and Fox, 1996). The time course of the early stage of response depression followed by a later response potentiation has also been discovered in primary visual cortex after monocular deprivation (Frenkel and Bear, 2004). In addition, 24 hr of single whisker experience (SWE), where all but the D1 whisker on one side have been removed in young mice (P13-16) could induce response potentiation in layer 2/3 to deflection of the principle spared whisker (Glazewski et al., 2007; Benedetti et al., 2009). This form of rapid response

potentiation to the spared whisker input within the spared barrel column is different from SWE-induced response potentiation to the surround spared input in adolescent rats (Glazewski and Fox, 1996) and involves synaptic plasticity at distinct cortical circuitry, i.e., within-columnar vs. trans-columnar. Response depression of layer 2/3 neurons to the deprived input disappears in adult rats (>6 months), but response potentiation to the surround spared whisker input is maintained, although longer deprivation period is required (Glazewski and Fox, 1996; Benedetti et al., 2009).

1.3 Synaptic mechanisms for map plasticity in barrel cortex

Hebbian forms of synaptic plasticity where correlated activity of pre- and postsynaptic cells strengthens the connection between them while uncorrelated activity depresses it, has been considered a general principle for cognitive functions of the brain including the cortex (Malenka and Bear, 2004). Indeed, LTP and LTD can be widely induced in visual and barrel cortex using canonical protocols as well as new forms of Hebbian plasticity, spike-timing dependent LTP or LTD (STDP or STDD) (Bi and Poo, 1998). Since experience-dependent map plasticity is thought to involve specific circuits and shows similar properties to long-term plasticity (LTP or LTD) in vitro, LTP or LTD has been considered a cellular mechanism for map plasticity in primary sensory cortices. Experimental evidence from recent years supports that LTP and LTD are important components of experience-dependent plasticity in primary visual and somatosensory cortex.

Activity-induced LTP at thalamocortical synapses in vitro displays a similar sensitive period of susceptibility to SWE-induced plasticity of cortical responses in barrel cortex during the first post-natal week (Fox, 1992; Crair and Malenka, 1995). The “barrelless” mice that do

not contain cortical barrel structures as a result of a loss-of-function mutation in the adenylyl cyclase I (*AC1*) gene, showed severe deficits in thalamocortical LTP and LTD and AMPAR trafficking in vitro, suggesting a critical role for LTP and LTD in the maturation of thalamocortical synapses (Lu et al., 2003). Recent evidence from barrel cortex and visual cortex strongly indicates that LTD is the major synaptic mechanism for sensory deprivation-induced response depression to the deprived inputs. Deprivation of a subset of whiskers after P12 causes robust depression of layer 2/3 response to the principle deprived whisker (Allen et al., 2003). At the same time, the input-output curve of layer 4-2/3 synaptic responses in the deprived barrel column displays a significant decrease in the deprived barrel column compared to the neighboring undeprived columns, suggesting synaptic weakening of layer 4-2/3 synapses induced by whisker deprivation in vivo (Allen et al., 2003). The Allen et al. study further shows that this deprivation-induced synaptic weakening occludes LTD and conversely enhances LTP in vitro at layer 4-2/3 synapses (Allen et al., 2003), a result that would be expected if in vivo synaptic weakening employs the same mechanism as LTD in vitro. In support of synaptic weakening at layer 4-2/3 excitatory synapses, circuit analysis by glutamate uncaging in all-whisker deprived rats shows that layer 2/3 pyramidal neurons receive much weakened input from within-column layer 4, compared to an increased input to layer 2/3 neurons above the septal region (Shepherd et al., 2003). In GluR1 knockout mice, STDD in vitro and response depression to the deprived whisker input in vivo are completely abolished in layer 2/3 neurons after SWE that lasts for 7 d (Wright et al., 2008), suggesting a dependence on AMPAR trafficking during in vivo plasticity. In addition to a postsynaptic mechanism mediated by AMPAR trafficking, other studies show that whisker deprivation induces a decrease in presynaptic release probability at layer 4-2/3 synapses of the deprived barrel

column that requires endocannabinoid signaling (Bender et al., 2006a; Bender et al., 2006b). No change in postsynaptic properties such as intrinsic excitability of layer 2/3 pyramidal cells (Allen et al., 2003), the amplitude of evoked minature EPSCs (mEPSCs) or AMPA:NMDA amplitude (A:N) ratio of layer 4-2/3 synapses (Bender et al., 2006a) was observed . These results indicate a presynaptic origin of LTD that may mediate synaptic weakening and response depression to the deprived input in barrel cortex (Bender et al., 2006a; Bender et al., 2006b). In addition, LTD is proposed to mediate ocular dominance plasticity in visual cortex. Brief monocular deprivation (24 hr) decreases surface expression of AMPARs and reduces AMPAR phosphorylation, akin to LTD induced by low-frequency LGN stimulation in vitro (Heynen et al., 2003). One of the predominant molecular mechanisms for in vitro LTD is internalization of postsynaptic AMPARs via the AP2-clathrin adaptor protein complex (Lee et al., 2002). When virally delivered into the slices or into the visual cortex in vivo, a peptide that interferes with the interaction between GluR2 c-tail and AP2 abolishes in vitro LTD and response depression to the deprived eye input in layer 4 cells after 3-d monocular deprivation (MD). Genetic knock-out or pharmacological depression of important pathways critical for in vitro LTD have been shown to prevent or reduce ocular dominance plasticity in vivo (Daw et al., 2004; Fischer et al., 2004; Liu et al., 2008), although experiments show the involvement of different molecules for LTD at different cortical layers.

Although LTD is a popular candidate for deprivation-induced response depression in both S1 and V1, exceptions remain where genetic manipulations of genes required for LTD in vitro failed to affect in vivo plasticity (Hanover et al., 1999; Hensch, 2005). Mechanisms other than homosynaptic LTD have received some experimental support. One of the other ways to decrease layer 2/3 firing is to decrease synaptic connectivity. Whisker deprivation has been

shown to decrease the connectivity between neighboring layer 2/3 pyramidal neurons and decrease the axonal arborization of layer 2/3 inputs from the non-deprived to the deprived column (Cheetham et al., 2007; Broser et al., 2008). The other important mechanism for experience-dependent plasticity in primary sensory cortices that has received extensive support is inhibitory plasticity (Hensch, 2005). Coritcal inhibitory interneurons are important components of local circuits that influence the precision of principle cell firing and coordinate information integration in a large population of principle cells. Like excitatory synapses, cortical inhibitory synapses made from interneurons to principle neurons undergo activity- or experience-dependent synaptic plasticity and their developmental maturation is experience-dependent (Gaiarsa et al., 2002; Kullmann and Lamsa, 2007). Visual deprivation could strengthen GABAergic transmission from fast-spiking (FS) basket cells to star pyramidal neurons (star pyramids) in layer 4. This deprivation-induced strengthening of FS cell-layer 4 star pyramid synapses in vivo could occlude pairing-induced potentation of these synapses in vitro (LTPi) (Maffei et al., 2006). These data suggest that response depression of layer 4 neurons to the deprived visual input might be in part mediated by potentiation of inhibitory synapses that in turn suppresses layer 4 firing.

In visual cortex, the maturation of cortical inhibitory circuits is required for both the onset and offset of the critical period of ocular dominance plasticity (Hensch et al., 1998; Rozas et al., 2001). On the other hand, inhibitory synapses have shown tremendous homeostatic plasticity, in that sensory over-stimulation increases while deprivation decreases inhibitory function. Continuous whisker stimulation in adult mice for 24 hrs causes a substantial increase in GABAergic synapse density on the spines of layer 4 neurons in barrel cortex, accompanied by weakened spiking of layer 4 neurons to deflection of the principle

whisker (Knott et al., 2002). Whisker deprivation started at P7 reduces inhibitory synaptic transmission onto layer 4 spiny neurons and this reduction in inhibitory function is mediated by parvabumin-expressing FS interneurons in layer 4 (Jiao et al., 2006). Together, these results suggest that homeostatic plasticity of inhibitory circuits may coexist with Hebbian forms of plasticity in regulating neuronal firing and thus map plasticity in primary sensory cortices. Future investigation will need to address the detailed mechanisms of how inhibitory plasticity mediates receptive field plasticity.

The second component of experience-dependent plasticity in barrel cortex is response potentiation to the spared whisker input, which is proposed to be LTP-like processes in vivo. Because trafficking of synaptic AMPARs is the prevalent form of LTP (Kessels and Malinow, 2009), sensory experience that implements LTP-like rules in vivo is expected to affect AMPAR trafficking. Whisker experience in vivo drives trafficking of not only the virally expressed GluR1s, but also the endogenous GluR1-containing AMPARs to layer 4-2/3 synapses as disruption of endogenous AMPAR trafficking by the recombinant c-tail of GluR1 prevents experience-dependent synaptic potentiation (Takahashi et al., 2003). Additional evidence supporting LTP in vivo has come from a study using transgenic mice that express a mutated form of Ca^{++}/calmodulin-dependent protein kinase II gene (*CaMKII*, T286A) which abolishes the ability of CaMKII to autophosphorylate, an important step required for LTP induction. These mice fail to express LTP in vitro at layer 4-2/3 synapses arising from the neighboring spared barrel and consistently do not show response potentiation of layer 2/3 neurons to the spared whisker input (Glazewski et al., 2000; Hardingham et al., 2003).

The within-column potentiation of layer 2/3 response to the principle spared whisker input in young postnatal animals (P13-16) was first described by our laboratory (Glazewski et

al., 2007; Benedetti et al., 2009). Consistent with the hypothesis of layer 4-2/3 LTP in mediating this type of plasticity, we observed a robust synaptic strengthening of layer 4-2/3 synapses after 24 hr SWE, associated with trafficking of GluR2-lacking, calcium-permeable AMPARs (CP-AMPARs) in some conditions (Clem and Barth, 2006; Wen and Barth, 2011). These studies indicate that LTP of layer 4-2/3 excitatory synapses via postsynaptic AMPAR trafficking is an important mechanism for response potentiation of layer 2/3 neurons to the spared whisker input in young postnatal animals. However, it is noteworthy that the same length (24 hr) of SWE had opposite effects on the firing rate (depression instead of potentiation) as well as on the response latency and precision of layer 2/3 neurons in adolescent mice, compared with young animals (Benedetti et al., 2009). These results suggest that different mechanisms might be employed to regulate experience-induced cortical plasticity in young vs. adult animals. In addition to a simple postsynaptic LTP mechanism for potentiation to the spared whisker input, recent evidence indicates an involvement of nitric oxide (NO) retrograde signaling that increases presynaptic release probability, which acts in concert with GluR1 trafficking to mediate response potentiation. GluR1 knock-out mice display partial deficiency (~33%) in response potentiation to the spared input while double knock-out mice that lack both GluR1 and neuronal nitric oxide synthase (αNOS1), lose almost all the potentiation (Dachtler et al., 2011), consistent with a requirement of NO for in vitro LTP at layer 4-2/3 synapses.

1.4 Critical periods for experience-dependent plasticity

General overview

Critical periods (CPs) are well-defined short time intervals during early development when the nervous system displays highly plastic changes in response to

sensory experience. Although it has been found that the nervous system in adults can be plastic, experience-induced changes within CPs are more pronounced in extent compared to in adults. Experience-dependent plasticity in adults often requires extended period or extreme form of sensory experience (Sawtell et al., 2003; Benedetti et al., 2009). Critical periods have been found in many species (mammals and non-mammals), sensory modalities (motor, visual, somatosensory, auditory), at different circuit levels (cortical or subcortical) and during naturally occurring experience as well as altered experience. As a result, the terminology of CP has been used broadly, despite the fact that it may refer to different phenomena (Hensch, 2004).

Plastic changes in neural circuits during early CPs can have profound impact on animal behavior as experience-induced learning within CPs can persist into adulthood. Second language learning in humans and song learning in songbirds are two well known examples of life-long behavior that is established during developmental CPs. Considering the functional importance of CPs in animal behavior and the widespread expression of CPs in the nervous system, a better understanding of the neural basis for CPs (onset and duration) during early development will provide important information to the cellular and molecular mechanisms regulating experience-dependent plasticity in the developing brain. In addition, these mechanistic investigations of CP regulation will help to find ways to reactivate plasticity and boost memory potential in the adult brain.

Because of the phenomenological complexity in the field of critical periods, we will primarily focus on the critical periods that were characterized in the neocortex (visual and somatosensory cortex) and present evidence that supports the main mechanisms proposed

to explain the regulation of the timing (onset and offset) of critical periods, during normal circuit maturation as well as during altered sensory experience.

Critical periods during normal circuit maturation

Some forms of CPs describe changes in neural circuits during naturally occurring experience without perturbation to sensory activity. Critical periods of these early maturation events are instrumental for circuit refinement and structural maturation.

Motor axonal pruning is found in a single target muscle fiber of neonatal neuromuscular junctions (NMJs), which display a CP (<P12) (Sanes and Lichtman, 1999). A similar CP for axonal pruning is observed in climbing fibers from the brainstem inferior olivary nucleus innervating cerebellar Purkinje cells, resulting in a one axon-one cell connectivity during the first two postnatal weeks (Crepel et al., 1976). The maturation of visual cortical functions, including the orientation selectivity, movement direction and receptive field size is also restrained in a CP (after P19, before P45 in rats) (Fagiolini et al., 1994).

Regulation of these CPs for circuit maturation has been suggested to involve experience-dependent LTP mechanisms in visual cortex (Kirkwood et al., 1995). Supporting the LTP hypothesis, the decline of the capacity for LTP induced in layer 3 cells in vitro is correlated with the CP closure in vivo. Alteration in visual experience (dark rearing from birth) results in an extended window for LTP induction (Kirkwood et al., 1995). These data indicate that activity-dependent LTP at visual cortical synapses is an important mechanism for CP regulation during normal cortical circuit maturation.

Critial periods for cortical plasticity following alteration in sensory experience: introduction

The most commonly studied forms of CPs are focused on plasticity induced by altered sensory experience, such as sensory deprivation or enhancement. Because alteration in sensory experience leads to rapid neural circuit plasticity that is often restricted to a developmental CP, the mechanisms regulating experience-dependent plasticity have thus been closely linked to those regulating CPs. A large body of work has been focused on studying experience-dependent plasticity in sensory pathways. The most intensively studied are visual, somatosensory and auditory pathways. Consistent with their complexity, CPs with different manifestations and involving different circuit components have been found across different sensory modalities, or even at different stages along the same sensory pathway. Therefore, I will present what is known about the CPs discovered in neocortical cells, which are the cells of interest in this thesis.

The first evidence of CP in neocortical cells has come from studies in primary visual cortex of kittens, where visual deprivation of one eye (monocular deprivation, MD) results in a loss of cortical responsiveness to the deprived eye input and a shift in the ocular dominance (OD) (Wiesel and Hubel, 1963). Like in cats, ocular dominance plasticity (ODP) in other species all exhibits a developmental CP. The shift of the balance in the cortical responsiveness towards the open eye input is accompanied by shrinkage of the cortical representation for the deprived eye and enlargement of that for the open eye in species that contain anatomical eye-specific domains in the cortex (such as cats and primates) (Hubel et al., 1977; Shatz and Stryker, 1978).

A great deal of the mechanistic studies on ODP during early CPs have been using the binocular zone of mouse primary visual cortex (V1) as a model system because of the accessibility to different transgenic and gene-targeting techniques in mice (Gordon and

Stryker, 1996). It was found that monocular deprivation-induced ocular dominance plasticity of single unit response can be detected in all layers of mouse V1, suggesting the presence of circuit level plasticity involving both thalamocortical (or geniculocortical) synapses and intracortical synapses (Gordon and Stryker, 1996).

Regulated synaptic plasticity as a mechanism for CP regulation

Thalamortical synapses

One main mechanism of CP regulation in primary sensory cortices that has received much experimental support is regulation of experience-dependent synaptic plasticity, i.e., LTP or LTD at distinct populations of excitatory synapses. Most studies supporting the synaptic basis for CP regulation were correlative in nature and the conclusions were based on the temporal correlation between the LTP or LTD induced in vitro and sensory experience-induced plasticity of cortical responses in vivo. Thalamocortical synapses and intracortical synapses have been the most studied synaptic pathways that are implicated in CP regulation in visual and barrel cortex.

The initial stage of cortical sensory processing involves thalamocortical synapses that terminate in layer 4 and plasticity of thalamocortical synapses is restricted to a CP during early development in both visual and barrel cortex. At thalamocortical synapses of barrel cortex, pairing-induced NMDAR-mediated LTP was present in P3-7, but not in P8-14 rats (Crair and Malenka, 1995) and a similar CP was present for LTD at the same synapses (Feldman et al., 1998). Similarly, LTD induced by white matter stimulation of a mixed population of synapses including thalamocortical synapses has shown a critical period in layer 4 of guinea pig visual cortex (Dudek and Friedlander, 1996). Furthermore, the temporal profile of thalamocortical LTP and LTD in rodent barrel cortex is similar to

that of the CP for whisker deprivation-induced plasticity of layer 4 response (with ~3 d offset) (Fox, 1992), supporting the role of experience-dependent synaptic plasticity of thalamocortical synapses in determining the CP for experience-dependent plasticity in layer 4 of barrel cortex.

Studies of the past two decades have investigated the molecular mechanisms for regulating the duration of the critical periods for LTP at thalamocortical synapses, changes in NMDARs being one attractive mechanism because of the developmental shift in NMDAR subunit composition (NR2B to NR2A). However, the transition of NR2 subunit composition did not coincide with the closure of the CP in barrel cortex (Barth and Malenka, 2001), neither can this CP be extended by preventing NR2B to 2A switch in NR2A knock-out animals (Lu et al., 2001). Recent evidence suggests that one molecule, fragile X mental retardation protein (FMRP) which is implicated in Fragile X syndrome, is required for maturation and CP regulation of thalamocortical synapses of barrel cortex. Knock-out mice of the encoding gene (*Fmr1*) display delayed maturation of synaptic properties as well as a delayed onset and offset of the CP for LTP at thalamocortical synapses, without affecting NMDAR currents or dendritic morphology of layer 4 neurons (Harlow et al., 2010). Despite the fact that most of the previous studies are correlative, the study of *Fmr1* knock-out animals indicates that specific molecules may be required for controlling the timing (onset and offset) of CPs for experience-dependent synaptic plasticity in sensory cortices.

Intracortical synapses

Several studies have shown that sensory deprivation induces plasticity within a CP not only in layer 4, but also in supragranular layers of primary sensory cortices,

suggesting the presence of CPs for synaptic plasticity at intracortical synapses. However, direct evidence for CPs at intracortical synapses in primary sensory cortices has been lacking.

Recently, our laboratory has discovered a new CP for rapid sensory experience-induced synaptic potentiation in layer 2/3 pyramidal cells of mouse barrel cortex (Wen and Barth, 2011). After a brief period (24 hr) of single whisker experience (SWE), the synaptic strength (amplitude of strontium-mediated mEPSCs) of two types of excitatory synapses (layer 4-2/3 and layer 2/3-2/3) innervating a single layer 2/3 pyramidal cell increases (Wen and Barth, 2011). Experience-induced synaptic potentiation at both synapses exhibits a postnatal CP; the CP at layer 4-2/3 synapses exhibits an earlier onset (P12) and earlier offset (P14) compared with that (P13-16) at layer 2/3-2/3 synapses (Wen and Barth, 2011).

This is the first study that demonstrates a CP for experience-induced synaptic plasticity of intracortical synapses in primary sensory neocortex. This study is remarkable because of the identification of synapse-specific CPs at two converging inputs terminating in the same postsynaptic cell, which no other study has previously reported. Furthermore, these two inputs occupy overlapping dendritic domains on the basal dendrites of postsynaptic layer 2/3 cells, yet exhibit incredibly specific onset timing of CPs (one day earlier for layer 4 inputs), supporting the presence of a precise mechanism that regulates the initiation of highly specific plasticity at neighboring synapses.

One possible explanation for the different onset timing of CPs at layer 4-2/3 vs. 2/3-2/3 synapses is the difference in the maturation of whisker-evoked responses of layer 4 vs. layer 2/3 cells. It has been shown that in developing rat barrel cortex, layer 4 cells

respond to whisker stimulation reliably at P12 compared with layer 2/3 cells that did not respond to whisker stimulation until P14 (Stern et al., 2001). The earlier maturation of layer 4 responses to sensory stimulation can drive an early onset of synaptic plasticity at layer 4-2/3 synapses. SWE-induced synaptic potentiation of both layer 4-2/3 and 2/3-2/3 synapses within the CPs coincides with an increase in the firing rate of layer 2/3 cells to deflection of the spared whisker input (Benedetti et al., 2009). Although the CP closes earlier for synaptic strengthening of layer 4-2/3 synapses (P14), the offset timing of the CP for potentiation of layer 2/3-2/3 synapses (P16) is correlated with a decline in the response potentiation of layer 2/3 cells (after P16) (Benedetti BL, unpublished data), suggesting a mechanistic link between experience-dependent LTP of layer 2/3-2/3 synapses and response potentiation of layer 2/3 neurons after brief sensory experience (24 hr SWE) in barrel cortex.

However, in contrast to a discrete CP for synaptic potentiation of layer 4-2/3 synapses induced by altered sensory experience in vivo, theses synapses maintain the capacity for spike-timing dependent potentiation (STDP) through adulthood when induced in vitro in wildtype animals (Banerjee et al., 2009). This discrepancy suggests that a developmental decline in the capacity for synaptic strengthening at the intracortical synapses is unlikely to explain the CP offset at layer 4-2/3 synapses induced in vivo, rather experience-dependent changes in synaptic or cortical functions might underlie the closure of the CP. The lack of a developmental regulation of STDP at layer 4-2/3 synapses also stands in contrast with the developmental decline of LTP at thalamocortical synapses that is correlated with experience-induced plasticity in layer 4, suggesting

distinct mechanisms for regulating experience-induced cortical plasticity and critical periods at different cortical layers.

Experiments performed in this thesis (Appendix C) suggest that NMDAR-dependent synaptic depression may regulate the closure of the CP at layer 4-2/3 synapses, as NMDAR blockade in vivo can briefly reopen the CP (for one day). In contrast, NMDAR-dependent synaptic depression seems to be not present at layer 2/3-2/3 synapses, suggesting remarkable input-specificity in regulation of the CP offset in a single layer 2/3 cell. A description of the detailed mechanisms to explain how the input-specificity of CP timing (onset and offset) regulation is achieved will require further investigation.

Structural plasticity as a mechanim for cortical plasticity and CP regulation

In addition to LTP and LTD, the plasticity at thalamocortical synapses that contributes to experience-dependent plasticity of cortical response can be manifested at the structural level. Recent evidence has shown that rapid reduction in the number and size of thalamocortical axonal butons occurs after 3 d monocular deprivation in layer 4 of mouse V1 within the CP (P21-28) for ocular dominance plasticity (Coleman et al., 2010). Passive tone-rearing can induce plasticity of tonographic map in primary auditory cortex (A1) that displays a critical period which involves pathways upstream of the thalamus (Barkat et al., 2011). Forebrain-specific deletion of the cell-adhesion molecule (Icam5) that normally slows spinogenesis can induce precocious spine maturation in layer 4 of A1 and result in an early CP (Barkat et al., 2011), suggesting that structural plasticity of thalamocortical synapses in A1 plays a critical role in controlling the onset timing of the CP for auditory cortical plasticity.

In barrel cortex, all-whisker deprivation induces rearrangement of the whisker map representation in layer 2/3 neurons, measured by whisker evoked subthreshold activity (Stern et al., 2001). This form of subthreshold receptive field plasticity of layer 2/3 neurons is limited to a brief CP (P12-14) (Stern et al., 2001), concomitant with the CP in experience-dependent motility of dendritic spines on layer 2/3 pyramidal cells in vivo (Lendvai et al., 2000). In addition, these brief CPs (P12-14) coincide with the developmental maturation of intrinsic properties of layer 2/3 pyramidal cells (Maravall et al., 2004), of whisker-evoked layer 2/3 responses (Stern et al., 2001) and the developmental onset of active whisking behavior in rodents (P13-14) (Welker et al., 1964). These developmental changes in neuronal, circuit properties and animal behavior could contribute to the initiation of experience-dependent plasticity for cortical responses.

Maturation of network inhibition as a mechanism for cortical CP regulation

The critical period onset for ocular dominance plasticity

Although activity-dependent synaptic plasticity (LTP and LTD) are attractive mechanisms for experience-induced cortical plasticity, a large body of evidence has suggested that maturation of network inhibition plays a critical role in controlling the timing of critical periods for cortical plasticity. Overexpression of BDNF in transgenic animals accelerates the maturation of GABAergic innervation and leads to an early onset as well as an early offset of ocular dominance plasticity (Huang et al., 1999). This study suggests a role of cortical inhibition as an alternative mechanism for cortical plasticity regulation. A substantial amount of work about CP regulation by the maturation of cortical inhibition has been done in visual cortex, strongly supporting the notion that

maturation of a selective component of inhibitory circuitry controls the onset timing of ocular dominance plasticity.

In a series of experiments designed to decrease or increase visual cortical inhibition, the onset of ocular dominance plasticity was shown to be delayed or accelerated. Mice carrying a deletion in one isoform of glutamic acid decarboxylase (GAD65), a GABA-synthesizing enzyme expressed in inhibitory terminals, failed to show MD-induced shift in cortical response to the open eye; conversely, acute infusion of a use-dependent $GABA_A$ receptor antagonist, benzodiazepine can rescue the plasticity in the GAD65 knock-out animals (Hensch et al., 1998). Consistently, a premature onset of plasticity in wildtype animals can be induced by a prolonged treatment of diazepam (Fagiolini and Hensch, 2000; Fagiolini et al., 2004). Additionally, dark rearing from birth that slows the maturation of cortical inhibitory network (Morales et al., 2002) delayed the CP onset (Mower, 1991), which can be prevented by diazepam infusion (Iwai et al., 2003) or BDNF expression (Gianfranceschi et al., 2003). A most recent study shows that transplantation of exogenous inhibitory neurons into visual cortex can induce a new critical period of ODP in adult animals past the normal CP (Southwell et al., 2010).

Further evidence indicates that specific types of GABAergic interneurons, possibly parvalbumin (PV)-expressing interneurons are required to initiate CP plasticity. Fagiolini et al. (2004) have found that inhibitory synapses expressing α1 subunit-containing $GABA_A$ receptors are specifically required for ODP. Because theses α1 subunit-containing, somatically localized $GABA_A$ receptors are opposite to axon terminals of PV-expressing large basket cells (Fagiolini et al., 2004), the inhibitory circuitry that regulates CP onset preferentially engages PV-expressing basket cells,

suggesting that the maturation of specific inhibitory circuitry underlies regulation of CP onset timing. Further supporting the specific involvement of PV cells, a recent study showed that cortical expression of an embryonic homeoprotein, Otx2, preferentially in PV cells of visual cortex can accelerate PV cell development and cause an early CP onset (Sugiyama et al., 2008).

The possible mechanisms for maturation of cortical inhibitory circuits in regulating the onset timing of CPs in visual cortex have been proposed to involve: 1) the requirement of a minimal level of inhibition to enhance the contrast between the deprived and non-deprived inputs by selectively reinforcing the deprived and damping the non-deprived activity at a time when cortical excitation is too high; 2) a role of a sustained level of inhibition to enhance precise timing of principle cell spiking and to facilitate associative plasticity, specifically LTD at the deprived inputs (Jiang et al., 2005).

The critical period offset for ocular dominance plasticity

Compared with a permissive level of inhibition that facilitates plasticity, saturation of inhibitory function might reduce cortical plasticity, presumably by reducing principle cell firing. Thus an increase in inhibitory function can play a role in the closure of CPs (Hensch, 2005; Jiang et al., 2005; Gandhi et al., 2008). However, whether cortical inhibition regulates the offset timing of cortical CPs is still under debate. For example, spike-timing dependent potentiaiton (STDP) at mouse layer 4-2/3 synapses (without blocking inhibition) does not exhibit a critical period in vitro and can still be induced in adult animals, whereas spike-timing dependent depression (STDD) at these synapses displays a critical period (<P25) (Banerjee et al., 2009). These results suggest that a

simple model of increased inhibition is not sufficient to explain both the offset of the CP for STDD and the lack of a CP for STDP at the same synapses. More complex circuit or synaptic mechanisms might be required to differentially control plasticity at these synapses.

Studies performed in adult visual cortex have suggested the involvement of other mechanisms, including epigenetic modulation of gene transcription and factors affecting neurite outgrowth (axons and dendrites). Histone acetylation, an epigenetic mechanism for gene transcription has been found to be greatly reduced in adult animals compared to young animals (Putignano et al., 2007). Enhancing the level of histone acetylation by applying histone deacetalyse inhibitor can reopen the critical period for ODP in adult animals (Putignano et al., 2007). It has been suggested that "structural consolidation" or slowdown of axonal or dendritic dynamics controls the closure of CPs (Hensch, 2004). Age-dependent increase of myelination in adult animals makes it less permissive for neurite outgrowth and in adult mice that do not express Nogo-66 receptor, receptor for myelin-derived factor Nogo that inhibits axonal outgrowth, the CP for ODP can be reactivated (McGee et al., 2005). Also, extracellular matrix (ECM) plays an important role in shutting the CP for plasticity. Chondroitin sulphate proteoglycans (CSPGs) are components of the extracellular matrix (ECM) shown to inhibit axonal sprouting and they form perineuronal nets. Degradation of CSPGs by chondroitinase-ABC can reactivate plasticity in adult animals (Pizzorusso et al., 2002).

Based on the above studies in adult cortex, it seems that the "structural consolidation" is necessary to maintain the adult cortex at a non-permissive state after the closure of the CP. However, whether it is the same mechanism that closes cortical CPs is

unknown. Future investigation will have to focus on events occurring at the time of CP closure instead of looking at the adult animals in general.

Summary

In summary, critical periods are a general phenomenon across species and sensory modalities, which describe a sensitive epoch during development with heightened susceptibility to plasticity of different types. Common mechanisms for regulating CPs in the neocortex involve: 1) experience-dependent synaptic plasticity (LTP and LTD) at thalamocortical and intracortical circuits, which likely regulates the offset of CPs; and 2) selective inhibitory circuitry, particularly PV interneurons and their targets which regulate the onset and possibly the offset of CPs. These two (synaptic and network) main mechanisms may interact with each other to contribute to cortical plasticity within the CPs and control CP timing and duration in neocortical circuits in a concerted way.

Overall, the inhibitory hypothesis seems to explain the causality better than the synaptic plasticity hypothesis for which most evidence is correlative. However, the detailed mechanism of how inhibition regulates the timing of critical periods is still speculative. Furthermore, since most studies supporting the inhibitory hypothesis are performed in visual cortex, the applicability to other cortical areas needs to be verified.

1.5 Calcium permeable AMPARs and synaptic plasticity

The α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA receptors, AMPARs) are ionotropic glutamate receptors that mediate the majority of fast excitatory synaptic transmission in the brain. AMPARs are tetramers that consist of a combination of four subunits GluR1-4 (GluRA-D or GluA1-4) (Dingledine et al., 1999), encoded by four different genes *GRIA1-4* (Wisden and Seeburg, 1993; Hollmann and

Heinemann, 1994). Each GluR subunit contains an extracellular N terminus (NTD), four membrane-associated hydrophobic domains (M1-4) including a re-entrant M2 loop, and a cytoplasmic carboxy-terminal tail (c-tail). Subunits GluR1, 4 and 2L (long-form of GluR2) have long c-tails, while GluR2, GluR3, and GluR4S (short-form of GluR4) have shorter c-tails. In the forebrain including the cortex and hippocampus, the predominant GluR subunits are GluR1 and GluR2 and the major type of AMPARs in these brain areas exist in the form of GluR1/GluR2 heteromers (Wenthold et al., 1996). Although GluR2/3 heteromers are also found in principle cells of hippocampus and cortex (Wenthold et al., 1996; Tsuzuki et al., 2001; Sans et al., 2003), these receptors only account for a minor population. Expression of different GluR subunits is brain region-specific and developmentally regulated.

Over the past decades, a large body of evidence has indicated that a prevalent mechanism for LTP depends on increased trafficking of AMPARs (Malenka and Bear, 2004; Kessels and Malinow, 2009). Trafficking of AMPARs has been associated with synaptic plasticity in vitro and behavioral plasticity in vivo. Experience-induced increase in AMPAR trafficking at layer 4-2/3 synapses in barrel cortex has also been observed and been considered an important mechanism for potentiation of firing response to the spared input. Disruption of GluR1 trafficking at layer 4-2/3 excitatory synapses in live rodents abolishes whisker experience-dependent synaptic potentiation in barrel cortex (Takahashi et al., 2003). Genetic knock-out of GluR1 in juvenile mice display deficits in SWE-induced response potentiation and layer 4-2/3 LTP in vitro (Hardingham and Fox, 2006; Dachtler et al., 2011).

One of the most interesting features about GluR2, the predominant GluR subunit in the brain is that more than 95% of GluR2 subunits contain an arginine residue (R607) within the re-entrant M2 loop that is located in the pore region of AMPARs. This occurs as a result of

hydrolytic RNA editing of an adenosine base to inosine mediated by adenosine deaminase enzyme ADARs (Higuchi et al., 1993). The presence of the R607 in the channel pore results in low Ca^{++} permeability, low single channel conductance and it prevents endogenous polyamine-mediated block of AMPARs. Thus edited GluR2-containing AMPARs show a linear current-voltage relationship (Boulter et al., 1990; Jonas and Burnashev, 1995; Swanson et al., 1997). In contrast, another type of AMPARs that lack edited GluR2, likely GluR1 homomers in the forebrain are permeable to Ca^{++}, and have higher single channel conductance and an inwardly rectifying current-voltage relationship due to voltage-dependent block by endogenous polyamines (Hestrin, 1993; Bowie and Mayer, 1995; Geiger et al., 1995; Jonas and Burnashev, 1995). The unique biophysical properties of GluR2-lacking CP-AMPARs are often used as tools to examine the abundance of these channels at a given synapse. A number of polyamines or polyamine derivatives and toxins such as philanthotoxin (Phtx), joro spider toxin (Jst) and 1-naph-thylacetyl-spermine (NASPM) have been used successfully to specifically block and probe the function of CP-AMPARs (Washburn and Dingledine, 1996). However, some studies show that these drugs can also have off-target effects by blocking other polyamine-modulated receptors such as nicotinic, kainate, and NMDARs (Washburn and Dingledine, 1996).

GluR2 subunit expression is low during early development but rapidly increases after the first post-natal week (Monyer et al., 1991), resulting in a developmental switch of AMPARs from GluR2-lacking to -containing (Kumar et al., 2002; Brill and Huguenard, 2008). Electrophysiological data show CP-AMPARs are the predominant form of AMPARs in layer 2/3 of barrel cortex before P11 and are replaced by GluR2-containing calcium-impermeable AMPARs (CI-AMPARs) afterwards (Brill and Huguenard, 2008). Because CP-AMPARs can

provide a Ca^{++} source that does not reply on depolarization to facilitate LTP induction, recent evidence has suggested a role of CP-AMPARs in synaptic strengthening. Supporting this hypothesis, brief high-frequency synaptic stimulation in Schaffer collateral synapses in GluR2 knockout animals results in LTP that is not blocked by NMDAR antagonist D-AP5 and requires activation of CP-AMPARs and postsynaptic Ca^{++} entry (Asrar et al., 2009). In wildtype animals, insertion of CP-AMPARs has been detected transiently (before 25 min) in CA1 pyramidal cells and soon replaced by CI-AMPARs after pairing-induced LTP at Schaffer collateral synapses (Plant et al., 2006). Blockade of CP-AMPARs during this period abolishes the maintenance of synaptic strength (Plant et al., 2006). In vivo experience to induce rapid synaptic strengthening mediated by trafficking of CP-AMPARs, has been documented in many brain areas including the nucleus accumbens, ventral tegmental area, and the amygdala (Rumpel et al., 2005; Bellone and Luscher, 2006; Matsuo et al., 2008). Single whisker experience (SWE) drives rapid synaptic strengthening at layer 4-2/3 synapses mediated in part by GluR2-lacking CP-AMPARs in some ages (at P12-13), associated with increased firing of layer 2/3 neurons in the spared barrel column (Clem and Barth, 2006; Glazewski et al., 2007; Benedetti et al., 2009). However, other studies have shown that CP-AMPAR insertion or activation is not essential for activity-induced LTP of hippocampal CA3-CA1 synapses in either young (2-3 weeks) or adult (2-3 months) rodents (Adesnik and Nicoll, 2007; Gray et al., 2007). In addition, the results from our laboratory have also shown that although incorporation of CP-AMPARs can be detected in some conditions during 24 hr SWE-induced synaptic strengthening, it is not necessary at either layer 4-2/3 or layer 2/3-2/3 excitatory synapses (Wen and Barth, 2011). However, since synaptic CP-AMAPRs were only examined after 24 hr of SWE, the possibility of a transient increase in synaptic CP-AMPARs during the early stages of

SWE-induced synaptic plasticity exists, which requires further investigation. Despite the fact that CP-AMPARs do not seem to play an essential role during LTP, they are shown to be associated with reversal of experience-dependent synaptic potentiation in some systems (Bellone and Luscher, 2006; Ho et al., 2007; Clem and Huganir, 2010; Yang et al., 2010; Lante et al., 2011). These data, combined with the transient nature of synaptic CP-AMPARs during in vitro LTP support the notion that these receptors might confer lability onto recently potentiated synapses that express these receptors and subject them to depression. However, the lability of synaptic strength or synaptic weakening does not always involve trafficking of CP-AMPARs. Recent evidence from our group shows that SWE-induced strengthening at layer 4-2/3 synapses is unstable and subject to depression by a brief (5 min) postsynaptic depolarization paired with presynaptic stimulation and CP-AMPARs are neither sufficient nor necessary for this form of synaptic weakening (Wen and Barth, 2012). Taken together, it seems that trafficking of CP-AMPARs can sometimes occur during Hebbian plasticity in certain brain regions but might not be a common principle of synaptic plasticity across the brain.

Moreover, CP-AMPARs have been proposed to mediate non-Hebbian forms of plasticity, homestatic synaptic plasticity or synaptic scaling after network inactivity. Chronic tedrototoxin (TTX) treatment of cultured cortical neurons leads to an increase in synaptic strength, mediated by preferential insertion of CP-AMPARs and blockade of these receptors by philanthotoxin-433 (PhTx) or NASPM abolishes this synaptic scaling (Man, 2011). Furthermore, in cortical interneurons where CP-AMPARs predominate amongst the synaptic AMPAR population of the excitatory synapses onto these neurons (Carter and Regehr, 2002; Walker et al., 2002), CP-AMPARs have been shown to control neuronal excitability, shape neuronal firing and engage in circuit function. Fast-spiking (FS) cells in layer 4 of barrel cortex

receive faster and greater excitation from the thalamus, compared to the neighboring regular spiking cells. The greater thalamic excitation onto FS cells is due to a larger quantal amplitude that is mediated by CP-AMPARs at thalamocortical synapses onto these cells (Hull et al., 2009). Blockade of CP-AMPARs leads to a complete loss of feed-forward inhibition onto RS cells, suggesting an essential role of CP-AMPARs in generating thalamocortical feed-forward inhibition that is critical for sensory information integration during the initial phases of cortical sensory processing (Hull et al., 2009). Thus, in non-principle neurons like some interneurons where GluR1 subunit expression is abundant and CP-AMPARs are the predominant population of synaptic AMPARs, these receptors could play critical roles in local microcircuit function.

1.6 NR2 subunits and implications for synaptic plasticity

N-Methyl-D-aspartate (NMDA) type of glutamtate receptors are critical mediators of neural circuit development, many forms of activity-dependent synaptic plasticity and important brain functions including learning and memory (Malenka and Bear, 2004). Dysregulation of NMDARs has been linked to various neurological diseases, such as acute and chronic pain, cerebral ischemia, Huntington's disease, Parkinson's disease, Alzheimer's disease and depression and thus NMDARs have been used as therapeutic targets to treat these diseases (Waxman and Lynch, 2005; Mony et al., 2009).

NMDA receptors consist of two obligatory NR1 subunits and two NR2 or NR3 subunits. There are four subtypes of NR2 subunit, i.e. NR2A, NR2B, NR2C and NR2D. The recently discovered NR3 family consists of NR3A and NR3B. NMDA receptors made of different subunit composition display distinct channel properties. Activation of postsynaptic NMDARs requires both glutamate binding and release of Mg^{++} block by depolarization, thus

they are a coincidence detector of pre- and postsynaptic activity, an attribute required for Hebbian forms of synaptic plasticity. NMDARs pass substantial amount of Ca^{++} (Burnashev et al., 1995) that in turn activate downstream Ca^{++}-dependent signaling cascades. All these properties of NMDARs make them well positioned to mediate and regulate synaptic plasticity.

NR2A and NR2B are the two predominant NR2 subtypes that are expressed in the hippocampus and neocortex. In contrast, NR2C and NR2D (mRNA and protein) are expressed at a much lower level in the forebrain compared with NR2A and NR2B (Monyer et al., 1994; Standaert et al., 1996; Laurie et al., 1997). Because NR2A and 2B are the major subtypes in the cerebral cortex where extensive NMDAR-dependent synaptic plasticity has been observed, most studies of NR2 subunits have been focused on NR2A and NR2B. Studies on regional distribution of NR2A and NR2B in developing rats have shown that NR2B expression predominates starting at the embryonic stages, peaks around the third postnatal week and is maintained at a constant level that persists into adulthood; in contrast, NR2A expression in the brain does not begin until the end of the first postnatal week and then rapidly increases and then stabilizes (Monyer et al., 1994; Laurie et al., 1997). Differential regulation of NR2B vs. NR2A during early development results in a decrease in the NR2B/NR2A ratio. Compared with NR2A-containing receptors, NR2B-containing NMDARs display slower rise and decay kinetics, lower channel open probability and probably carry different amount of total charge transfer and Ca^{++} influx, given a specific synaptic activity (Yashiro and Philpot, 2008). In addition, NR2A- and NR2B-containing NMDARs can be blocked by distinct antagonists that show high (at least hundreds of fold difference) selectivity towards one subunit vs. the other. These differences in channel properties allow detection of NR2A and NR2B using electrophysiological techniques. Consistent with a developmental loss of NR2B prevalence,

electrophysiological data show a faster decay time of NMDAR-mediated currents and decreased sensitivity to NR2B antagonist, ifenprodil (Carmignoto and Vicini, 1992; Hestrin, 1992; Sheng et al., 1994; Roberts and Ramoa, 1999; Barth and Malenka, 2001). Both synaptic and extrasynaptic NMDARs in postsynaptic cells have been found in the brain, although they can also be expressed presynaptically (Corlew et al., 2008). It has been suggested that NR2A-containing NMDARs are synaptic while NR2B-containing receptors are extrasynaptic (Stocca and Vicini, 1998; Tovar and Westbrook, 1999; Scimemi et al., 2004). However, this view has been controversial because of synaptic NR2B and extrasynaptic NR2A could also be detected in some conditions (Mohrmann et al., 2000; Harris and Pettit, 2007).

Research in the past decade has been focused on understanding precise functional roles of NR2B vs. NR2A and the significance of the developmental switch of NR2 subunit. The NR2B to NR2A switch has been once proposed to regulate the critical period for experience-dependent plasticity at thalamocortical synapses. Although the coincidence between the critical period of thalamocortical plasticity and subunit switch of NR2 may suggest a causal relationship, NR2A knockout animals that do not undergo such subunit switch still show the critical period, indicating that the NR2 subunit switch is not required for the critical period closure at thalamocortical synapses (Barth and Malenka, 2001; Lu et al., 2001). Recent evidence suggests that a high level of NR2B during early development might facilitate normal neural development by maintaining a proper level of homeostatic plasticity (Wang et al., 2011). Genetic replacement of NR2B by NR2A prematurely leads to deficits in homeostatic synaptic plasticity induced by brief activity blockade and reduces social exploratory behavior with hyper-locomotion, akin to schizophrenia-like behaviors (Wang et al, 2011), suggesting a required role of NR2B in facilitating normal synaptic function during cortical development that

could not be replaced by NR2A. Interestingly, NR2B-containing NMDARs inhibit surface expression of GluR1 subunit and reduce AMPAR currents in developing synapses of cultured cortical neurons (Hall et al., 2007). A more recent study performed in developing synapses of CA1 neurons further demonstrates that basal level of NR2B activation prevents maturation of silent synapses thus limiting the total number of functional synapses before the arrival of correlated activity, whereas NR2A activation decreases synaptic strength thus preventing further potentiation (Gray et al., 2011). This study suggests that NMDARs may mediate different form of plasticity in mature synapses compared with young synapses and that during early development NR2B and NR2A both act to suppress AMPAR synaptic transmission, however via different means. The mechanism that underlies the NR2B to NR2A conversion has been shown to involve mGluR5-mediated signaling in hippocampal CA1 pyramidal cells (Matta et al., 2011).

Because NR2B- and NR2A-containing NMDARs show distinct expression profiles during early development and display different channel properties, speculation has thus arisen as to what specific roles NR2A and NR2B may play in synaptic plasticity, that is, one NR2 subunit may be linked specifically to LTP or LTD. Although some studies showed a specific requirement of NR2B for LTD and of NR2A for LTP (Liu et al., 2004; Massey et al., 2004), other studies failed to replicate theses results (Morishita et al., 2007). On the contrary, a number of studies have clearly demonstrated that NR2B subunit mediates LTP and facilitates learning and memory in prefrontal cortex (Zhao et al., 2005), hippocampus (Tang et al., 1999) or amygdale-dependent tasks (Miwa et al., 2008), despite the fact that blocking NR2B only has partial effects in LTP that was induced in these brain areas.

In addition to the NR2B to NR2A switch that occurs during normal development, altered sensory experience has been shown to bidirectionally regulate the relative abundance of NR2B vs. NR2A (Quinlan et al., 1999a; Philpot et al., 2001). Dark rearing rapidly decreases NR2A expression thus decreasing the NR2A/NR2B ratio in visual cortex, an effect that could be reversed upon subsequent light exposure (Quinlan et al., 1999b), suggesting bidirectional modulation of NR2 subunit composition by alterations of sensory experience. Bienenstock et al. (1982) proposed a theoretical model, termed "Bienenstock, Cooper, and Munro" (BCM) model which postulates that the frequency thresholds for inducing LTP vs. LTD are modifiable; the final outcome of synaptic plasticity under certain condition is determined by the thresholds for LTP vs. LTD and thus the shape of the BCM curve as defined by the two thresholds. An important finding from dark-reared mice is that the frequency thresholds for inducing LTP and LTD in vitro change, resulting in a left shift in the BCM curve which allows LTP to be induced with lower stimulation frequency (Kirkwood et al., 1996; Philpot et al., 2003; Philpot et al., 2007). The electrophysiological data of visual cortical NR2A vs. NR2B content and LTP vs. LTD in vitro after dark rearing suggest that the change in NR2A/NR2B ratio is correlated with the change in plasticity induction thresholds, suggesting that the relative balance between NR2A and NR2B might determine the direction of synaptic plasticity, in the way predicted by the BCM model. The NR2 subunit switch also occurs during ocular dominance plasticity (ODP) in the binocular zone of mouse visual cortex. The bi-phasic plasticity of the early response depression to the deprived-eye inputs (3 d) followed by a slower potentiation to the open-eye inputs (5 d) (Frenkel and Bear, 2004) has been proposed to involve LTD in the deprived-eye pathway and LTP in the open-eye pathway. However, the intermingled nature of eye-specific inputs in visual cortex makes the investigation of pathway-specific plasticity impossible.

Recent biochemical analysis during monocular deprivation shows a reduction in NR2A/NR2B ratio during the slower phase of ODP (potentiation), caused by coordinated changes in both NR2B and NR2A proteins in synaptoneurosomes prepared from bionocular visual cortex (Chen and Bear, 2007). Since NR2A knock-out animals exhibit a much lower LTP induction threshold (Philpot et al., 2007), the reduction in NR2A/NR2B ratio following monocular deprivation may lower the threshold for LTP induction and facilitate response potentiation of cortical neurons to the open-eye inputs. However, whether the changes in NR2A/NR2B occur at the open-eye inputs/synapses or globally in the cortical neurons, remains unknown. Therefore, complete understanding of the molecular mechanisms regulating experience-dependent plasticity will require synapse-specific analysis of NR2 subunit composition.

There are several hypotheses regarding how NR2A- and NR2B-containing NMDARs might differentially regulate synaptic plasticity. The most studied are the cytoplasmic carboxy-terminal tails (c-tails) of NR2A and NR2B (Fetterolf and Foster, 2011). The c-tails of NMDARs have been implicated in NMDAR-dependent synaptic plasticity and learning because of the presence of various phosphorylation sites and PDZ domains that offer points of modification as well as linkage to interact with scaffolding proteins within postsynaptic density (PSD). Truncation of c-tails of NR2A, 2B and 2C has caused severe defects in animals ranging from lethality to deficits in synaptic plasticity and learning (Sprengel et al., 1998). NR2A and NR2B each preferentially interact with distinct sets of proteins and kinases, the tight coordination of which determines the outcome of plasticity (Kennedy et al., 2005). Ca^{++}/calmodulin-dependent protein kinase II (CaMKII) that plays a well established role in LTP, binds to the c-tail of NR2B more potently compared with NR2A and its preferential

binding to NR2B specifically associates NR2B with LTP (Barria and Malinow, 2005). These results also suggest that synaptic NR2B not only acts as a coincidence detector and channel that passes Ca^{++}, but as a critical signaling molecule to recruit downstream effectors necessary for LTP induction. In addition, NR2B has been shown to preferentially interact with other proteins including Ras-guanine nucleotide-releasing factor 1 (Ras-GRF1) (Krapivinsky et al., 2003) and Ras GTPase activating protein (RasGAP) (Kim et al., 1998). On the other hand, NR2A preferentially binds to nitric oxide synthase (NOS) (Al-Hallaq et al., 2007). NR2A and NR2B might also bind synaptic scaffolding proteins with different affinity, for example, membrane-associated guanylate kinases (MAGUKs), which link them to distinct intracellular signaling pathways and thus lead to different outcome of plasticity (Kennedy et al., 2005). Recent evidence shows that in contrast to a critical role of NR2B in LTP, NR2A seems to inhibit LTP by recruiting negative regulators via its c-tail (Foster et al., 2010).

Finally, the stability of synaptic NR2A vs. NR2B might be different, likely due to different surface diffusion rate (Tovar and Westbrook, 2002), which could be modified during synaptic plasticity and sensory experience in vivo. The relative stability of NR2A vs. NR2B affects the dwelling time of these subunits in synapses and influences the ratio of distinct interacting proteins specifically associated with the c-tail of each subunit, resulting in distinct polarity of synaptic plasticity.

In summary, alteration of sensory experience can induce rapid changes in the relative abundance of NR2A vs. NR2B in cortical principle neurons. The resulting change in the NR2A/NR2B ratio can shift the plasticity thresholds for induction of LTP vs. LTD and lead to distinct outcome of plasticity, in that a higher NR2A/NR2B ratio favors LTD whereas a lower ratio favors LTP. Therefore, the experience-induced change in NR2A/NR2B ratio can serve as

a molecular mechanism for synaptic metaplasticity (plasticity of synaptic plasticity), a phenomenon that describes the change in the capacitiy for LTP or LTD as a result of prior neural activity (Abraham, 2008).

1.7 Presynaptic NMDARs

Presynaptic receptors that modulate neurotransmitter release have been detected in the brain, including ligand-gated ion channels such as $GABA_A$ receptors, glycine receptors, kainite receptors and G protein coupled receptors such as $GABA_B$ receptors and metabotropic glutamate receptors (Corlew et al., 2008).

NMDARs have been added to this presynaptic receptor list by the initial finding in the glutamatergic synapses onto layer 2 of entorhinal cortex, where the frequency of spontaneous miniature EPSCs was reduced by NMDAR antagonist D-APV (Berretta and Jones, 1996), when postsynaptic NMDARs were blocked. This finding suggests that tonic activation of putative presynaptic NMDARs enhances spontaneous glutamate release. Following the first discovery, electrophysiological evidence has shown the presence of functional non-postsynaptic NMDARs in many other glutamatergic synapses, including layer 5 of entorhinal cortex, layer 2/3, 4 and layer 5 of visual cortex, layer 2/3 of barrel cortex, CA1 and dendate gyrus of hippocampus (Corlew et al., 2008). In almost all of these cases, presynaptic NMDARs act to enhance spontaneous glutamate release. In addition, presynaptic NMDARs also modulate evoked neurotransmitter release. Bath application of D-APV while postsynaptic NMDARs are blocked decreases the amplitude of evoked AMPA-EPSC and increases paired pulse ratio between layer 5 pyramidal neurons in primary visual cortex (Sjostrom et al., 2003) and layer 4 to 2/3 pyramidal neurons in barrel cortex (Brasier and Feldman, 2008).

Interestingly, in both cortex and hippocampus, NR2B-containing NMDARs are the predominant presynaptic NMDAR subtype (Berretta and Jones, 1996; Sjostrom et al., 2003; Bender et al., 2006b; Yang et al., 2006; Corlew et al., 2007; Brasier and Feldman, 2008; Rodriguez-Moreno and Paulsen, 2008), although NR2D (Mameli et al., 2005; Suarez et al., 2005; Suarez and Solis, 2006) and NR3 subunits (Larsen et al., 2011) have also been found. Additionally, electrophysiological data suggest synapse-specific distribution of presynaptic NMDARs. In an elegant study performed in barrel cortex (P14-22) (Brasier and Feldman, 2008), focal application of D-APV in layer 2/3 decreases the frequency of miniature EPSCs (mEPSCs) in layer 2/3 pyramidal cells, however, D-APV application in layer 4 of the same column does not, suggesting presynaptic NMDARs that promote spontaneous release are not located in the somatodendritic portion of layer 4 cells but in the axonal terminals or nearby sites of layer 4 inputs. Furthermore, this study demonstrates layer 4-4 and cross-columnar 2/3-2/3 excitatory synapses do not show an effect of APV on mEPSC frequency, suggesting synapse specificity of presynaptic NMDARs. Taken together, these results are consistent with a likely axonal location of presynaptic NMDARs at layer 4-2/3 synapses and a synapse-specific nature of these receptors in barrel cortex.

Presynaptic NMDARs may also play a role in activity-dependent synaptic plasticity, in particular spike timing-dependent depression (STDD). Induction of STDD by a post- followed by pre- activity sequence could be blocked by NMDAR antagonist while postsynaptic NMDARs are blocked by either hyperpolarization or intracellular MK801 at layer 5-5 (Sjostrom et al., 2003) and layer 4-2/3 synapses (Corlew et al., 2007) in visual cortex, as well as layer 4-2/3 synapses in barrel cortex (Bender et al., 2006b). In both cortical areas, putative presynaptic NMDARs are required for STDD in young animals (<P20); however, STDD in

older animals (>P20) at layer 4-2/3 synapses in visual cortex requires post- but not presynaptic NMDARs (Corlew et al., 2007), suggesting a strict age dependence of STDD. More direct evidence of the involvement of presynaptic NMDARs in STDD in primary sensory cortices has come from paired recordings between connected layer 4 and layer 2/3 cells, where blockade of NMDARs in the layer 4 but not layer 2/3 cell by intracellular MK801 abolishes STDD (Rodriguez-Moreno and Paulsen, 2008). These data suggest that presynaptic NMDARs play an important role in STDD specifically during early development (<P20) and could be implicated in experience-dependent map plasticity.

Despite the electrophysiological evidence suggesting the presence of presynaptic NMDARs at axon terminals, anatomical evidence is needed for an accurate characterization of these receptors. Immuno-eletron microscopy (EM) studies using antibodies against NR1 or NR2 subunits show NR1- and NR2B-positive immunoreactivity in presynaptic compartments of neocortex (Aoki et al., 1994; DeBiasi et al., 1996; Charton et al., 1999; Corlew et al., 2007), hippocampus (Siegel et al., 1994; Charton et al., 1999; Jourdain et al., 2007), amygdala (Farb et al., 1995; Pickel et al., 2006), spinal cord (Liu et al., 1994) and the cerebellum (Petralia et al., 1994; Bidoret et al., 2009). Although these studies suggest axonal expression of presynaptic NMDARs, the expression level of these receptors is usually low. Other studies have suggested that the mechanism for NMDAR regulating release probability is via somatodendritic NMDAR-mediated depolarization that activates voltage-gated calcium channels (VSCCs) in the terminals, rather than via axonally localized presynaptic NMDARs. One of such studies by Christie and Jahr (2008) shows that in cerebellar stellate interneurons, activation of dendritically localized NMDARs by exogenous NMDA application or synaptic stimulation triggers depolarization that passively propagates to axon terminals and regulates

neurotransmitter release through activation of VSCCs (Christie and Jahr, 2008). Further supporting the non-axonal localization of NMDARs, they find that focal NMDA application at the axon terminals of layer 5 pyramidal cells in visual cortex, fails to elicit Ca^{++} entry in the terminals, arguing against the presence of axonal NMDARs (Christie and Jahr, 2009).

Expression of presynaptic NMDARs has been found to be regulated by development. In hippocampal CA1 cells, there is a developmental decline in presynaptic NMDAR function after P5, due to the loss of NR2D subunit (Mameli et al., 2005). The low Mg^{++} sensitivity of NR2D-containing presynaptic NMDARs makes these receptors more likely to be activated at hyperpolarized membrane potentials. Similar loss of presynaptic NMDAR function that is correlated with a developmental decline in its expression, has been found in layer 5 of entorhinal cortex (Yang et al., 2006) and layer 2/3, 4 and 5 pyramidal cells of visual cortex (Corlew et al., 2007). In addition, a unique population of presynaptic NMDARs has been recently identified containing NR3A (Larsen et al., 2011). Earlier research has shown that NR3A expression exhibits a strict temporal window (before P12) (Wong et al., 2002). The presence of NR3A subunit in NMDARs reduces Mg^{++} sensitivity and Ca^{++} permeability of the channels (Perez-Otano et al., 2001; Sasaki et al., 2002). Postsynaptic NR3A expression in early development acts as a "brake" to inhibit premature synaptogenesis and synaptic strengthening (Roberts et al., 2009). Interestingly, the newly identified NR3A-containing presynaptic NMDARs confer low Mg^{++} sensitivity and thus facilitate tonic activation of presynaptic NMDARs during early development (Larsen et al., 2011). In addition, STDD induced in young animals (P13-18) requires both NR2B and NR3A (Larsen et al., 2011), suggesting that presynaptic NMDARs exist as NR1/NR2B/NR3A triheteromers during this developmental period. In addition to normal developmental regulation, presynaptic NMDAR function could

be influenced by changes in neural activity. Blockade of NMDAR activation in the visual cortex of anesthetized rats acutely reduces NR2B immunoreactivity at both pre- and postsynaptic compartments of layer 1 (Fujisawa and Aoki, 2003). Epileptic activity has been found to reactivate presynaptic NMDARs in the entorhinal cortex of mice being treated chronically with seizure-inducing agents (Yang et al., 2006).

What are the mechanisms for presynaptic NMDAR-mediated regulation of neurotransmitter release? Several hypotheses have been proposed that revolve around the increase in Ca^{++} concentration within the axonal terminal. The most parsimonious hypothesis is direct depolarization by NMDARs, as with other presynaptic ionotropic receptors (Engelman and MacDermott, 2004). Indeed, activation of somatodendritically localized NMDARs is sufficient to depolarize the axonal terminals that in turn activate VSCCs to increase transmitter release (Christie and Jahr, 2008). On the other hand, other hypotheses suggest NMDAR-mediated increase in terminal Ca^{++} that directly enhances release probability. Evidence supporting this hypothesis has come from studies where the increase in neurotransmitter release is independent of VSCCs (Glitsch, 2008). However, the signaling pathways that lie downstream of the increase in terminal Ca^{++} concentration after presynaptic NMDAR activation remain largely unknown. Whether the initial increase in Ca^{++} further activates other intracellular Ca^{++} source leading to further Ca^{++} increase, or it activates protein kinase pathways that in turn modulate efficacy of molecules involved in vesicle release machinery, or it works by saturating intracellular Ca^{++} buffers and then increases free Ca^{++}, remain largely unknown. Taken together, the detailed mechanisms of presynaptic NMDARs might be more complicated than originally thought and might involve age-specific and brain region-specific regulations.

1.8 Lability of synaptic strength and metaplasticity

One long-lasting and hotly pursued topic in neuroscience and psychology is memory and amongst the most commonly asked questions about memory is its stability. The early experiments done by psychologists have shown that memory, at least some forms of it are labile and that the ability to recall the initially learnt material decays over time. In the past decade, memory consolidation research that largely focuses on hippocampus-dependent memory tasks has demonstrated that new memories are labile shortly after their acquisition and are sensitive to disruption by a wide variety of manipulations, such as hippocampal damage, inhibition of new protein synthesis, electroconvulsive shock (ECS) or learning a different task (Nader et al., 2000). After the initial labile phase with heightened sensitivity to disruption, memories then enter into a stabilization phase when they are not sensitive to the same disruptive forces, a phase that is commonly known as "consolidation" (Nader et al., 2000; Nader and Einarsson, 2010). However, the "consolidated" memories do not remain stabilized forever; instead, they can re-enter the labile state during later memory retrieval or reactivation, when the same manipulations capable of disrupting new memories can lead to the loss of reactivated memories. This phase where reactivated memories are subject to degradation is called "reconsolidation". In addition to hippocampus-dependent spatial memory and amygdala-dependent emotional memory, procedural motor learning in humans displays a similar labile phase during reactivation that seems to depend upon sleep and wakeful states (Walker et al., 2003). Similary, reconsolidation was reported in episodic memories in humans as well (Hupbach et al., 2007). The similarities between the late phase of LTP (L-LTP) and memory reconsodilation have proposed L-LTP as a possible physiological mechanism for memory reconsolidation (Nader and Einarsson, 2010).

Consistent with a labile nature of memories, the maintenance of enhanced synaptic strength after LTP induction has been shown to be unstable in vivo and in vitro. At the medial perforant path-dendate gyrus synapse, LTP induced by theta burst stimulation (TBS) protocol in awake, freely moving rats decays within 3-5 days (Villarreal et al., 2002). Sustained injection (over a 7-d period, once per day) of NMDAR antagonist, CPP intraperitoneally into the animals after LTP induction can block the decay (Villarreal et al., 2002). Consistent with the effect of prolonged LTP, sustained CPP injection has been shown to enhance spatial memory retention in an 8-arm radial task (Villarreal et al., 2002). These results indicate that activity-dependent increase in synaptic strength is labile and subject to NMDAR-mediated depression in vivo. They also indicate that in contrast to the intial role of NMDARs in mediating LTP induction, subsequent NMDAR activation can weaken synaptic strength and degrade memory retention. In hippocampal CA1 neurons, the magnitude of tetanus-induced LTP was greatly reduced by seizure activity (Hesse and Teyler, 1976). These results showing a labile nature of LTP in vivo can be supported by the in vitro data as well. In retinotectal synapses of tadpoles, an increase in spontaneous activity can reverse LTP induced by either repetitive electrical stimulation or visual stimulation (Zhou et al., 2003).

Metaplasticity, or plasticity of plasticity is a higher order form of synaptic plasticity, which describes the change in the capacity or direction of synaptic plasticity after prior activity (Abraham, 2008). The standard induction paradigms of metaplasticity involve a priming stimulus, either biochemical signals or synaptic activation before the second round of plasticity induction. Sometimes the priming stimulus only changes the ability of synapses to undergo subsequent LTP or LTD without changing the basal synaptic strength. In other cases where LTP is initially induced, the priming stimulus results in synaptic depression. Whether this

subsequent synaptic depression represents “depotentiation” where synaptic gain from prior LTP simply reverses or whether LTD is induced remains unknown (Abraham, 2008). In terms of the molecular mechanisms, some evidence suggests that depotentiation might be a distinct cellular phenomenon compared with de novo LTD, evidenced by different cellular processes involved. For example, depotentiation involves dephosphorylation at different serine sites on GluR1 of synaptic AMPARs (Lee et al., 2000; Huang et al., 2001). Calcineurin Aα is only required for depotentiation, but not for LTD (Zhuo et al., 1999). Also, NMDARs may be differentially involved during depotentiation vs. LTD. Long-term potentiation (LTP) in CA1 neurons of cultured hippocampal slices by pairing postsynaptic depolarization with 2 Hz synaptic stimulation, results in a robust swapping of NR2B by NR2A; low frequency stimulation (0.1 Hz) shortly after LTP induction (5 min), a depotentiation protocol reverses both the synaptic strengthening and changes the NR2 subunit composition from NR2A back to NR2B (Bellone and Nicoll, 2007). However, the change in NR2 subunit composition could not be detected during LTD induction (Bellone and Nicoll, 2007) on the same synapses, suggesting that depotentiation and LTD may employ distinct mechanisms by differentially affecting NR2 subunit composition in this experimental system. On the other hand, some evidence suggests that depotentiation and LTD may employ overlapping mechanisms, since both types of plasticity have been shown to require NMDAR activation (Fujii et al., 1991; Wagner and Alger, 1995) and mGluRs (Bashir and Collingridge, 1994; Bolshakov and Siegelbaum, 1994; Oliet et al., 1997; Fitzjohn et al., 1998; Huber et al., 2000). Because the mechanisms for LTD and depotentiation are complicated and it is difficult to differentiate these processes, they are often used interchangeably in the literature.

A body of in vitro evidence shows that depotentiation of potentiated synapses could occur by either low frequency or theta-frequency stimulation (Barrionuevo et al., 1980; Staubli and Lynch, 1990; Fujii et al., 1991; Larson et al., 1993; O'Dell and Kandel, 1994; Staubli and Chun, 1996). Furthermore, in vivo physiological activity has also been shown to cause depotentiation as electrically induced LTP in hippocampus could be rapidly weakened after adult rats entered a novel environment within an hour after LTP induction and these changes in synaptic strength were accompanied by a concomitant increase in network spontaneous activity (Xu et al., 1998; Manahan-Vaughan and Braunewell, 1999). To support the role of spontaneous activity in depotentiation or lability of synaptic strengthening, it has been shown that an increase in spontaneous activity at retinotectal synapses could reverse electrical stimulation- or visual experience-induced LTP (Zhou et al., 2003). These studies suggest that a heterosynaptic mechanism mediated by a general increase in network activity could potentially explain depotentiation observed in the above studies Furthermore, in vivo physiological activity has also been shown to cause depotentiation as electrically induced LTP in hippocampus could be rapidly weakened after adult rats entered a novel environment within an hour after LTP induction and these changes in synaptic strength was accompanied by a concomitant increase in network spontaneous activity (Xu et al., 1998; Manahan-Vaughan and Braunewell, 1999). To support the role of spontaneous activity in depotentiation or lability of synaptic strengthening, it has been shown that an increase in spontaneous activity at retinotectal synapses can reverse theta burst stimulation (TBS) or visual stimulation-induced LTP (Zhou et al., 2003). These studies suggest that a heterosynaptic mechanism mediated by a general increase in network activity could potentially explain depotentiation, as has been found with LTD and depotentiation (Doyere et al., 1997). However, synaptic weakening has been shown

to only occur at potentiated synapses, suggesting input specificity of this type of plasticity (Staubli and Lynch, 1990; Larson et al., 1993; Staubli and Chun, 1996).

1.9 Summary of aims and findings

In this thesis, we use mouse barrel cortex as a model system to study experience-dependent synaptic plasticity in anatomically defined cortical circuitry. The experiments presented in this thesis were aimed at understanding the cellular and molecular mechanisms of experience-dependent plasticity in layer 2/3 pyramidal neurons, the principle cells of the supragranular layer in barrel cortex, during early development.

Single whisker experience (SWE) has been shown to increase synaptic strength of layer 4-2/3 excitatory synapses. To examine if this synaptic strengthening is developmentally regulated, we show that rapid experience-dependent changes in the strength of excitatory synapses exhibit a critical period that is input specific and mechanistically distinct in layer 2/3 pyramidal neurons. These results are presented in Chapter 2.

Recently potentiated layer 4-2/3 synapses are labile and subject to experience-dependent depression in vivo and in vitro. To examine if CP-AMPARs confer lability to recently potentiated synapses, we develop an assay to depress the strength of individual layer4-2/3 excitatory synapses after SWE, using strontium (Sr^{++})-replaced artificial cerebrospinal fluid (ACSF) solution (Sr-depression). We show that CP-AMPARs are neither sufficient nor required for synaptic depression after in vivo plasticity. These results are presented in Chapter 3.

Lastly, to examine if ongoing experience progressively increases synaptic strength in vivo, we measure synaptic strength at layer 4-2/3 excitatory synapses at different time points after the onset of experience. We show that ongoing whisker experience triggers an early phase of plasticity where synaptic strength progressively increases, followed by a labile phase where synaptic strength weakens and a subsequent stabilization phase where synaptic strength does not change. We also show that change in NMDAR function is tightly correlated with experience-induced alteration in synaptic strength at layer 4-2/3 synapses, suggesting an NMDAR-dependent metaplasticity mechanism in determining the direction of plasticity in vivo. These results are presented in Chapter 4.

2. Input-specific critical periods for experience-dependent plasticity in layer 2/3 pyramidal neurons

2.1 Introduction

Layer 2/3 neurons are major integrators of sensory input. Alterations in sensory experience can alter the properties of these neurons, increasing firing output in response to sensory stimulation (Fox, 1992; Diamond et al., 1993; Diamond et al., 1994; Glazewski and Fox, 1996; Barth et al., 2000; Glazewski et al., 2007; Benedetti et al., 2009). This is due in part to potentiation of excitatory synapses from layer 4, the input layer of the cortex, onto layer 2/3 neurons (Clem and Barth, 2006; Clem et al., 2008; Clem et al., 2010). In addition, increased firing output after sensory stimulation may also be due to changes in synaptic drive from other layer 2/3 neurons, which themselves are strongly interconnected (Lefort et al., 2009).

SWE-driven increases in whisker-evoked firing occur quickly (within 24 hrs of SWE) in young postnatal animals. In this case, increased firing output to stimulation of the single spared whisker can be localized to the spared whisker barrel column itself, as well as in surrounding barrel columns (Glazewski and Fox, 1996; Glazewski et al., 2007; Benedetti et al., 2009). In older animals, longer periods of SWE (>7 days) are required to potentiate sensory-evoked firing in the spared barrel column, indicating that the threshold for this plasticity is developmentally regulated.

At least part of the mechanism underlying rapid increases in sensory-evoked firing in young animals is increased excitatory drive resulting from the post-synaptic addition of AMPARs to layer 4-2/3 synapses (Clem and Barth, 2006; Clem et al., 2008). However, it is unknown whether the experience-dependent strengthening of excitatory inputs onto layer 2/3 neurons declines during postnatal development and might account for the reduced capacity of

these neurons to exhibit SWE-induced increases in sensory-evoked responses in adult animals (Glazewski et al., 2007; Benedetti et al., 2009).

Here we show that synaptic identity controls a capacity for in vivo, experience-dependent plasticity. The rapid strengthening of layer 4-2/3 inputs by SWE has been well-characterized (Clem and Barth, 2006; Clem et al., 2008; Clem et al., 2010). Previous work indicates that this potentiation occurs via the post-synaptic addition of calcium-permeable AMPARs (CP-AMPARs; (Clem and Barth, 2006), requiring NMDAR-activation for initiation (Clem et al., 2008). Here, we show that SWE also potentiates putative layer 2/3-2/3 synapses within the spared whisker barrel column, and that the mechanisms of this in vivo plasticity are differentially regulated compared to layer 4-2/3 inputs.

Although both types of synapses exhibit developmentally regulated, experience-dependent synaptic strengthening, critical period timing is both delayed and prolonged for layer 2/3 inputs compared to layer 4 inputs. Additionally, the molecular mechanisms underlying this plasticity are distinct between the two pathways. SWE is sufficient to induce the delivery of CP-AMPARs at layer 4 but not layer 2/3 inputs, although plasticity at both inputs can occur without the trafficking of CP-AMPARs. Despite the prolonged presence of ifenprodil-sensitive, NR2B-containing NMDARs at layer 4 inputs, these synapses display a shorter critical period than layer 2/3-2/3 inputs, which contain more NR2A. These data indicate that *in vivo*, a capacity for synaptic potentiation is regulated with extraordinary synaptic specificity.

2.2 Materials and Methods

Animals

Wild-type or heterozygous mice (males and females) from a fosGFP (1-3 line, C57Bl6 background) transgenic line (fosGFP+/-) ages P11-P17 were used. Bilateral whisker deprivation was performed where all but the D1 whisker on one side were removed (Barth et al., 2000). Animals were returned to their home cages for 24 hrs before recording. Control animals were whisker-intact littermates of the deprived animals. Since there was no significant difference between control wild-type C57Bl6 and fosGFP+/-, data from these animals were grouped. Recordings in control animals were not restricted to the D1 barrel column, since all columns were equivalent in whisker-intact animals. The barrel column representing the "spared" D1 whisker was identified by enhanced fosGFP expression and relative position to the hippocampus in acute brain slices.

Whole-cell recording

Animals were anesthetized with isoflurane and decapitated. Coronal slices with 350 μm thickness were vibratome sectioned in regular artificial cerebrospinal fluid (ACSF) at 2-6^{o}C composed of (mM): 119 NaCl, 2.5 KCl, 2.5 $CaCl_2$, 1.3 $MgSO_4$, 1 NaH_2PO_4, 26.2 $NaHCO_3$, 11 glucose and equilibrated with 95/5% O_2/CO_2. Slices were maintained and whole-cell recordings were carried out at room temperature. Somata of lower layer 2/3 pyramidal neurons in barrel cortex were targeted for whole-cell recording with borosilicate glass electrodes with a resistance of 6-8 MOhm. Electrode internal solution was composed of (in mM): 130 cesium-gluconate, 10 HEPES, 0.5 EGTA, 8 NaCl, 4 Mg-ATP and 0.4 Na-GTP, at pH 7.25-7.30, 290-300 mOsm and contained trace amounts of Alexa-568. Pyramidal cell identity was confirmed after the recording session by pyramidal soma morphology and the presence of

dendritic spines. Only cells with $R_{series} \leq 20$ MΩ and $R_{input} \geq 200$ MΩ, where changes in either measurement were less than 20% were included for analysis. Stimulation of presynaptic afferents was applied at ~0.1 Hz by placing glass monopolar electrodes in the center of layer 4 barrels or mid-layer 2 pyramidal cell layer. Although this method cannot exclusively isolate layer 2/3 inputs, the high density of within-layer inputs indicates that the majority of inputs are likely to come from other layer 2/3 neurons. We refer to these layer 2/3 inputs as "putative" to indicate this uncertainty. Postsynaptic responses from layer 2/3 pyramidal neurons within the same barrel column were recorded. Electrophysiological data were acquired by Multiclamp 700A (Axon Instruments, Foster City, CA) and a National Instruments acquisition interface. The data was filtered at 3 kHz and digitized at 10 kHz and collected by Igor Pro 6.0 (Wavemetrics, Lake Oswego, Oregon). Extracellular simulation was controlled by a Master-8 (A.M.P.I, Israel).

AMPA EPSC measurement and rectification index (RI)

To isolate AMPAR-mediated excitatory post-synaptic currents (EPSCs), D-APV (50 μM) and picrotoxin (Ptx, 50 μM) were included to block NMDAR and $GABA_AR$ activation in regular ACSF that contained 1 mM $MgSO_4$. Spermine (100 μM) was included in the internal solution to avoid washout of endogenous polyamines. Layer 2/3 pyramidal neurons were voltage-clamped at -70 mV and stimulus intensity was adjusted until a clear monosynaptic response (2-7 ms latency, consistent across trials for a given response) was visible for every sweep. For a series of holding potentials at -70, -40, -20, 0, 20, 40 mV, 10-20 sweeps were collected and averaged. The peak amplitudes of the averaged current trace at each holding potential was normalized to that at -40 mV, based on which current-potential (I-V) curve was constructed for each cell. A mean I-V curve was generated by averaging across all cells in a

group. Liquid junction potential (~+10 mV) was not corrected for. Reversal potential for the AMPA-EPSC, or E_{rev} was obtained for each cell from its I-V curve. The rectification index (RI) was calculated based on the following formula:

$$RI=I_{+40}/(40-E_{rev})/I_{-40}(E_{rev}+40)$$

Evoked Sr-AMPA mEPSCs measurement

To measure the amplitude of stimulus-evoked miniature AMPAR-EPSCs, Sr^{++} (3 mM) was substituted for Ca^{++} in regular ACSF to drive asynchronous glutamate release. D-APV (50 μM) and picrotoxin (Ptx, 50 μM) were included to pharmacologically isolate AMPAR-mediated EPSCs and 5 mM QX314 was included in the Cs-gluconate internal solution to reduce noise in recordings of miniature events. Layer 2/3 pyramidal neurons were voltage-clamped at -70 mV. The evoked response has an initial synchronous component (~50 ms post the stimulus artifact) which was excluded in the analysis. Isolated, asynchronous events that occurred from 50-500 ms after the stimulus were manually selected and analyzed using Minianalysis software (Synaptosoft, Inc. Decatur, GA). The detection threshold for events was set at 2x RMS noise (usually around 4-5 pA) and data were filtered with a low-pass filter at 1 kHz. Approximately 100 random events were selected for each cell and then grouped for each condition. Comparisons were made between groups. Averaged traces for each experimental condition were obtained by grouping average traces from selected events for all cells.

NMDA EPSC measurement and ifenprodil sensitivity

To pharmacologically isolate NMDAR-mediated EPSCs, NBQX (5 μM) and Ptx (50 μM) were included in the bath solution (regular ACSF). Cells were held at +40 mV and synaptic EPSCs were collected in voltage clamp mode. A single exponential function was fit to the average NMDA EPSC trace from its peak to 200 ms after the stimulus onset and a decay constant tau (τ)

was determined from the fit. To assess the content of NR2B-containing NMDARs, ifenprodil (10 μM), an NR2B specific antagonist was infused to the recording chamber locally since the action of ifenprodil was poorly reversible (Kumar and Huguenard, 2003) after a stable baseline was achieved for 5 mins. A post response was collected 5 mins after infusion to allow complete diffusion.

Ratio of AMPAR:NMDAR-mediated EPSCs

Cells were held at -70 mV and +40 mV to isolate the AMPA-EPSC and NMDA-EPSC, respectively. The amplitude of the AMPA-EPSC was taken at the peak of synaptic response recorded at -70 mV, and the amplitude of the NMDA-EPSC was taken 50 ms after the stimulus onset at +40 mV, when the AMPA-EPSC component decays to baseline. A ratio of AMPA-EPSCs and NMDA-EPSC amplitudes were obtained from these values.

Dual-pathway recordings

For experiments where synaptic responses from layer 4-2/3 and layer 2/3-2/3 excitatory pathways were recorded from the same cell, two stimulating electrodes were set up in layer 4 and layer 2 of the same barrel column. Stimulation frequency was the same for both pathways (0.l Hz).

Statistics

For all non-pairwise comparisons, a non-parametric Mann Whitney U test (two-tailed) was used. For all pairwise comparisons (dual-pathway and ifenprodil sensitivity experiments), a paired Student t-test was used. For Sr-EPSC amplitude comparisons between control and SWE conditions (Figs 1 and 3), cell values were averaged within an animal and then averaged across animals for that age group. To determine whether SWE-induced plasticity was developmentally regulated, mean Sr-EPSC amplitudes were statistically evaluated using a

Mann Whitney U test followed by a Bonferroni correction (groups P12-14 for layer 4-2/3 synapses, P13-16 for layer 2/3-2/3 synapses) to compare control and SWE conditions. The corrected p value was derived from the Mann Whitney p value multiplied by the number of possible contiguous groupings (15 in this study).

2.3 Results

2.3.1 A critical period for experience-dependent plasticity at layer 4-2/3 synapses

Layer 4 excitatory neurons make multiple contacts onto layer 2/3 pyramidal cells, providing strong and reliable input that varies with stimulus strength. In order to assess the mean amplitude of an individual synaptic contact, we stimulated layer 4 neurons in ACSF where Ca^{++} had been replaced with Sr^{++} to desynchronize neurotransmitter release (Goda and Stevens, 1994; Abdul-Ghani et al., 1996; Xu-Friedman and Regehr, 1999) while recording the response of layer 2/3 neurons (Fig.1A). Under these conditions, the delayed occurrence of AMPAR-mediated miniature EPSCs (mEPSCs) can be observed (Fig. 1D), events that are thought to reflect the release of a single neurotransmitter vesicle.

Previous work from the lab has shown that 24 hrs of SWE results in an increase in the mean amplitude of AMPAR-EPSCs evoked by layer 4 stimulation in the presence of Sr (Sr-EPSCs), compared to control undeprived animals. To determine whether the magnitude of plasticity at layer 4-2/3 synapses was developmentally regulated, we initiated 24 hrs of SWE at different postnatal ages. In order to identify the cortical representation of the spared whisker, acute brain slices from fosGFP transgenic mice that had undergone SWE were prepared (Fig. 1C), and stimulating and recording electrodes were placed in the spared barrel column.

Twenty-four hours of SWE from postnatal day 10 (P10)-P11 was insufficient to induce an increase in the quantal amplitude of AMPAR-EPSCs (control 10.8±0.81 pA versus SWE 9.0±0.70 pA; Fig. 1E,F). At this time, the overall input strength from layer 4 to layer 2/3 is weak (Lendvai et al., 2000; Maravall et al., 2004). However, a single day later, SWE was sufficient to drive a significant increase in the amplitude of layer 4-2/3 excitatory (P12 control 9.5±0.48 versus SWE 12.6±0.61 pA). SWE-induced increases in excitatory synaptic strength were significant at P13 and P14 (Fig. 1E,F). However, at P15 SWE was no longer capable of triggering an increase in Sr-EPSC amplitude (P15 control 10.5±0.32 versus SWE 10.4±0.39), and this result was observed for all subsequent ages examined (Fig.1F). Thus, we find that at layer 4-2/3 synapses, rapid experience-dependent increase in synaptic strength can be induced only for a short time window at the end of the second postnatal week, from P12-P14 (P12-14 control versus SWE $p=0.0001$ with Bonferroni Correction).

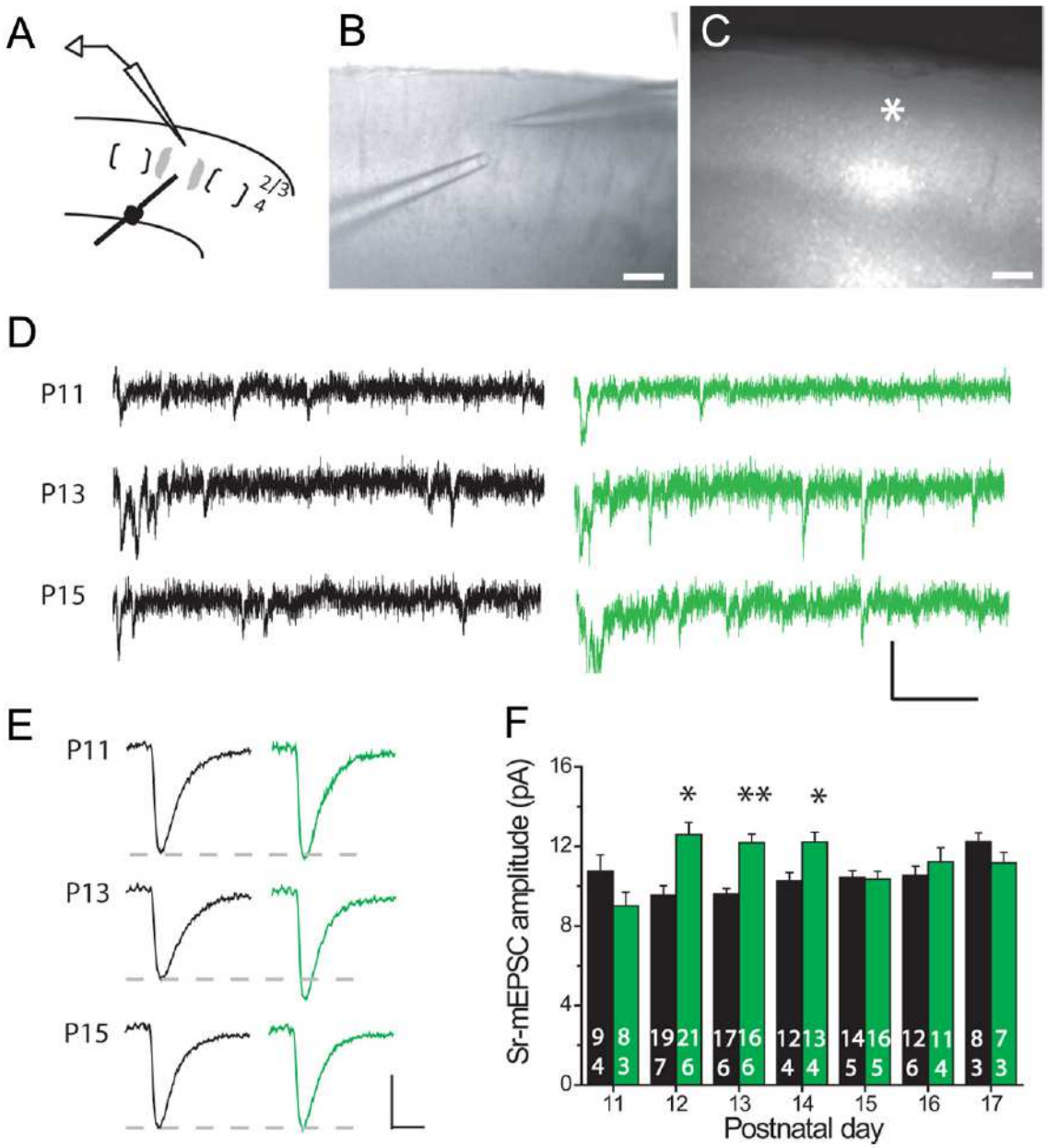


Figure 1. SWE induces rapid increase in evoked Sr-mEPSC amplitude at layer 4-2/3 excitatory synapses only during a short developmental window. ***A***, Schematic of stimulating and recording electrode placement for measuring layer 4-2/3 Sr-mEPSCs. ***B***, Brightfield image of recording configuration in one barrel column. Scale bar: 200 µM. ***C***, Low-magnification fluorescence image of the spared barrel column with strong signal located in layer 4 (barrel column indicated with asterisk). Scale bar: 200 µM. ***D***, Example traces from control (black) and SWE (green) at postnatal age P11, 13 and 15. Scale bars: 20 pA, 100 ms. ***E***, Averaged traces of evoked Sr-mEPSCs from all cells at P11, 13 and 15. Scale bars: 5 pA, 5 ms. Dashed line indicates the control amplitude for comparison. ***F***, Mean amplitude of evoked Sr-mEPSCs recorded at postnatal ages P11-17 from control (black) and SWE-treated animals (green) at layer 4-2/3 synapses. The number of cells (top) and animals (bottom) used are indicated on the bar graph. *p<0.05, **p<0.01 between age-matched control and SWE-treated cell groups by age using Mann-Whitney U test

2.3.2 CP-AMPARs and SWE-induced plasticity

We and others have shown that addition of CP-AMPARs can occur during potentiation of excitatory synapses, both *in vivo* and *in vitro* (Shi et al., 1999; Hayashi et al., 2000; Zhu et al., 2000; Takahashi et al., 2003; Bellone and Luscher, 2006; Clem and Barth, 2006; Plant et al., 2006; Matsuo et al., 2008). Under control, undeprived conditions, layer 4-2/3 synapses show a linear I-V relationships (Fig. 2B,C) and slow EPSC decay kinetics (Fig. 2G-H), characteristic of GluR2-containing AMPARs. After 24 hrs of SWE, AMPARs with fast decay kinetics and rectifying I-V relationships characteristic of CP-AMPARs can be detected (Clem and Barth, 2006); P13 rectification index (RI; control 0.81±0.06; SWE 0.57±0.05; Fig. 2C, F, see also Sr-EPSC decay time G-H). Because CP-AMPARs possess a long-C-terminal tail that promotes activity-dependent mobilization to the synapse (Sheng and Lee, 2001) and also exhibit higher single-channel conductance than calcium-impermeable AMPARs (Swanson et al., 1997), trafficking of these receptors to the excitatory inputs can increase synaptic strength in several ways. Furthermore, the developmental regulation of CP-AMPARs has been noted in previous studies (Kumar et al., 2002; Brill and Huguenard, 2008). Thus, it was tempting to speculate that SWE-induced potentiation of layer 4-2/3 synapses might require CP-AMPARs, and the unavailability of these AMPARs might trigger the closure of the critical period.

To determine whether the end of rapid, SWE-induced plasticity was associated with a reduction in the SWE-dependent trafficking of CP-AMPARs, we evaluated the electrophysiological properties of potentiated layer 4-2/3 inputs at P14 after SWE. Surprisingly, we observed robust potentiation even in the absence of CP-AMPARs (P14

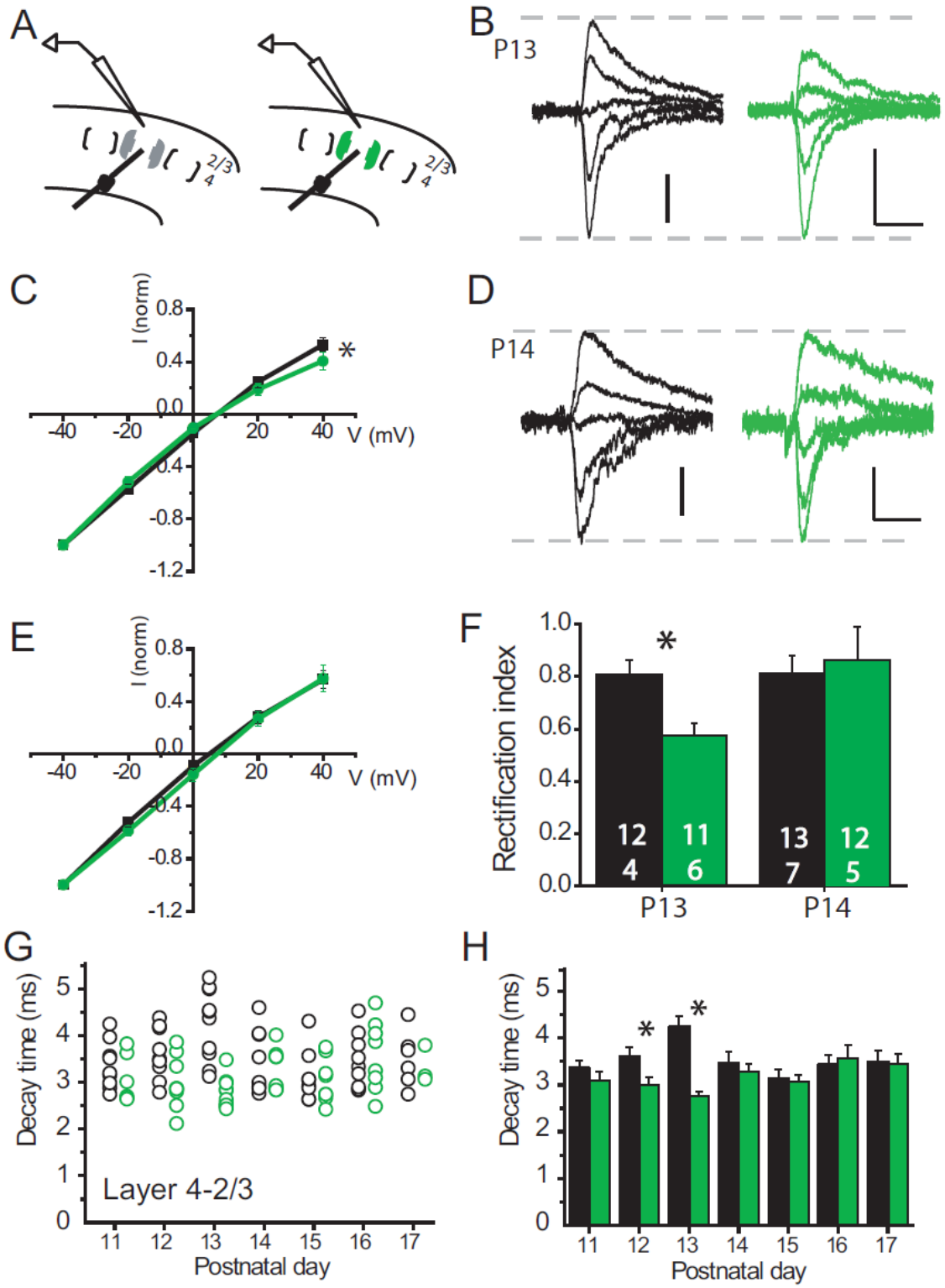


Figure 2. SWE drives CP-AMPARs to layer 4-2/3 synapses only at P13 but not P14. ***A,*** **S**chematics of electrode placement for control (black) and SWE-treated tissue (green) at layer 4-2/3 synapses. ***B***, Example layer 2/3 pyramidal cells from a P13 control (black) and a SWE-treated (green) animal showing layer 4-evoked AMPA-EPSCs recorded at -40, -20, 0, +20 and +40 mV holding potentials.

Scale bars: 20 pA, 20 ms. The SWE cell shows clear EPSC rectification at positive holding potentials compared to the control cell. ***C***, Normalized AMPA-EPSC amplitude I (to amplitude at -40 mV) as a function of holding membrane potential V (I-V curve) for control and SWE animals at P13. ***D***, Example cells from a P14 control and a SWE animal as in ***B)***. Scale bars: 10 pA, 20 ms. ***E,*** I-V curves for control and SWE at P14 as in ***C)***. ***F,*** Rectification index for control and SWE animals at P13 and P14 at layer 4-2/3 synapses. ***G,*** Scatter plot of Sr-mEPSC decay time for control and SWE-treated animals at layer 4-2/3 pathway from P11-17. ***H***, Mean Sr-mEPSC decay time for layer 4-2/3 pathway. *$p<0.05$.

RI for control 0.81±0.07 versus SWE 0.86±0.13, Fig. 2D-F, also G-H). Indeed, increased rectification of AMPAR-EPSCs after SWE was prominent for only one postnatal day, at P13. These data indicate that strong experience-dependent synaptic strengthening can occur without the trafficking of CP-AMPARs, and that a decline in the availability of CP-AMPARS is unlikely to explain the close of the critical period at layer 4-2/3 synapses.

2.3.3 Experience-dependent potentiation of layer 2/3-2/3 excitatory synapses

In our studies to characterize SWE-induced changes in sensory-evoked firing across development, the rapid potentiation of firing rates in layer 2/3 neurons can be observed into the third postnatal week, although it declines soon afterward (Benedetti et al., 2009). Since this time period extends past the critical period for SWE-induced potentiation at layer 4-2/3 synapses, it is likely that other processes must be at work to facilitate increased firing output after stimulation of the spared whisker.

Previous work has not addressed whether other excitatory inputs onto layer 2/3 pyramidal neurons also undergo SWE-induced increases synaptic strength. To examine whether intralaminar excitatory inputs might also show SWE-induced potentiation, AMPAR-mediated Sr-EPSCs were compared between control and SWE-treated animals. In this case, the stimulation electrode was placed in layer 2, within the same barrel column (Fig 3A,B). This form of stimulation will primarily activate intralaminar connections from other

layer 2/3 neurons since these neurons are densely interconnected and receive little input from other cortical layers (Lefort et al., 2009), although excitatory synapses from other areas may also be driven by this form of stimulation. Similar to layer 4-2/3 inputs, SWE assessed at P11 was not sufficient to drive increases in Sr-EPSC amplitude (control 12.0±0.54 pA versus SWE 11.0±0.42 pA). However, unlike at layer 4-2/3 inputs, SWE assessed at P12 was also unable to drive a significant change in Sr-EPSC amplitude (control 11.9±0.80 pA versus SWE 12.2±0.51 pA, Fig. 3C-E).

SWE-driven increases in Sr-EPSC amplitude at the layer 2/3-2/3 inputs were first observed at P13 (control 10.8±0.54 pA versus SWE 13.4±0.97 pA). Thus, altered sensory input in the form of SWE can drive the strengthening of intralaminar, putative layer 2/3-2/3 inputs, although the timing of this phenomenon is distinct from that observed at layer 4-2/3 inputs.

Developmental analysis of SWE-induced changes in Sr-EPSC amplitude at layer 2/3-2/3 synapses revealed that this plasticity was prolonged by several days compared to layer 4-2/3 synapses. At P16, SWE led to a significant increase in synaptic strength (control 10.0±0.37 pA versus SWE 12.4±0.75 pA), two days after the closure for rapid, SWE-induced increases in the amplitude of layer 4-2/3 inputs (Fig. 3E) (P13-16 control versus SWE $p=0.01$ with Bonferroni correction). These results show that onset timing, duration, and end of SWE-induced plasticity are distinct for layer 4-2/3 and layer 2/3-2/3 synapses and indicate that experience-dependent plasticity is regulated in an input-specific manner.

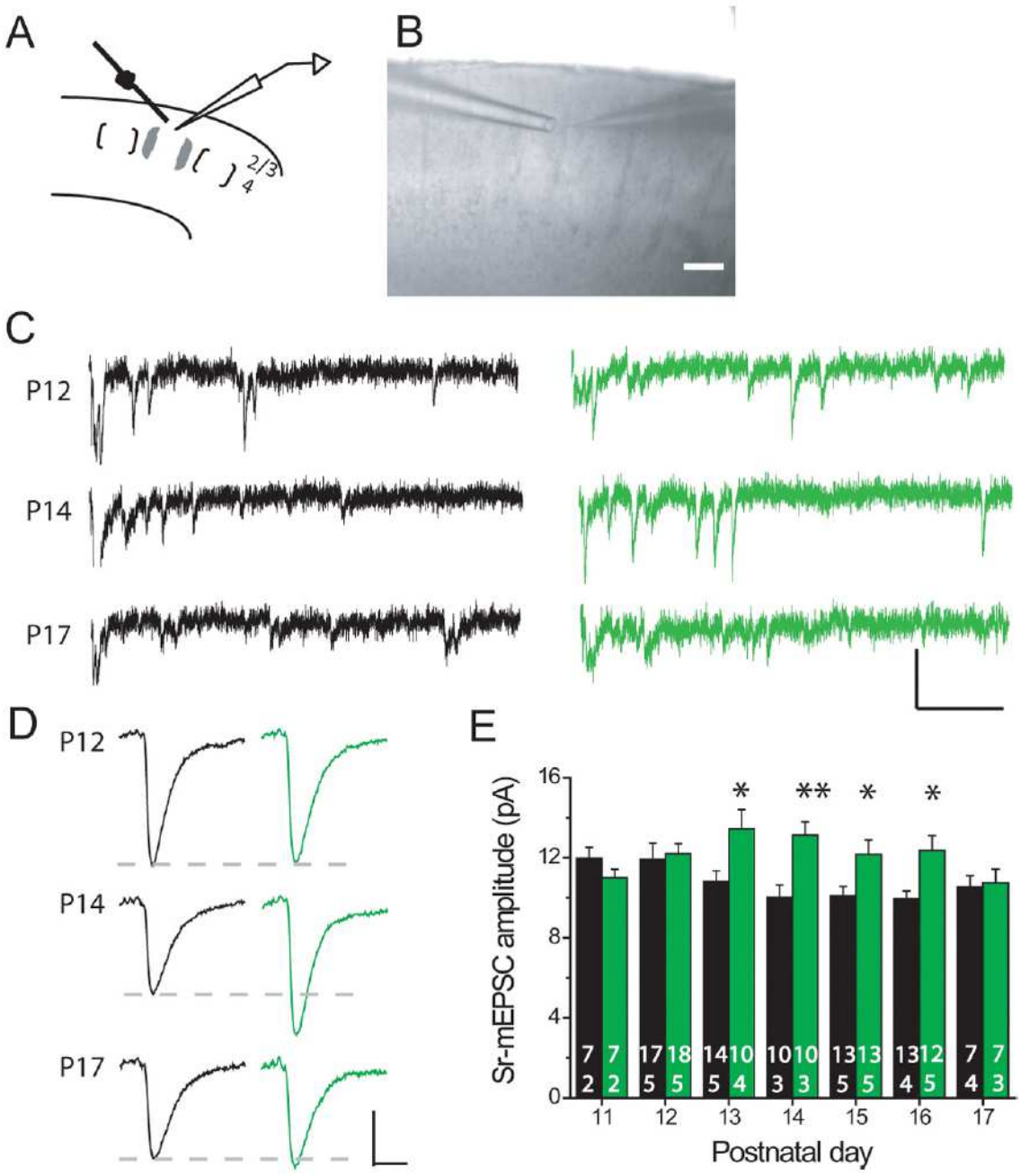


Figure 3. SWE triggers a rapid increase in evoked Sr-mEPSC amplitude at layer 2/3-2/3 excitatory synapses. ***A***, Schematic of stimulating and recording electrode placement for measuring layer 2/3-2/3 Sr-mEPSCs. ***B***, Brightfield image of recording configuration in one barrel column. Scale bar: 200 µM. ***C***, Example traces from control (black) and SWE (green) at postnatal age P12, 14 and 17. Scale bars: 20 pA, 100 ms. ***D***, Average traces of evoked Sr-mEPSCs for control and SWE animals. Scale bars: 5 pA, 10 ms. ***E***, Mean amplitude of evoked Sr-mEPSCs recorded at postnatal ages P11-17 for control and SWE animals at layer 2/3-2/3 synapses. The number of cells (top) and animals (bottom) used are indicated on the bar graph. *p<0.05, **p<0.01 between age-matched control and SWE-treated cell groups by age using Mann-Whitney U test. *p<0.05, **p<0.01.

2.3.4 Potentiated layer 2/3-2/3 synapses do not mobilize CP-AMPARs

To determine whether plasticity at layer 2/3-2/3 inputs was mechanistically similar to that observed at layer 4-2/3 synapses, we examined whether CP-AMPARs were present at potentiated intralaminar synapses, as had been observed for layer 4-2/3 inputs.

Analysis of AMPAR-EPSC rectification and Sr-EPSC decay kinetics suggested that CP-AMPARs were not trafficked to layer 2/3-2/3 synapses under any conditions (Fig. 4B-E, 4I). Similar to layer 4-2/3 inputs, no significant rectification was observed in slices from control, undeprived animals. This was also observed at P13, when SWE was effective at trafficking CP-AMPARs to layer 4-2/3 synapses (Fig. 4B,C). To determine whether there might be an offset in the timing of CP-AMPAR delivery that might be later at layer 2/3-2/3 synapses, we also examined SWE-induced changes in rectification at P14. However, SWE was not associated with evidence for rectifying AMPARs at this age, either (Fig. 4D,E).

To verify that this was not due to subtle differences in our recording conditions across different experiments, recordings were carried out to directly compare rectification properties in the same cell (Fig. 4F,G). Stimulation electrodes were simultaneously placed in layer 4 and layer 2/3 of the spared barrel column, and the EPSCs from the post-synaptic cell were collected across a range of holding potentials in the presence of D-APV and picrotoxin to pharmacologically isolate AMPAR-EPSCs. In 6/8 cases, rectifying AMPAR-EPSCs could clearly be observed at layer 4-2/3 but not layer 2/3-2/3 inputs (Fig. 4G). On average, no significant increase in AMPAR rectification was observed after SWE at intralaminar inputs (Fig. 4H). Thus, we conclude that layer 2/3 excitatory inputs are distinct from layer 4 in both their developmental timing and the synaptic mechanisms underlying SWE-induced plasticity.

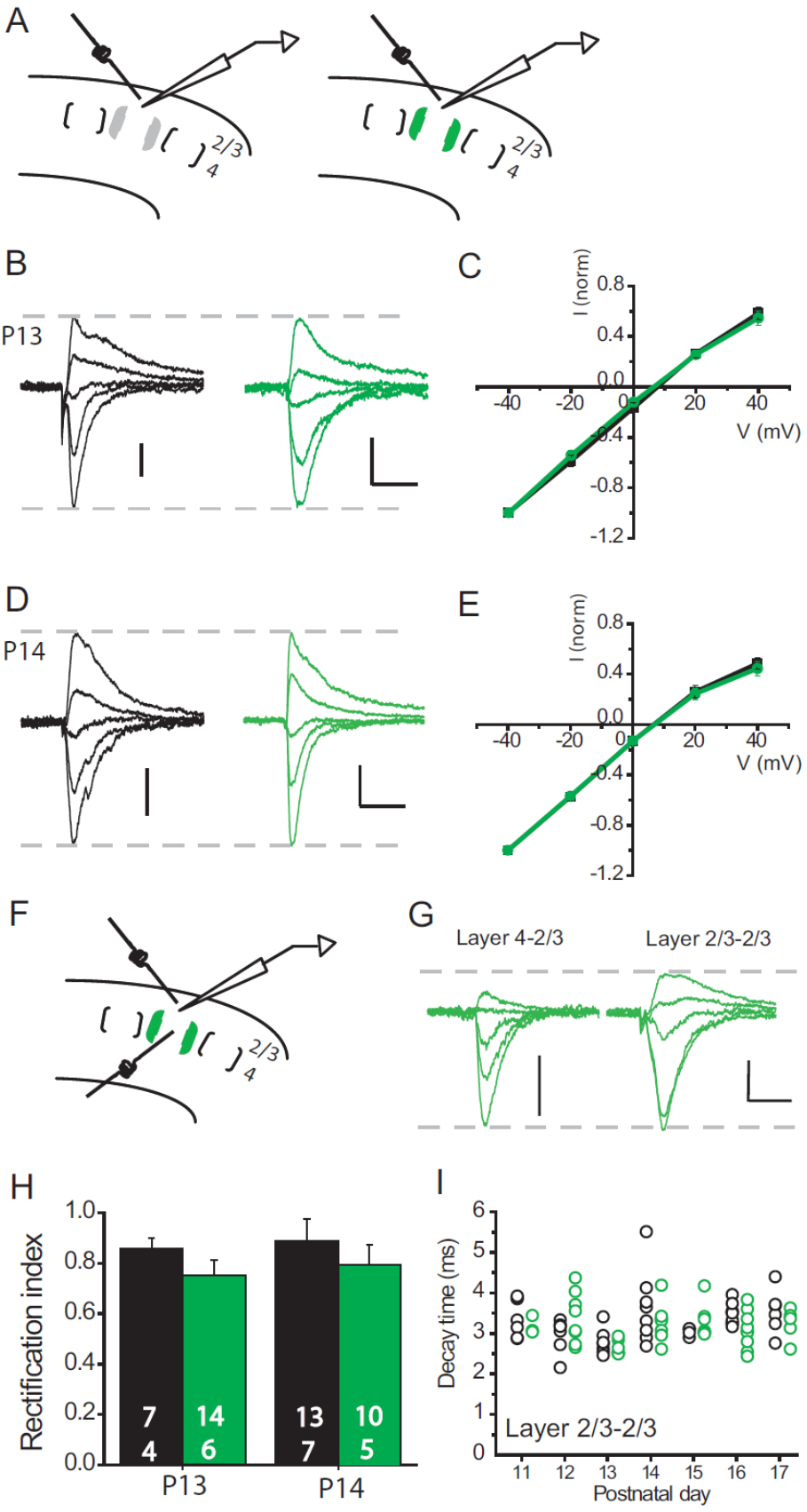

A
2/3
4
B
P13
C
I (norm)
0.8
0.4
0.0
-0.4
-0.8
-1.2
-40
-20
0
20
40
V (mV)
D
P14
E
I (norm)
V (mV)
F
2/3
4
G
Layer 4-2/3
Layer 2/3-2/3
H
Rectification index
1.0
0.8
0.6
0.4
0.2
0.0
7
4
14
6
13
7
10
5
P13
P14
I
Decay time (ms)
6
5
4
3
2
1
0
Layer 2/3-2/3
11
12
13
14
15
16
17
Postnatal day

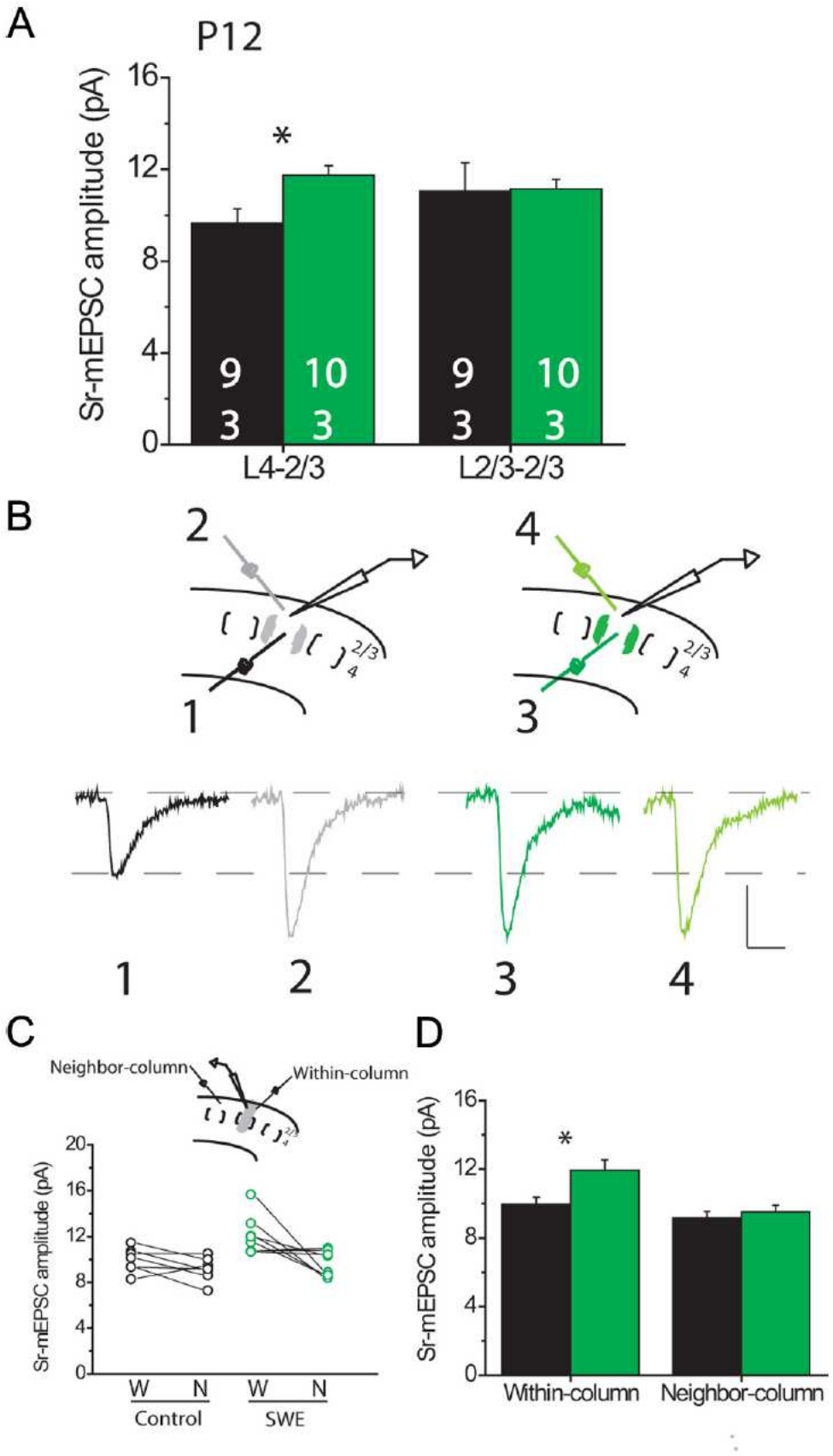
Figure 4. SWE does not drive CP-AMPARs to layer 2/3-2/3 synapses. ***A***, Schematics of electrode placement for control (black) and SWE-treated tissue (green) at layer 2/3-2/3 synapses. ***B***, Example cells from P13 control and SWE animals showing layer 2/3-evoked AMPA-EPSCs at holding potentials -40, -20, 0, +20 and +40 mV. Scale bars: 20 pA, 20 ms. ***C***, I-V curves for control and SWE animals at P13. ***D***, Example cells from P14 control and SWE animals as in ***A)***. Scale bars: 30 pA, 20 ms. ***E***, I-V curves for control and SWE animals at P14. ***F***, Configuration of dual-pathway recording in SWE-treated tissue. One stimulation electrode is placed in layer 4 and the other is in layer 2 of the same barrel column while recording from the same layer 2/3 neuron. ***G***, Example traces (peak-scaled) from dual-pathway recordings of a P13 SWE-treated animal at holding potentials -40, -20, 0 +20 and +40 mV. Scale bars: 30 pA, 10 ms. Layer 4-2/3 synapse shows more rectification than layer 2/3-2/3 synapse within the same postsynaptic neuron. ***H***, Rectification index for P13 and P14 control and SWE-treated animals at layer 2/3-2/3 synapses. ***I***, Scatter plot of Sr-mEPSC decay time at layer 2/3-2/3 pathway from P11-17.

2.3.5 Precise, synapse-specific onset of experience-dependent plasticity

Given experimental uncertainty in gestation period and the precise timing of birth (birthdate was approximated by inspection 1-2x/day), it is remarkable that we were able to indentify a discrete time window by which layer 4 and layer 2/3 inputs are differentially regulated by sensory experience. To control for potential differences between groups of animals, we undertook a second series of experiments whereby littermate control and SWE-treated animals were examined on the same postnatal day. Furthermore, both input pathways were evaluated in the same cell, providing additional strength to this method of comparison. Because SWE is capable of triggering synaptic strengthening at layer 4 but not layer 2/3 inputs at P12, this age was selected for more extensive analysis.

Dual-pathway recordings of Sr-EPSCs from pairs of P12 control and SWE-treated littermates were carried out (Fig. 5A,B). In these littermates with identical postnatal ages, SWE induced a robust and significant increase in Sr-EPSC amplitude only at layer 4 inputs (control 9.7±0.6 pA versus SWE 11.7±0.4 pA, $p<0.05$ by Mann Whitney) but not at layer 2/3 inputs (control 11.0±1.2 pA versus SWE 11.1±0.4 pA, $p>0.3$ by Mann Whitney). In addition, stimulation in layer 2/3 of adjacent, deprived barrel did not reveal any SWE-dependent change

in synaptic strength, indicating that these effects are specific to the spared barrel column (Fig. 5C,D). These results are consistent with previous findings from the single pathway-stimulation recordings and strongly support the conclusion that critical period timing is regulated in an input-specific manner.


A
P12
Sr-mEPSC amplitude (pA)
16
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8
4
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L4-2/3
L2/3-2/3
B
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2
3
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C
Neighbor-column
Within-column
Sr-mEPSC amplitude (pA)
20
16
12
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W
N
W
N
Control
SWE
D
Sr-mEPSC amplitude (pA)
16
12
8
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0
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Within-column
Neighbor-column

Figure 5. Dual pathway recordings show that SWE-induced plasticity is age- and pathway-specific. ***A,*** Mean amplitude of evoked Sr-EPSCs in dual-pathway recordings from control (black) and SWE (green) littermates at P12. ***B***, Example experiment from control and SWE animals showing average Sr-EPSC traces at two inputs onto the same post-synaptic cell. Top, schematics of experimental recording configuration. **1**, layer 4-2/3 control; **2**, layer 2/3-2/3 control; **3**, layer 4-2/3 SWE; **4**, layer 2/3-2/3 SWE. Scale bars: 5 pA, 5 ms. SWE leads to a pronounced increase in Sr-EPSC amplitude at layer 4-2/3 synapses (3 compared to 1) but minimal change at layer 2/3-2/3 synapses (4 compared to 2). ***C,*** Scatter plot of dual-pathway recorded Sr-EPSC amplitudes for layer 2/3 neurons with inputs arising from within the spared barrel column and from neighboring, deprived columns. Top, schematic of dual-pathway recording configuration. Bottom, scatter plot. **W**: Within column, **N**: Neighboring column. ***D,*** Mean Sr-EPSCs amplitude for layer 2/3-2/3 synapses with inputs from within the spared barrel column and from neighboring deprived column. *$p<0.05$.

2.3.6 NMDARs at layer 2/3-2/3 synapses contain less NR2B than layer 4 inputs

Because developmental plasticity is associated with elevated NR2B content at thalamocortical synapses (Carmignoto and Vicini, 1992; Barth and Malenka, 2001; Lu et al., 2001) we hypothesized that the extended duration of SWE-induced plasticity at layer 2/3-2/3 inputs might be associated with increased NR2B at these connections. To assess this, we examined the ifenprodil sensitivity and decay kinetics of the pharmacologically-isolated NMDAR-EPSC in control, undeprived animals at a time where SWE was capable of inducing an increase in Sr-EPSC amplitude at both types of synapses, P13.

Layer 2/3-2/3 and layer 4-2/3 inputs were analyzed in the same post-synaptic cell, using the dual-pathway stimulation set-up. At this age, NMDAR-mediated EPSCs at layer 2/3-2/3 synapses were moderately but significantly less sensitive to the NR2B-specific antagonist ifenprodil than layer 4-2/3 synapses within the same cell (percent reduction in peak NMDAR-EPSC current at +40 mV for layer 2/3-2/3: 51.2±5.4 % versus layer 4-2/3: 57.7±5.7 %, Fig. 6C,D). Consistent with a higher NR2A content at layer 2/3-2/3 synapses, layer 2/3-2/3 NMDAR-EPSCS also displayed faster decay kinetics than those at layer 4-2/3 synapses (layer 2/3-2/3 τ=59.8±2.7 ms versus layer 4-2/3 τ=93.3±11.4 ms; Fig. 6A,B). These results could

not be attributed to differences in presynaptic release properties between the two pathways, since the degree of pair-pulse depression was identical at the two inputs (0.67±0.06 at layer 4-2/3 synapses versus 0.68±0.07 at layer 2/3-2/3 synapses, $p>0.8$).

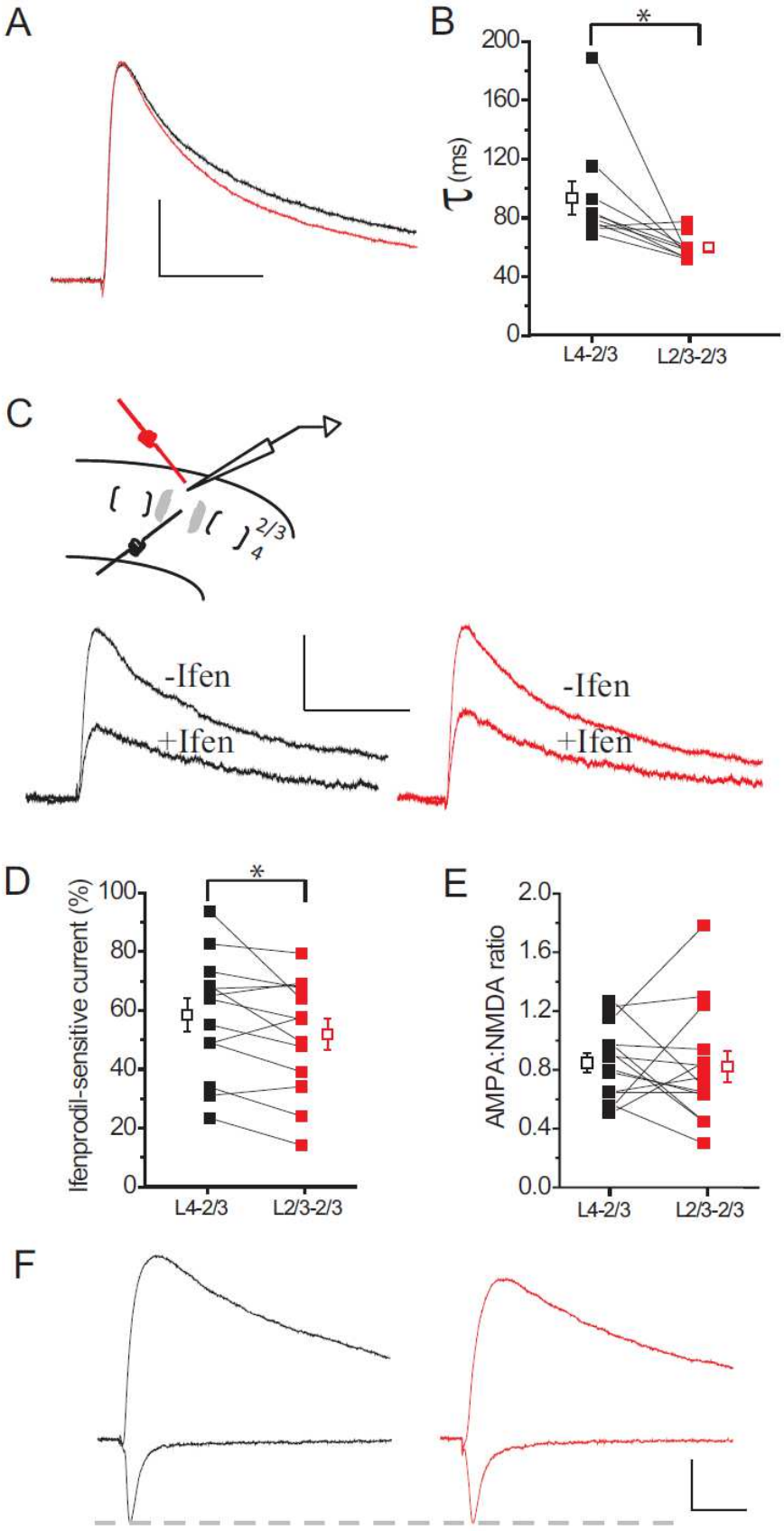
In addition to comparing the properties of NMDARs at these two different synapses, we also examined the ratio of AMPAR- and NMDAR-current amplitudes (A:N ratio) as an indicator of synaptic maturation (Wu et al., 1996; Isaac et al., 1997; Chiu et al., 2008). Experiments were carried out by stimulating both pathways and recording from a single cell in the presence of picrotoxin to pharmacologically isolate glutamatergic currents. The A:N ratio was not significantly different at control layer 2/3-2/3 versus layer 4-2/3 synapses (P12-13 layer 2/3-2/3: 0.82±0.1 versus layer 4-2/3: 0.85±0.07, Fig. 6E,F).

A similarity in the A:N ratio was observed despite a small but reproducible difference in the amplitude of control layer 2/3-2/3 Sr-EPSCs compared to layer 4-2/3 synapses at the end of the second postnatal week (P13 layer 2/3-2/3 11.2±0.7 pA versus layer 4-2/3 9.9±0.4 pA; Fig. 7A,B). This difference in Sr-EPSC amplitude is not likely to be due to differential electrical filtering of the EPSC signal due to the location of layer 2/3 and layer 4 inputs along the layer 2/3 pyramidal cell dendrite, since the rise and decay times for these events were identical for the two inputs (Fig. 7E). This difference in the amplitude of Sr-EPSCs did not appear to persist over time, as the two pathways became similar at later developmental timepoints (P14-15 layer 2/3-2/3 9.12±0.4 pA versus layer 4-2/3 9.52±0.3 pA; Fig. 7C,D for two-pathway experiments in the same cell and for all values pooled across a given day from P11-17, Fig. 7F).

Taken together, these data provide additional support for the finding that different excitatory synapses onto the same layer 2/3 neuron can display markedly different properties.

In addition, these findings indicate that relatively high NR2B content is linked to a capacity for experience-dependent plasticity *in vivo*, consistent with previous developmental studies.

Figure 6. NMDAR properties are different between layer 4-2/3 and layer 2/3-2/3 excitatory synapses. *A*, Peak-scaled averaged traces of NMDA-EPSC recorded at +40 mV for layer 4-2/3 (black) and layer 2/3-2/3 (red) pathways. Scale bars: 100 pA, 100 ms. ***B***, Decay kinetics of NMDA-EPSCs (+40 mV) for dual-pathway recordings at layer 4-2/3 and 2/3-2/3 pathways. Single exponential decay function was fitted to NMDA-EPSC traces from peak to 200 ms after the stimulus artifact and decay constant τ was plotted. Filled squares are values for individual cells. Open squares are the mean τ. Error bars: s.e.m. *$p<0.05$ by paired Student's t-test. ***C***, Ifenprodil (Ifen) sensitivity. Top, schematic of dual-cell recording in control tissue. Bottom left, example traces of NMDA-EPSC before (-Ifen) and after ifenprodil (+Ifen) treatment for dual recordings from both pathways. Scale bars: 50 pA, 100 ms. ***D***, Percent ifenprodil-sensitive currents for layer 4-2/3 and 2/3-2/3 pathways. Scatter plot and the mean (±s.e.m.) are presented. ***E***, AMPA:NMDA ratio for both pathways. ***F***,. Example traces of EPSCs recorded at -70 and +40 mV for AMPA:NMDA ratio in dual-pathway recording configuration. Scale bars: 50 pA, 20 ms.


A
B
*
200
160
120
80
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0
τ (ms)
L4-2/3
L2/3-2/3
C
2/3
4
-Ifen
+Ifen
-Ifen
+Ifen
D
*
Ifenprodil-sensitive current (%)
100
80
60
40
20
0
L4-2/3
L2/3-2/3
E
AMPA:NMDA ratio
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L4-2/3
L2/3-2/3
F

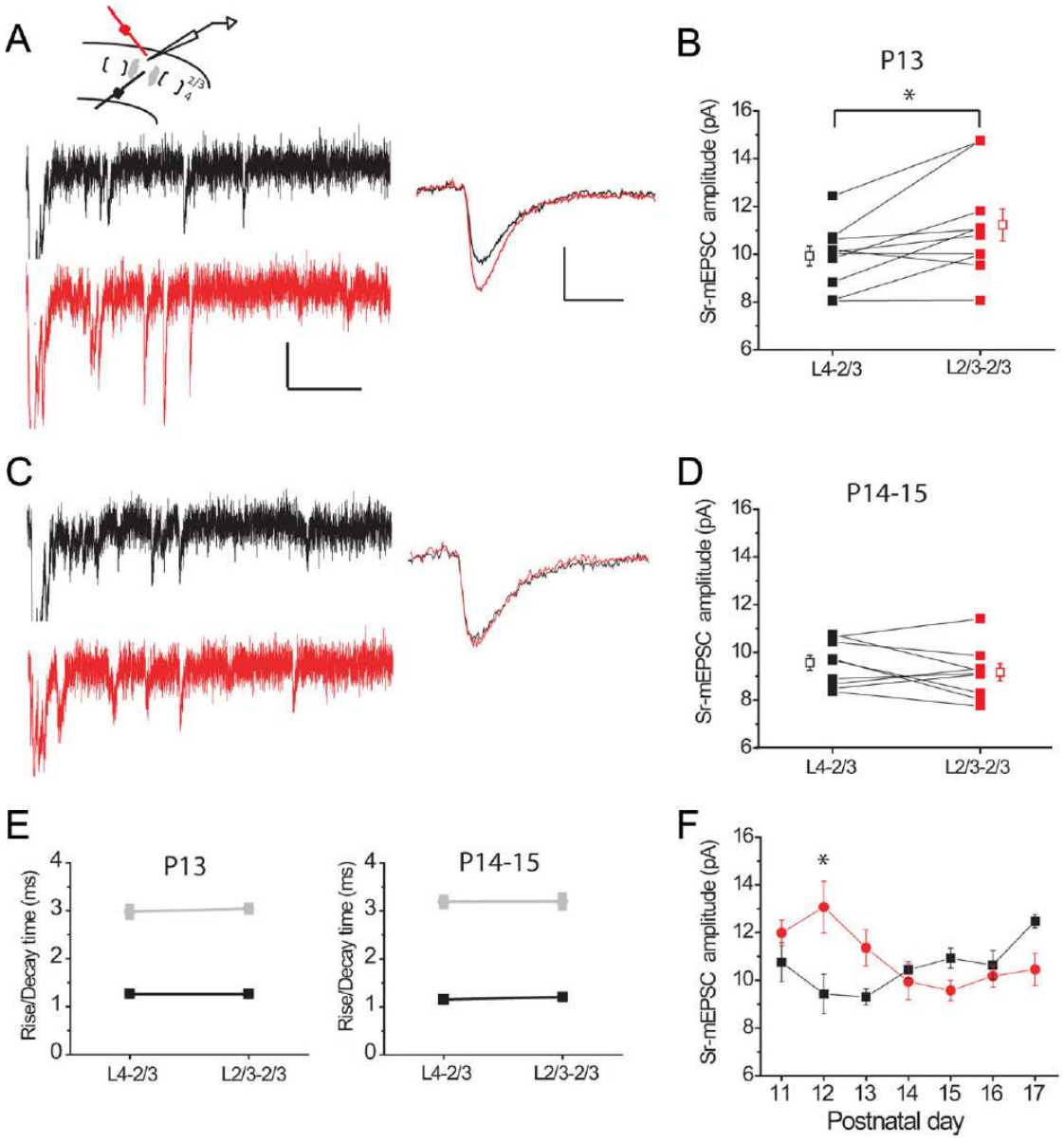


Figure 7. Layer 4-2/3 and 2/3-2/3 synapses show different developmental maturation of Sr-mEPSCs. ***A***, Example traces of evoked Sr-mEPSCs from dual-recording of two pathways in a P13 control cell. Top: schematics of dual-recording. Bottom left: example traces of evoked Sr-mEPSCs at layer 4-2/3 (black) and layer 2/3-2/3 (red) synapses from the same postsynaptic cell. Scale bars: 10 pA, 100 ms. Bottom right: average traces. Scale bar: 5 pA, 5 ms. ***B***, Scatter plots of Sr-mEPSC amplitude recorded at P13 at both pathways. ***C***, Example traces of a P14 control cell. Scale bars: the same as in ***A)***. ***D***, The same as in ***B)*** for P14-15. ***E***, Mean rise time (black) and decay time (gray) of Sr-mEPSCs at P13 (left) and P14-15 (right). ***F***, Mean Sr-mEPSC amplitude from P11-17 at l.ayer 4-2/3 and layer 2/3-2/3 pathways from single pathway experiments. *p<0.05.

2.4 Discussion

Here we have used SWE-induced potentiation to evaluate how plasticity at different synapses can be regulated by *in vivo* sensory experience, identifying pathway-specific critical periods for excitatory synaptic strengthening in layer 2/3 pyramidal neurons. Compared to layer 2/3-2/3 synapses, layer 4-2/3 synapses show an earlier and shorter period during which alteration in sensory input induced by the removal of all but a single whisker can increase synaptic strength. The timing of this critical period, for SWE initiated at P11-13, is similar to what has been observed for the effects of sensory deprivation on layer 2/3 neurons in rat barrel cortex for dendritic spine motility (Lendvai et al., 2000), and the maturation of sensory-evoked responses in vivo (Stern et al., 2001; Shoykhet et al., 2005). In addition, the maturation of intrinsic firing properties in layer 2/3 neurons has been localized to this brief developmental window (Maravall et al., 2004).

In comparison, we found a delayed and prolonged period for experience-dependent plasticity at layer 2/3-2/3 synapses. Given that mice mature slightly more quickly (based upon different gestation periods and time to sexual maturity), the critical period for plasticity at layer 2/3-2/3 synapses appears offset from that observed in previous studies in rats. However, we note that previous studies uniformly focused on the effects of sensory deprivation, not sensory-induced potentiation, and that critical periods for these two conditions may not proceed in parallel.

Synaptic segregation and pathway-specific plasticity

Input-specific difference in experience-dependent plasticity is of particular interest because layer 4-2/3 and layer 2/3-2/3 inputs are typically interdigitated across the same regions of the dendritic arbor in layer 2/3 neurons. For example, more than 75% of inputs from layer 4 terminate on the secondary and tertiary basal dendrites of layer 2/3 pyramidal cells (Lubke et

al., 2003), compared to about 70% of inputs from layer 2/3 pyramidal cells (Feldmeyer et al., 2006). The combination of overlapping synaptic input domains with temporally dissociated critical periods in layer 2/3 neurons suggests that dendritic gradients of ion channels that influence electrical coupling of the synapse to the soma (Magee, 1998; Lorincz et al., 2002; Notomi and Shigemoto, 2004; Kole et al., 2006; Johenning et al., 2009; Shah et al., 2010) are unlikely to regulate SWE-induced plasticity.

Previous studies support the notion that the mechanisms underlying *in vitro* plasticity at layer 4-2/3 and layer 2/3-2/3 inputs are distinct. For example, the rules for spike-timing dependent plasticity (STDP and STDD), a prominent hypothesis to explain how coincident pre- and post-synaptic activity might lead to synaptic potentiation or depression (Markram et al., 1997; Bi and Poo, 1998; Debanne et al., 1998) that has been evaluated at cortical synapses (Feldman, 2000; Banerjee et al., 2009; Zilberter et al., 2009). Previous experiments at layer 2/3-2/3 synapses suggest that long-term depression (LTD) predominates at these contacts (Zilberter et al., 2009). At layer 4-2/3 synapses, STDP follows more conventional rules, where LTP occurs with presynaptic spiking before postsynaptic depolarization (Banerjee et al., 2009). In addition, spike-timing dependent LTD at layer 4-2/3 synapses involves presynaptic NMDARs (Bender et al., 2006a; Bender et al., 2006b; Rodriguez-Moreno and Paulsen, 2008) but not at layer 2/3-2/3 inputs (Brasier and Feldman, 2008). Although both layer 4 and layer 2/3 inputs can be modified *in vitro* in adult tissue using spike-timing plasticity protocols, we find rapid *in vivo* plasticity is constrained to a discrete window of time during early development.

Synapse-specific critical periods described here were assessed by a postsynaptic measure of synaptic strength, using Sr-desynchronized neurotransmitter release.Presynaptic

changes may also contribute to a critical period for experience-dependent response potentiation in vivo.

Developmental changes in EPSC amplitude

Analysis of Sr-EPSCs in control animals indicates that there is no set-point for the amplitude of unitary excitatory synaptic contacts in layer 2/3 cells during early development. For example, EPSC amplitudes at layer 2/3-2/3 synapses become progressively smaller during the second postnatal week. Conversely, control layer 4-2/3 EPSCs become larger by the end of the time windows examined (P17). Remarkably, mean Sr-EPSC amplitudes in control animals could differ by as much as 2 pA across several days, comparable to the maximal effect of SWE at a given postnatal day. In addition, layer 4-2/3 and layer 2/3-2/3 inputs were not of similar amplitude on a given postnatal day (P12). Because the effects of SWE were compared to control values at a specific postnatal day (not to an average across multiple days), it is unlikely that our estimate of the timing of these two critical periods is affected by this developmental phenomenon. The regulation of mean synaptic strength at different inputs and across normal development has received considerable attention (Desai et al., 2002), and these data may warrant further investigation.

CP-AMPARS are not required for plasticity

Trafficking of CP-AMPARs has been observed following *in vitro* potentiation (Shi et al., 1999; Hayashi et al., 2000; Zhu et al., 2000; Plant et al., 2006; Clem et al., 2008); but see (Adesnik and Nicoll, 2007) as well as *in vivo* synaptic strengthening in the neocortex, the nucleus accumbens, the ventral tegmental area, and the amygdala (Takahashi et al., 2003; Rumpel et al., 2005; Bellone and Luscher, 2006; Clem and Barth, 2006; Matsuo et al., 2008). In the barrel cortex, the trafficking of CP-AMPARs has been observed in the spared barrel

column following SWE (Clem and Barth, 2006), suggesting that these receptors might have some essential role in the induction or maintenance of plasticity. However, we find that CP-AMPARs are not required for plasticity at layer 4-2/3 synapses, nor is their selective mobilization an adequate explanation for the closure of the critical period.

In addition, it does not appear that CP-AMPARs are ever present in significant amounts at layer 2/3-2/3 synapses, even when these synapses display robust SWE-induced potentiation. The selective trafficking or maintenance of CP-AMPARs to layer 4-2/3 synapses suggests that plasticity-inducing processes mobilize distinct pools of AMPARs within the same post-synaptic cell (Zhu, 2009). Although the present data do not rule out the possibility that CP-AMPARs might be transiently present (Plant et al., 2006), we think that it is unlikely that they are required for neocortical synaptic strengthening, even at layer 4-2/3 inputs (see also (Clem et al.).

Our finding that AMPAR-content can be differentially regulated at layer 4 and layer 2/3 inputs is similar to what has been observed at different inputs onto neurons in stellate cells within layer 4 (Zhu, 2009), the lateral geniculate nucleus (Kielland et al., 2009) or the basal amygdala (Humeau et al., 2007), where distinct pools of AMPAR subunits participate in activity-dependent synaptic modifications. Thus, these data provide additional evidence that the identity and activity of the presynaptic input can influence post-synaptic receptor subtype.

Patterns of activity at different excitatory inputs to layer 2/3 pyramidal neurons

What types of activity are required to drive excitatory synaptic strengthening in vivo? At the developmental ages examined here, layer 4 neurons fire reliably to whisker deflection (Benedetti et al., 2009). However, layer 2/3 neurons do not, and response rates of less than 0.5 spikes per stimulus by single-unit recording in the anaesthestized animal have been

observed (Benedetti et al., 2009). Thus, it is likely that at early developmental ages, layer 4-2/3 synapses are activated more robustly by incoming sensory activity than intralaminar inputs onto layer 2/3 pyramidal neurons, which require strong and reliable firing of connected layer 2/3 neurons.

Strong and reliable input from layer 4 neurons observed during the second postnatal week may provoke the earlier maturation and experience-dependent plasticity of layer 4-2/3 synapses. In contrast, layer 2/3-2/3 inputs may mature more slowly, consistent with the fact that 2/3-2/3 Sr-EPSCs displayed a longer window for SWE-induced synaptic strengthening. However it is not consistent with the high NR2B content at layer 4-2/3 synapses, a feature typically seen in more immature synapses (Monyer et al., 1994; Sheng et al., 1994; Flint et al., 1997; Stocca and Vicini, 1998; Tovar and Westbrook, 1999; Barth and Malenka, 2001).

Both layer 4-2/3 and layer 2/3-2/3 inputs can undergo synaptic strengthening *in vitro* well past the *in vivo* critical periods described here. For example, STD-LTP can be evoked into adulthood at layer 4-2/3 synapses (Banerjee et al., 2009), and changes in synaptic strength and connectivity have also been observed in mature animals (Cheetham et al., 2007; Cheetham et al., 2008). Developmental changes in circuit function, such as the emergence of feedback and feedforward inhibition (Kiser et al., 1998; Porter et al., 2001; Swadlow, 2002, 2003; Sun et al., 2006; Helmstaedter et al., 2008), may be important regulators of plasticity *in vivo* (Hensch et al., 1998; Iwai et al., 2003; Fagiolini et al., 2004; Hensch, 2005). Our data indicate that, *in vivo*, critical period plasticity is not likely to be regulated solely by the postsynaptic cell, or by the proximal/distal location of the synapse on the dendrite. Synapse-specific plasticity might be the result of distinct patterns of activity induced that arrive at layer 4 versus layer 2/3 inputs during

the stimulation of sensory inputs, or by the synapse-specific presence of plasticity-promoting factors.

3. Synaptic lability after experience-dependent plasticity is not mediated by calcium-permeable AMPARs

3.1 Introduction

The activity-dependent trafficking of AMPA receptor (AMPAR)-type glutamate receptors to enhance synaptic strength has been observed in many experimental preparations (Shi et al., 1999; Takahashi et al., 2003; Rumpel et al., 2005; Bellone and Luscher, 2006; Clem and Barth, 2006; Plant et al., 2006; Sutton et al., 2006; Clem and Huganir, 2010; Lante et al., 2011). AMPARs are tetrameric receptors, the vast majority of which contain an RNA-edited form of the GluA2 subunit that renders the complex impermeable to calcium (calcium-impermeable AMPARs; CI-AMPARS). However, under some circumstances, it has been possible to detect the presence of calcium-permeable AMPARs (CP-AMPARs) at synapses, based upon their unique electrophysiological and pharmacological properties (Man, 2011). These receptors typically lack GluA2, and may be homomers of GluA1. The uncommon subunit composition of these receptors, as well as their unusual calcium permeability, has made these receptors the source of great interest in understanding the mechanisms that modulate synaptic function in health and disease. For example, it has been hypothesized that CP-AMPARS can provide a source of Ca^{++} entry that might regulate excitotoxicity or subsequent plasticity (Deng *et al.*, 2003; Wiltgen *et al.*, 2010).

In a number of cases, the trafficking of native CP-AMPARs has been associated with recent synaptic potentiation (Bellone and Luscher, 2006; Clem and Barth, 2006; Plant et al., 2006; Clem and Huganir, 2010), suggesting that these receptors may play an important role in the initiation or maturation of synaptic plasticity. For example, the presence of CP-AMPARs can be detected after altered sensory experience at layer 4-2/3 synapses in somatosensory cortex (Clem and Barth, 2006), as well as in the amygdala after fear conditioning (Clem and

Huganir, 2010). However, other reports indicate that the trafficking of CP-AMPARs does not occur during in vitro plasticity induction (Adesnik and Nicoll, 2007; Gray et al., 2007; Clem et al., 2008). Thus, the requirement for CP-AMPARs in synaptic potentiation and depression is the center of debate.

A model that has some experimental support is that CP-AMPARs are specifically responsible for rapid and early, but not long-term, changes in synaptic strength. They may serve as "placeholders" for eventual substitution by CI-AMPARs (Plant et al., 2006; Clem and Huganir, 2010; Yang et al., 2010), or might provide a substrate to rapidly retune synaptic strength to maintain some dynamic range of synaptic drive (Thiagarajan *et al.*, 2005; Sutton *et al.*, 2006; Hou *et al.*, 2008). Indeed, a number of reports indicate that the *removal* of CP-AMPARs can be triggered by synaptic stimulation or *in vivo* activation, leading to synaptic depression (Bellone and Luscher, 2006; Ho et al., 2007; Clem and Huganir, 2010; Yang et al., 2010; Lante et al., 2011). Taken together, these data suggest that CP-AMPARs might serve as an intermediate step in synaptic modifications, where their persistence or removal can determine how long-lasting synaptic strength may be.

The addition of CP-AMPARs to layer 4-2/3 synapses during experience-dependent plasticity in somatosensory cortex has been well-established (Clem and Barth, 2006; Clem et al., 2008; Wen and Barth, 2011). However, increasing evidence indicates that they are not *required* for this form of plasticity, since synaptic strengthening can be observed without CP-AMPARS, either at later developmental ages or in transgenic mice that are mutant for the GluA2 trafficking molecule PICK-1 (Clem et al., 2010; Wen and Barth, 2011). Despite the fact that they are not required, their presence might nonetheless confer specific properties onto recently strengthened synapses, such as the ability to erase prior modifications. This has been

proposed from previous work, and has important therapeutic implications for reducing pathological changes in synaptic strength in addiction, seizure disorders, or anxiety disorders (Bellone and Luscher, 2006; Rakhade et al., 2008; Clem and Huganir, 2010).

Here we test the hypothesis that CP-AMPARs are associated with synaptic lability, whereby recently modified synapses might be susceptible to synaptic weakening due to the removal of CP-AMPARs. Previous work from our lab has established that plasticity at excitatory layer 4-2/3 synapses undergoes an early, NMDAR-dependent phase of synaptic strengthening, followed by a later, NMDAR-dependent phase of synaptic weakening (Clem *et al.*, 2008). We show that this NMDAR-dependent depression can be triggered at individual synaptic contacts in vitro, using a novel protocol that triggers a reduction of AMPAR-EPSCs by pairing post-synaptic depolarization with presynaptic stimulation in a Sr^{++} based ACSF solution.

This form of synaptic depression only occurs at previously potentiated synapses from animals with altered whisker input, requires NMDAR-activation, and can occur in cells where CP-AMPARs are undetectable or have been pharmacologically blocked. Further dissociating a role for CP-AMPARs in this phenomenon, Sr-depression was never observed in control, whisker-intact animals although CP-AMPARs could be detected at layer 4-2/3 synapses in some cells. Thus, we conclude that CP-AMPARs do not necessarily confer synaptic lability at layer 4-2/3 synapses, and that they are not essential for the induction or expression of synaptic depression in this assay.

3.2 Materials and Methods

Animals

Wild-type or heterozygous mice (males and females) from a fosGFP (1-3 line, C57Bl6 background (Barth *et al.*, 2004)) transgenic line at postnatal days 13 or 14 (P13-14) were used. Bilateral whisker deprivation was performed where all but the D1 whisker on one side were removed (Glazewski *et al.*, 2007). Animals were returned to their home cages for 24 hrs before recording. Control animals were whisker-intact littermates of the deprived animals. Since there was no significant difference between control wild-type C57Bl6 and fosGFP+/-, data from these animals were grouped. Recordings in control animals were not restricted to the D1 barrel column, since all columns were equivalent in whisker-intact animals. The barrel column representing the "spared" D1 whisker was identified by enhanced fosGFP expression and relative position to the hippocampus in acute brain slices.

Whole-cell recording

Animals were anesthetized with isoflurane and decapitated. Coronal slices (350 μm thick) were vibratome sectioned in artificial cerebrospinal fluid (ACSF) at 2-6^{o} C composed of (mM): 119 NaCl, 2.5 KCl, 2.5 $CaCl_2$, 1-1.3 $MgSO_4$, 1 NaH_2PO_4, 26.2 $NaHCO_3$, 11 glucose and equilibrated with 95/5% O_2/CO_2. Slices were maintained and whole-cell recordings were carried out at room temperature in ACSF. In all experiments, postsynaptic glutamatergic responses from layer 2/3 pyramidal neurons within the same barrel column were pharmacologically isolated using the $GABA_A$ antagonist picrotoxin (Ptx; 50 μM) in the bath solution. Somata of lower layer 2/3 pyramidal neurons in barrel cortex were targeted for whole-cell recording with borosilicate glass electrodes with a resistance of 4-8 MOhm. Electrode internal solution was composed of (in mM): 130 cesium-gluconate, 10 HEPES, 0.5 EGTA, 8 NaCl, 4 Mg-ATP and 0.4 Na-GTP, 5 QX-314, at pH 7.25-7.30, 290-300 mOsm and contained trace amounts of the fluorescent dye Alexa-568. Pyramidal cell identity was

confirmed after the recording session by pyramidal soma morphology and the presence of dendritic spines. Only cells with $R_{series} \leq 20$ MΩ and $R_{input} \geq 200$ MΩ, where changes in either measurement were less than 20% were included for analysis.

Stimulation of layer 4 afferents was applied at 0.1 Hz by placing glass monopolar electrodes in the center of a layer 4 barrel, and stimulation intensity was adjusted to isolate monosynaptic EPSCs without recurrent activity. Electrophysiological data were acquired by Multiclamp 700A (Axon Instruments, Foster City, CA) and a National Instruments acquisition interface. The data were filtered at 3 kHz and digitized at 10 kHz and collected by Igor Pro 6.0 (Wavemetrics, Lake Oswego, Oregon). Extracellular simulation was controlled by a Master-8 (A.M.P.I, Israel).

Depression of evoked Sr-EPSCs

To measure the amplitude of stimulus-evoked miniature AMPAR-EPSCs, Sr^{++} (3 mM) was substituted for Ca^{++} in ACSF to drive asynchronous glutamate release. Layer 2/3 pyramidal neurons were voltage clamped at -70 mV, where the contribution of NMDARs to the EPSC is minimal. This was experimentally verified with ACSF containing 0.5 mM Mg^{++}, where the mean Sr^{-}EPSC amplitude was not altered by bath application of D-APV. To induce synaptic depression of Sr-EPSCs, layer 2/3 pyramidal neurons were voltage-clamped at -70 mV for ≥5 min (baseline response), then to -20 mV for 5 min, returning to -70 mV holding potential (post- response). Stimulation frequency (0.1 Hz) and intensity were not altered during the experiment, which allowed a comparison of event frequency before and after pairing.

The evoked response has an initial synchronous component (~50 ms post the stimulus artifact) which was excluded in the analysis of Sr-EPSC events. Isolated, asynchronous events that occurred from 50-500 ms after the stimulus were manually selected and analyzed using

Minianalysis software (Synaptosoft, Inc. Decatur, GA). The detection threshold for events was set at 2x RMS noise (usually around 4-5 pA) and data were filtered with a low-pass filter at 1 kHz. Approximately 50-100 events were randomly selected from the pre- and post-response and then grouped to generate average traces. Within-cell comparisons were made between the events from the baseline and post- responses for each cell. An average trace was generated from grouping 50-100 randomly chosen events from each cell. Selected events were grouped across all cells within an experimental condition and ranked ordered to generate cumulative distribution plots.

Decay time of individual Sr-EPSC was analyzed online as the difference between the time at the peak and 1/3 of the peak and the mean decay time was obtained by averaging all selected events from all cells within a group.

AMPA EPSC measurement and rectification index (RI)

To isolate the multiquantal AMPAR- EPSCs, D-APV (50 μM) and Ptx were included in the bath solution. Spermine (100 μM) was included in the internal solution to ensure a sufficient amount of intracellular polyamine. Layer 2/3 pyramidal neurons were voltage-clamped at -70 mV and stimulus intensity was adjusted until a clear monosynaptic response (2-5 ms latency, consistent across trials for a given response) was visible for every sweep. For holding potentials at -70, 0, 40 mV, 10-20 sweeps were collected and averaged. The rectification index (RI) was calculated based on the following formula:

$$RI=abs(I_{+40} - I_0) / abs(I_{-70} - I_0)$$

$I_{-70,}$ I_{+40} and I_0 refer to the peak amplitude of the averaged responses for a given cell. If there is no rectification at positive holding potentials or the current-voltage relationship is linear, the RI in these measurements should be 4/7 (0.57).

NASPM/PhTx application

1-Naphthylacetyl spermine trihydrochloride (NASPM, 50 μM) or Philanthotoxin (PhTx, 10 μM) were applied in the bath (containing D-APV) to assay the contribution of CP-AMPARs. Antagonist was applied for at least 10 minutes while the cell was being held at -70 mV, and the amplitude of the post-drug response was calculated by averaging 10-20 sweeps immediately prior to drug application versus 10 min after the onset of application. Because NASPM/PhTx is hard to wash out, data from only one cell per slice was collected. In some cases, AMPA-EPSCs increased in amplitude after NASPM application; data from these cells was included in our analysis.

To verify the reliability of using NASPM to block CP-AMPARs, the RI before and after NASPM application (10 min after wash-in) was compared. Synaptic responses from recurrent excitatory synapses onto layer 5 pyramidal cells of young postnatal C57Bl6 mice (P9-11) were obtained by stimulating layer 5. Consistent with the finding that CP-AMPARs are highly expressed around this developmental age in layer 5 (Brill and Huguenard, 2008), we observed that some AMPAR-EPSCs were rectifying. EPSC amplitude and RI before and after NASPM application was determined.

The effect of NASPM on Sr-EPSC amplitude was also evaluated. For within-cell comparisons, Sr-EPSCs were collected for ~5 min before wash-in of NASPM-containing Sr-ACSF. Only one cell per slice was included for such NASPM wash-in experiments. For across cell comparisons of Sr-EPSC amplitude, slices were bathed in NASPM-containing ACSF for at least 20-120 minutes with afferent stimulation before analysis of Sr-EPSC amplitude.

In experiments where the requirement of CP-AMPARs in Sr-depression was tested, slices were bathed in NASPM-containing ACSF for 20-120 min with some afferent stimulation before recordings to allow complete drug diffusion and blockade of CP-AMPARs.

Within-cell recording of RI and Sr-depression

To more accurately explore the necessity of CP-AMPARs in Sr-depression, the RI was examined and then Sr-depression was induced in the same cell. In these experiments, AMPA-EPSCs were recorded at -70, 0 and +40 mV in Ca^{++} based ACSF, then a Sr^{++} based ACSF was washed in and Sr-depression was induced as described above. In a subset of cells, the Sr-based ACSF was washed out after the Sr-depression protocol, and Ca-ACSF was reapplied to obtain a second, post-depression RI measurement.

Statistics

Specific statistical tests used are indicated in the results. For Sr-EPSC amplitude comparisons before and after Sr-depression or NASPM treatment within the same cell and all other non-pair wise comparisons between two conditions (control vs. SWE), a non-parametric Mann-Whitney U test (two-tailed) was used. For Sr-EPSC event frequency comparisons before and after Sr-depression, NASPM treatment in control and SWE conditions, and RI comparisons within the same cell before and after some manipulation, a paired t-test was used. For comparisons of distribution of Sr-EPSC amplitude before and after pairing for Sr-depression experiments, a Kolmogorov-Smirnov test was used. Summary data are presented as mean±sem.

3.3 Results

Previous work has shown that modified whisker input, where all but a single whisker (single whisker experience; SWE) has been removed from one side of the mouse face, leads to the potentiation of synapses at layer 4-layer 2/3 excitatory inputs in the neocortical representation of the spared whisker. This potentiation can be accompanied by an increase in presence of CP-AMPARs, determined by their electrophysiological and pharmacological properties. The trafficking of CP-AMPARs is associated with age and input identity, where they are not implicated for plasticity induced at later developmental ages (when SWE begins at P13 or older ages) or at layer 2/3-layer 2/3 inputs (Wen and Barth, 2011).

Assays to demonstrate the experience-dependent increase in excitatory synaptic strength have relied upon a method to isolate the post-synaptic response in a pathway-specific manner, using an ACSF where Sr^{++} replaces Ca^{++}. Under these conditions, neurotransmitter release is triggered by electrical stimulation to a specific input, but vesicle release is slowed such that the post-synaptic response to individual quanta can be evaluated (Goda and Stevens, 1994; Xu-Friedman and Regehr, 1999). In previous analyses (Clem and Barth, 2006; Clem et al., 2008; Clem et al., 2010; Wen and Barth, 2011), the AMPAR-EPSC was pharmacologically isolated from NMDAR currents by the application of D-APV. However, during the course of our investigations, we discovered that when both NMDAR- and AMPAR-mediated currents were present, there was sometimes a voltage-dependent run-down in the amplitude of average Sr-EPSC for a given cell. This run-down was only present at layer 4-2/3 synapses from SWE-treated animals, suggesting that it might be related to the recent potentiation at these synapses.

3.3.1 Sr-depression is induced by a modest post-synaptic depolarization

We formalized a method to examine this synaptic lability, named Sr-depression, by comparing mean Sr-EPSC amplitude before and after postsynaptic depolarization. We use the term Sr-depression to indicate specifically that this depression was not what has typically been considered "short-term depression" in other studies, since its onset is immediate and it is stable for many minutes following pairing. Experiments were carried out in acute brain slices from SWE-treated animals at postnatal day 13 (P13), a time when experience-dependent plasticity is pronounced (Figure 8A,B) (Clem and Barth, 2006; Clem et al., 2008; Clem et al., 2010; Wen and Barth, 2011). Typically, Sr-EPSC amplitude from a given cell was calculated from the average of a 50-100 individual, well-isolated events (Figure 8C). The mean amplitude of these events was constant over the recording period when the post-synaptic cell is maintained at hyperpolarized holding potentials. Sr-EPSCs are primarily mediated by AMPARs, since application of NMDAR-antagonists does not change Sr-EPSC amplitude at hyperpolarized potentials when NMDARs exhibit a characteristic Mg^{++}-dependent voltage block.

Depolarization of the post-synaptic cell (-20 mV, 5 min, 0.1 Hz) leads to a rapid change in Sr-EPSC amplitude (Figure 8D), without any change in event frequency between the baseline and post-pairing period (Figure 8E, before frequency 2.85±0.35, vs. after 2.63±0.21, n=8 cells, p=0.65 by paired t-test). Since stimulation strength is not altered during the experiment, and since individual Sr-EPSCs are thought to represent individual release events at distinct synaptic contacts, these results indicate that this depression is likely to be post-synaptic in origin.

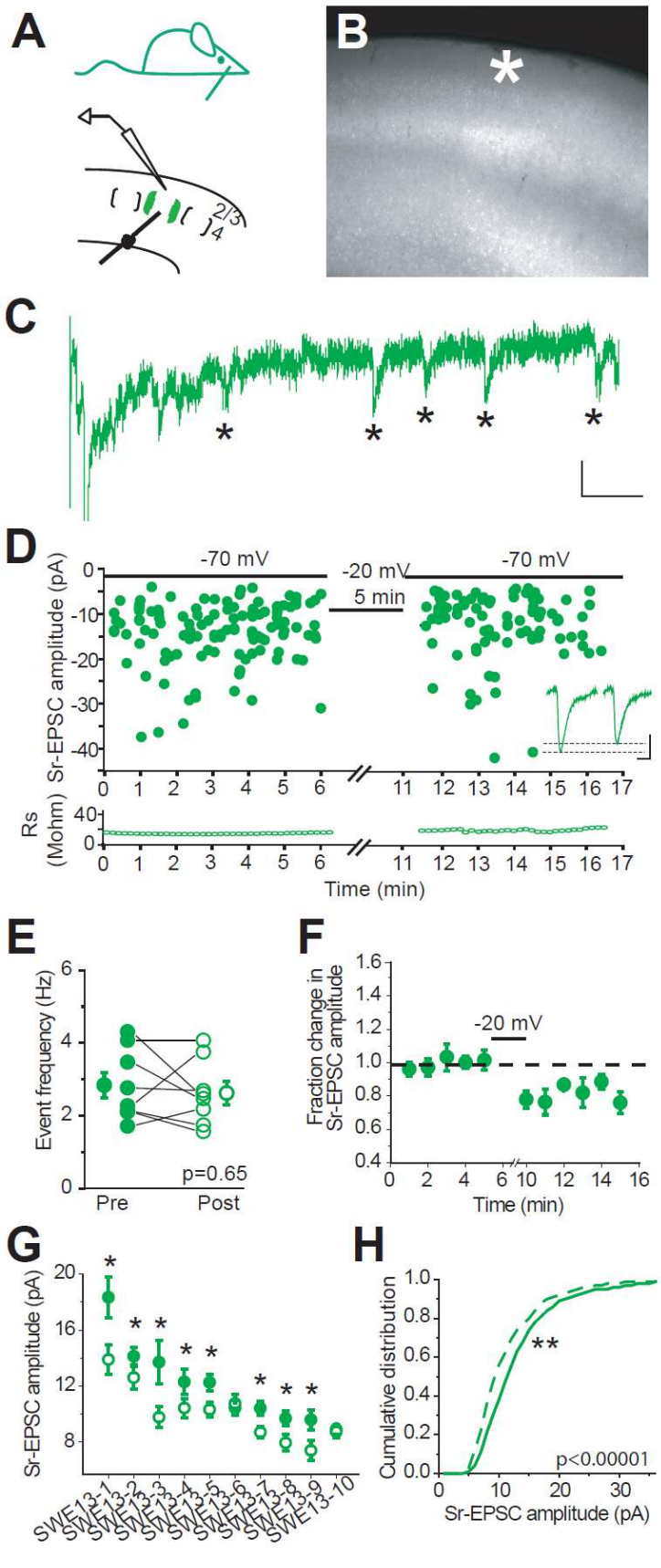

A
B
C
D
E
F
G
H
2/3
4
-70 mV
-20 mV
5 min
-70 mV
Sr-EPSC amplitude (pA)
Rs (Mohm)
Time (min)
Event frequency (Hz)
p=0.65
Pre
Post
Fraction change in Sr-EPSC amplitude
-20 mV
Time (min)
Sr-EPSC amplitude (pA)
SWE13-1
SWE13-2
SWE13-3
SWE13-4
SWE13-5
SWE13-6
SWE13-7
SWE13-8
SWE13-9
SWE13-10
Cumulative distribution
p<0.00001
Sr-EPSC amplitude (pA)

Figure 8. Sr-depression can be elicited at layer 4-2/3 synapses from SWE-treated mice. A) Schematic of an SWE animal (top) and recording configuration in the spared barrel column (bottom). **B)** Fluorescence image of a slice that contains the spared barrel column, visualized by expression of GFP fluorescence in a fosGFP transgenic mouse (*). **C)** An example Sr-EPSC trace (* indicates individual Sr-EPSC event). Scale: 10 pA, 50 ms. **D)** Scatter plot of individual Sr-EPSC amplitudes before and after Sr-depression induction, in a SWE-treated animal. Inset: average traces of Sr-EPSCs before (left) and after (right) Sr-depression induction. Scale bar: 5pA, 5 ms. Bottom: Electrode series resistance from the same cell, which does not change between baseline vs. post-pairing window. **E)** Sr-EPSC event frequency does not change after Sr-depression induction. **F)** Mean change in Sr-EPSC amplitude normalized to the baseline period, for the 8 cells that showed significant Sr-depression. **G)** Comparison of Sr-EPSC amplitude between pre- (filled circle, green) and post-pairing window (open circle, green) for individual cells. Cells were rank ordered according to their mean amplitude of the baseline responses. Mean±sem is plotted for each cell. *$p<0.05$ by Mann-Whitney U test. **H)** Cumulative distribution histogram of Sr-EPSC amplitude before (solid line) and after (dotted line) pairing at -20 mV with presynaptic stimulation (n=10 cells).

Because statistical comparisons were carried out for a large number of events before and after pairing, this method was very sensitive to small changes in Sr-EPSC amplitude. In tissue from SWE animals, 16/20 (Figure 8G, 8/10 cells; also see Figure 15A showing 8 cells that express Sr-depression in a separate experiment, 2 cells not included in the figure do not express Sr-depression, therefore 8/10 cells) cells showed a significant reduction in Sr-EPSC amplitude, with a mean depression of ~20% (Figure 8F,G, Figure 14; average of 16 cells 20±2%; p value range for individual cells 0.025-0.00002 for baseline vs. post-pairing window by Mann-Whitney U-test). A cumulative distribution histogram for all cells from the spared barrel column showed a highly significant reduction in event amplitude in the post-pairing window (Figure 8H; Kolmogorov-Smirnov test $p<0.00001$, n=10 cells).

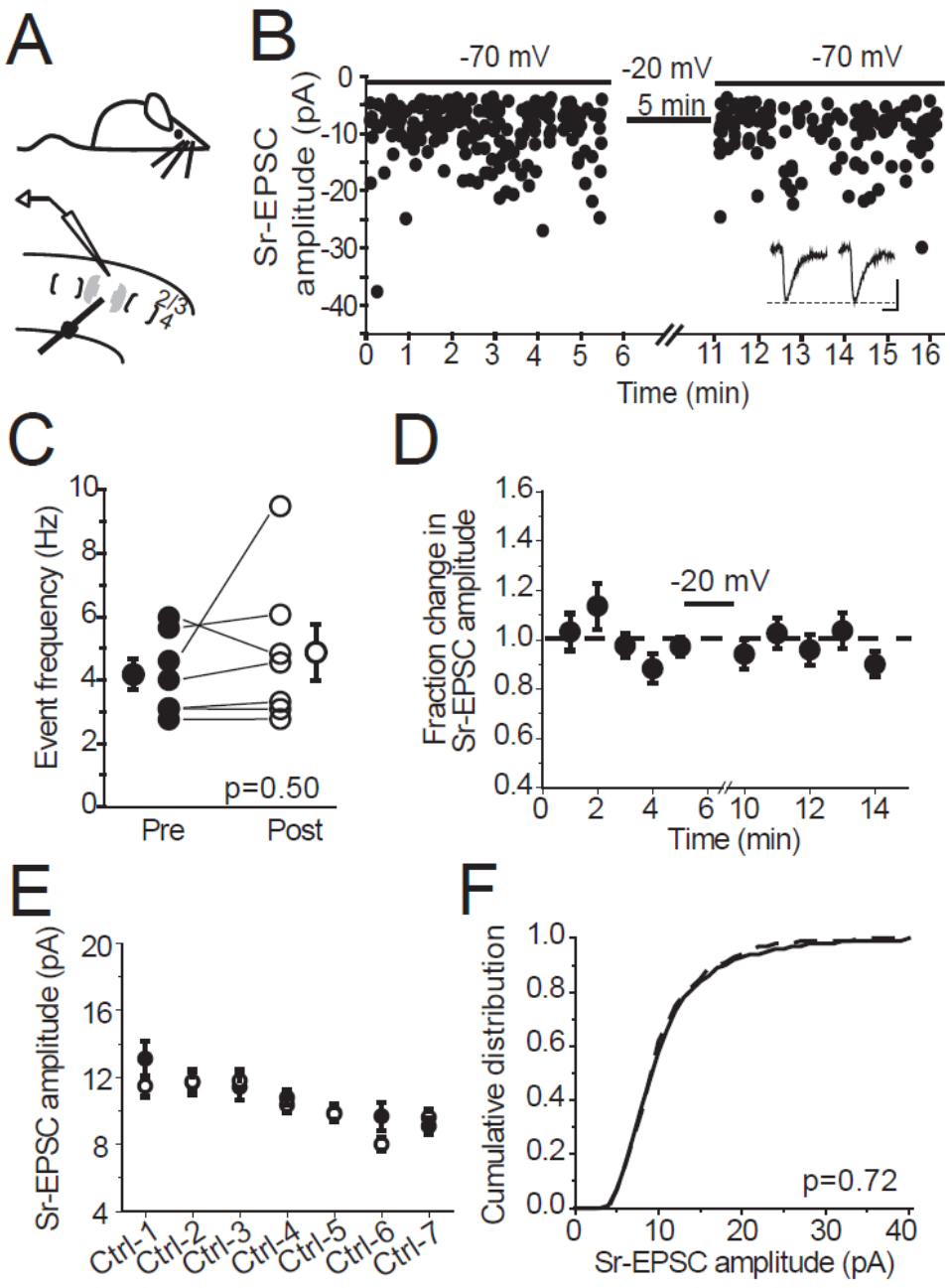


Figure 9. Layer 4-2/3 synapses from control animals did not exhibit Sr-depression. A) Schematics of a control animal (top) and electrode configuration (bottom). **B)** Example scatter plot of individual Sr-EPSC amplitudes from a control cell using the same pairing protocol. Inset: average traces, before and after pairing. Scale: 5 pA, 5 ms. **C)** Sr-EPSC event frequency does not change after pairing in Sr^{++}. **D)** Mean change in Sr-EPSC amplitude normalized to the baseline period before and after pairing in control animals. **E)** Within-cell comparison of Sr-EPSC amplitude between pre- (filled circle, black) and post-depression induction (open circle, black). Cells were rank ordered according to their mean amplitude of the baseline responses. **F)** Cumulative distribution histogram of Sr-EPSC amplitude before (solid line) and after (dotted line) pairing at -20 mV with presynaptic stimulation (n=7 cells).

In control animals, 0/7 cells showed a change in Sr-EPSC amplitude after pairing, (p value range for individual cells 0.19-0.89 for baseline vs. post-pairing window; Figure 9A-E). Depolarization did not change event frequency in the post-pairing window (Figure 9C). An absence of synaptic depression was confirmed by analysis of a cumulative distribution histogram of Sr-EPSCs from control cells, where depolarization failed to trigger any shift in event distribution (Figure 9F, Kolmogorov-Smirnov test p=0.715, n=7 cells).

3.3.2 Requirements for Sr-depression

Many forms of synaptic depression, including depolarization-induced plasticity at layer 4-2/3 synapses in the spared barrel column (Clem *et al.*, 2008), require NMDAR-activation. To determine whether Sr-depression requires NMDARs, we examined whether bath application of the NMDAR-antagonist D-APV was sufficient to block depression in cells from SWE-treated animals. In the presence of D-APV, 0/5 cells showed a significant reduction in Sr-EPSC amplitude after pairing (Figure 10A,B, p value range 0.11-0.65, baseline vs. post-pairing window by Mann-Whitney U test). Analysis of a cumulative distribution histogram of Sr-EPSC events from all cells before and after pairing in Sr^{++} showed a small, but still significant reduction in amplitude (Figure 10B; Kolmogorov-Smirnov test p=0.021 for baseline vs. post-pairing window; note comparison from Figure 8H where $p<0.00001$).

We also found that a more modest depolarization to -40 mV was sufficient to block Sr-depression in most cells (Figure 10C; 4/6 did not show significant depression, within-cell p value range for these 4 cells, 0.14-0.73, for baseline vs. post-pairing window by Mann-Whitney U test), consistent with a role for NMDAR-activation which remains partially blocked at this holding potential. As above, the cumulative distribution of Sr-EPSC event amplitudes showed

a small shift following pairing at -40 mV (Figure 10D; Kolmogorov-Smirnov test p=0.01 for baseline vs. post-pairing window). Thus, Sr-depression requires post-synaptic depolarization and NMDAR activation. Based on the small but statistically significant reduction in Sr-EPSC amplitudes shown in the cumulative distribution histograms, there may be additional pathways that are involved in this depression.

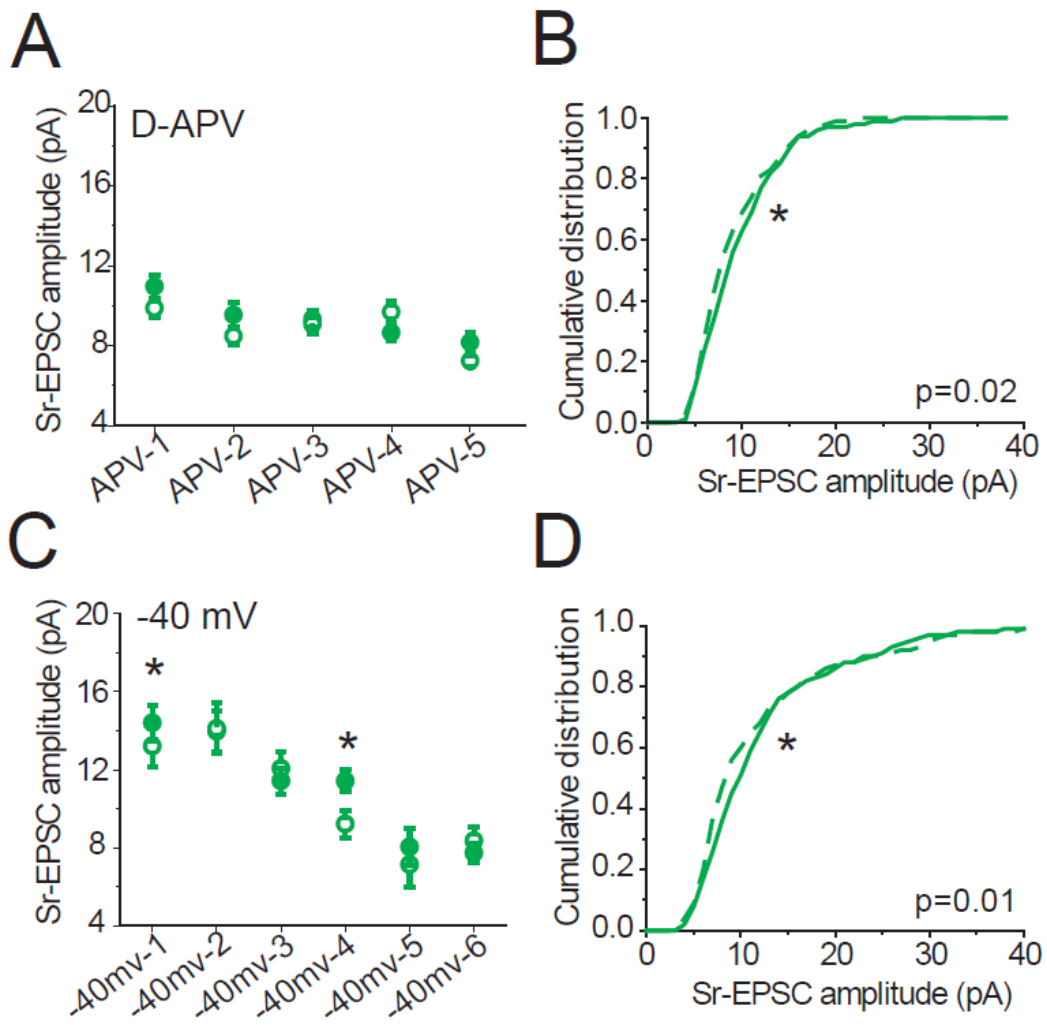


Figure 10. Sr-depression in SWE-treated animals requires NMDAR activation. **A)** Within-cell comparison between pre- (filled) and post-pairing (open) windows in the presence of D-APV. **B)** Cumulative distribution histogram of Sr-EPSC amplitude before (solid line) and after (dashed line) pairing at -20 mV with presynaptic stimulation in D-APV (n=5 cells). **C)** Within-cell comparison between pre- (filled) and post- responses (open) by pairing postsynaptic depolarization to -40 mV with presynaptic stimulation. *p<0.05 by Mann-Whitney U test. **D)** Cumulative distribution histogram of Sr-EPSC amplitude before (solid line) and after (dashed line) pairing at -40 mV with presynaptic stimulation (n=6 cells).

3.3.3 The role of CP-AMPARs in Sr-depression

Previous work from our lab has shown that CP-AMPARs are trafficked to layer 4-2/3 synapses after SWE. Because other investigations have found that these receptors can be highly labile at the synapse, we hypothesized that the rapid depression observed might be due to the

NMDAR-dependent removal of CP-AMPARs. Previous Sr-depression experiments were carried out in tissue from P13 animals, since CP-AMPARs have been detected after SWE at this age (Wen and Barth, 2011). Consistent with our previous findings, we observed significant rectification of pharmacologically-isolated AMPAR-EPSCs after SWE at this age (Figure 11A,B; Control rectification index (RI) 0.57±0.06 n=14 cells vs. SWE RI 0.42±0.04 n=10 cells, Mann-Whitney U test p=0.03). To calculate the RI, EPSC amplitude was recorded at -70, 0 and +40 mV (see Methods). Cells with a RI smaller than 0.57 were classified as rectifying, and those with an RI equal to or larger than 0.57 were classified as non-rectifying.

As a second method to quantify the contribution of CP-AMPARs at layer 4-2/3 inputs, we used Philanthotoxin (PhTx) or a synthetic analog of the CP-AMPAR antagonist Joro spider toxin, 1-Naphthylacetyl spermine trihydrochloride (NASPM) (Koike *et al.*, 1997)) to block CP-AMPARs (Figure 11C). Although layer 4-2/3 inputs from SWE animals show significant rectification compared to age-matched controls, the PhTx/NASPM-sensitive current was not significantly different between the two groups (Figure 11D, reduction in EPSC amplitude for control 0.11±0.09 n=7 vs. SWE 0.24±0.12 n=7, p=0.32 by Mann-Whitney U test), likely due to large variability in magnitude of NASPM-sensitive current across cells in both control and SWE conditions. This is in contrast to previously published results showing minimal Joro spider toxin block at layer 4-2/3 synapses in control animals (Clem & Barth, 2006), which were not focused on the specific developmental age (P13) tested here. It is notable that a subset of cells in control animals showed strong rectification (5/14 cells show rectification index less than 0.55) and NASPM blockade (3/7 control cells showed >15% block), despite the fact that we could not induce Sr-depression in cells from this group.

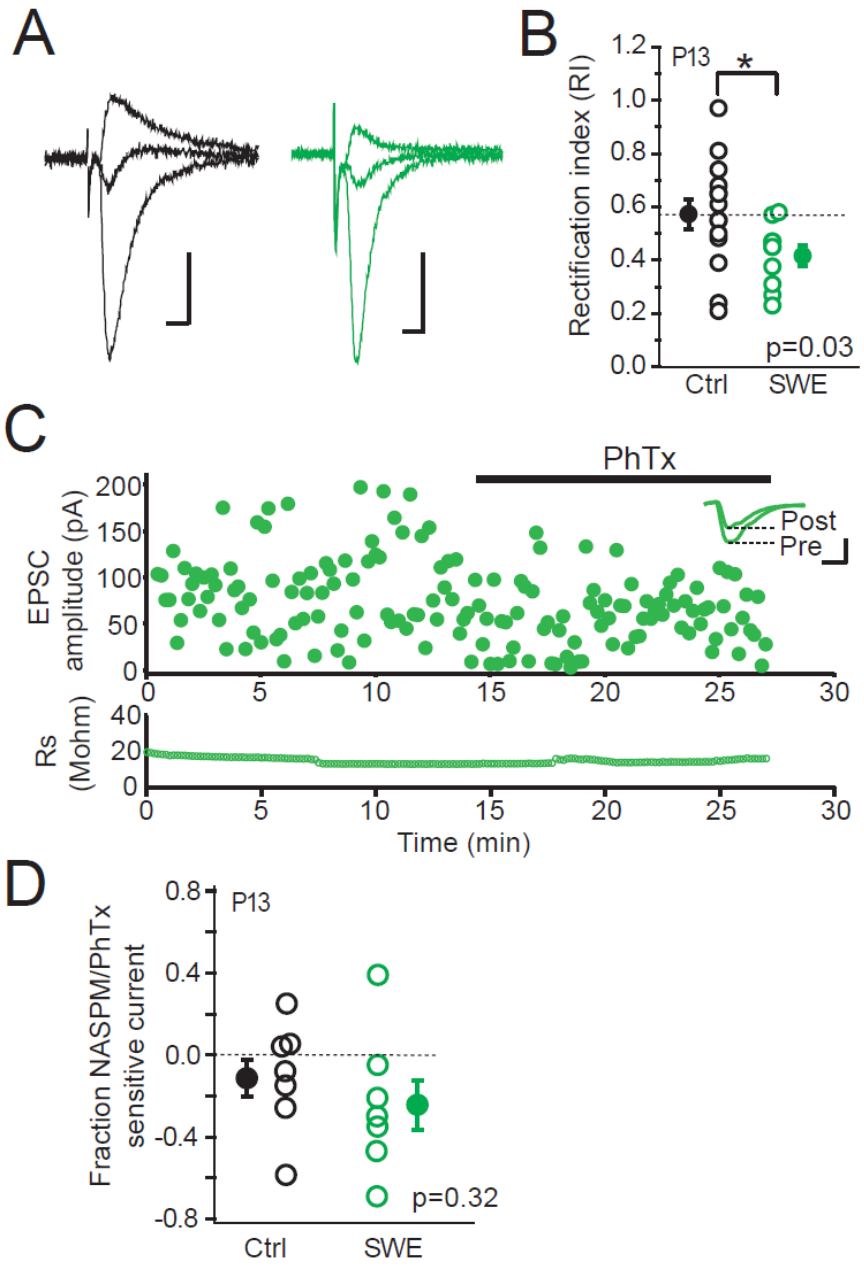


Figure 11. CP-AMPARs are present at layer 4-2/3 excitatory synapses after 24 hr SWE at P13. A) Example AMPA-EPSC traces recorded at -70, 0 and +40 mV from a control animal (left, black) and a SWE-treated animal (right, green). Scale: 20 pA, 10 ms. **B)** RI in control (black) and SWE-treated (green) animals. *p<0.05 by Mann-Whitney U test. **C)** PhTx blockade of AMPA-EPSC amplitude from a cell of P13 SWE-treated animal. Top: scatter plot of AMPA-EPSC amplitude before and after PhTx wash in. Inset: average AMPA-EPSC traces for the pre- and post-wash in periods. Scale: 50 pA, 5 ms. Bottom: series resistance that does not change over time. **D)** Fraction NASPM/PhTx sensitive current for control and SWE-treated animals at P13.

3.3.4 CP-AMPAR blockade results in a small decrease in Sr-EPSC amplitude

To determine whether we could detect a contribution of CP-AMPARs in Sr-EPSC amplitude, we determined the effect of NASPM on layer 4-evoked Sr-EPSCs from SWE-treated animals. We predicted that if there were a small number of CP-AMPARs at individual layer 4-2/3 synapses, NASPM blockade should reduce the mean amplitude of these events. A comparison across cells from SWE-treated animals showed that mean Sr-EPSC amplitude was 11.62±0.5 pA (n=19), compared to the amplitude of Sr-EPSCs in NASPM at 10.65±0.34 pA (n=17), a difference that was not significant (Figure 12A, p=0.099 by Mann-Whitney U test). Sr-EPSC amplitude before and after NASPM application within the same cell was compared for a smaller group of neurons. NASPM did not consistently decrease event amplitude (Figure 12B,C; mean EPSC amplitude before drug application, 11.94±0.4 pA vs. after 10.95±0.9 pA, n=4, p=0.43 by paired t-test).

Because CP-AMPARs, specifically those that are homomeric for GluA1, have been shown to have moderately faster decay kinetics than GluA1-GluA2 heteromers or GluA2 homomers (Oh and Derkach, 2005), we also examined whether NASPM would slow the decay constant of the Sr-EPSC. There was no significant reduction in decay kinetics in the presence of NAPSM when compared across all cells (P13 SWE Sr-EPSC 3.27±0.09 ms, n=17 versus in NAPSM 3.19±0.08 ms, n=15, p=0.31 by Mann-Whitney U test) and also when compared before and after drug application in the same cell (before 3.12±0.16 ms versus after 2.94±0.19 ms, n=4, p=0.19 by Mann-Whitney U test). Although this difference might become significant with a larger sample size, the lack of a pronounced effect suggests that decay kinetics of the Sr-EPSC is not a reliable indicator for CP-AMPARs.

If there were some synapses that contained primarily CP-AMPARs, NASPM application might reduce the apparent frequency of layer 4-triggered Sr-EPSCs without influencing the mean amplitude of events. Such a scenario might explain why the multi-quantal EPSC amplitude might be reduced by antagonist application, but the single-quantal response would not be markedly affected. However, a comparison of event frequency before and after NASPM application showed that event frequency was significantly increased (Figure 12D, baseline 3.96±0.21 Hz vs post-drug 4.73±0.40 Hz, n=4 cells), even when the mean amplitude of the Sr-EPSC was significantly reduced (Figure 12B, bottom panel).This increase in frequency suggests that NASPM might have some presynaptic targets that normally suppress presynaptic neurotransmitter release. Thus, we ascribe the lack of statistical significance for NASPM-blockade of multiquantal EPSCs between control and SWE layer 4-2/3 synapses (Figure 11D) to large cell-to-cell, not simply synapse-to-synapse, heterogeneity in the distribution of CP-AMPARs.

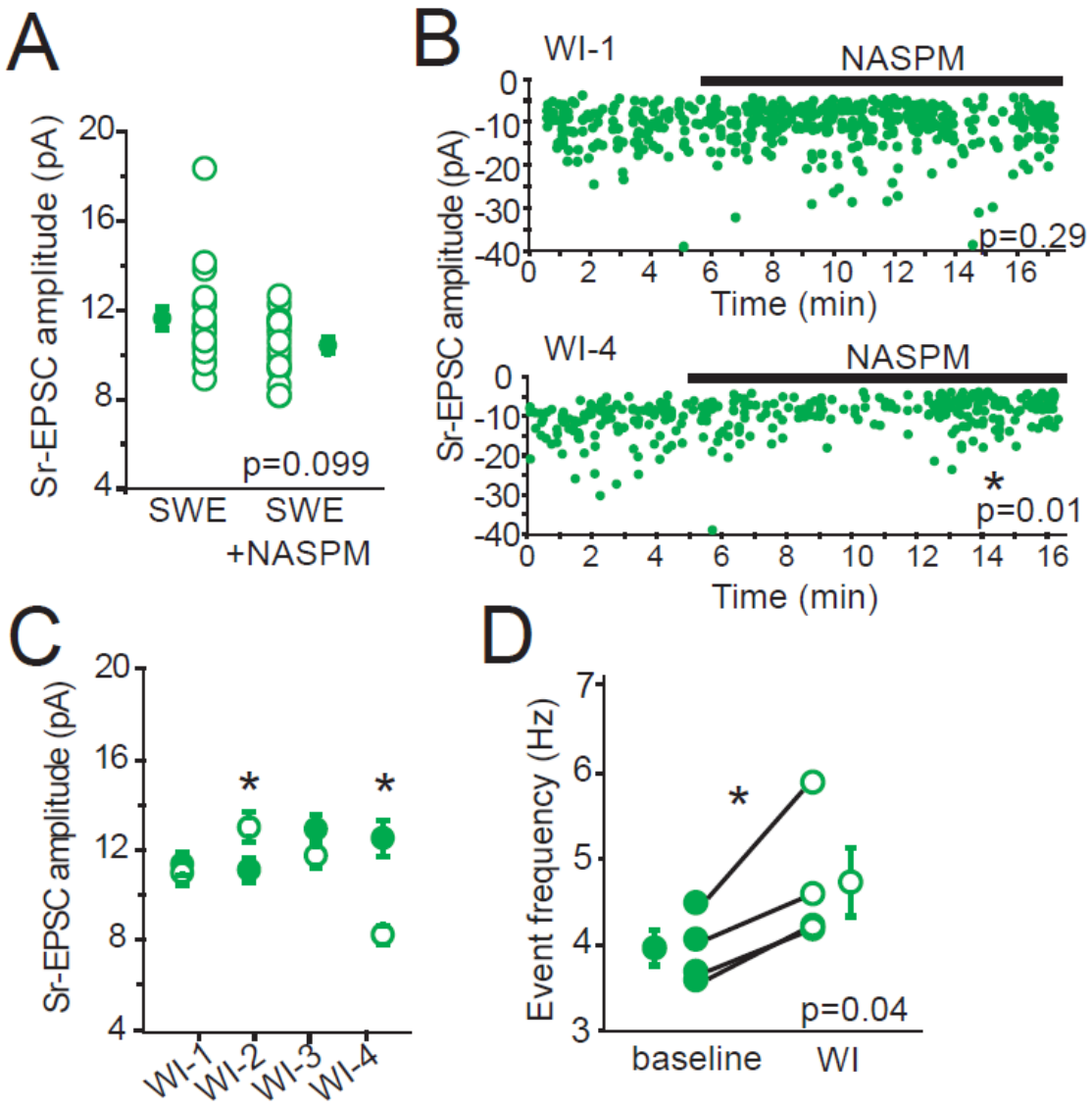


Figure 12. NASPM as a tool to block CP-AMPARs. A) Cross-cell comparison of Sr-EPSC amplitude between SWE-treated animals (SWE) and SWE-treated animals in the presence of NASPM (SWE+NASPM). **B)** Example experiments of a cell that showed no reduction in Sr-EPSC amplitude (top) and a cell that was reduced after NASPM wash in (bottom). *p<0.05, comparing the baseline and 5 min post-NASPM wash in window by Mann-Whitney U test. **C)** Within-cell comparison of Sr-EPSC amplitude between baseline and 5 min post-NASPM wash in (WI-1-4). **D)** Comparison of Sr-EPSC event frequency between baseline and post-NASPM wash in window (WI). *p<0.05 by paired t-test.

3.3.5 NASPM effectively blocks CP-AMPARs

A critical assumption behind using NASPM to block CP-AMPARs is that this compound is sufficient to fully block these receptors under our recording conditions. To verify that this was indeed the case, the RI was determined before and after drug application in the same cell. If NAPSM is sufficient to eliminate the contribution of rectifying AMPARs, the RI should become linear after drug application. This is indeed what was observed in pharmacologically-isolated AMPAR-EPSCs (Figure 13). Cells were divided into two groups (rectifying vs. non-rectifying), based upon their RI values. Cells with a rectifying I-V showed a 23±8% (n=5) block in peak AMPAR-EPSC amplitude, compared to cells with a linear I-V with a 3.5±5% (n=5) block. As expected, blockade of CP-AMPARs made the I-V more linear (Figure 13C,D, pre RI 0.4±0.07 vs post RI 0.59±0.03, n=5, $p<0.05$ by paired t-test). Taken together, these data indicate that NASPM application is sufficient to eliminate rectification, most likely through the selective blockade of CP-AMPARs.

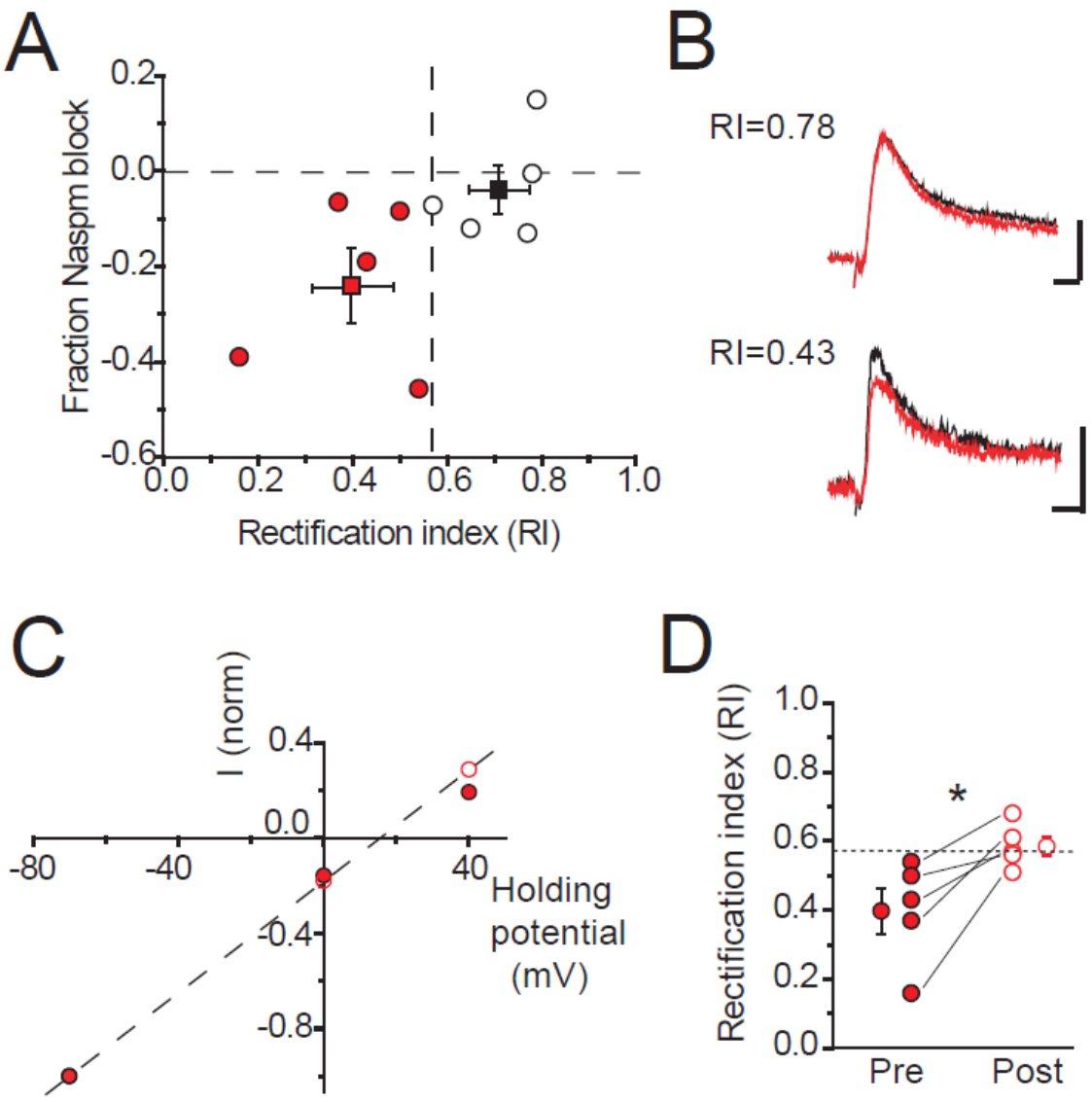


Figure 13. NASPM application blocks rectifying AMPARs. **A)** Fraction AMPA-EPSC amplitude blocked by NASPM wash-in at +40 mV in rectifying cells (red) and non-rectifying cells (black). Dashed lines: horizontal, fraction NASPM=0; vertical, RI=0.57. Mean RI and fractional NASPM block with corresponding S.E. are shown in the same plot. **B)** Example EPSC traces showing selective NASPM blockade of rectifying cells. Black: baseline EPSC before NASPM wash-in; red: 10 min after NASPM wash-in. Top: a non-rectifying cell (RI 0.78); scale bars: 20 pA, 5 ms. Bottom: a non-rectifying cell (RI 0.43); scale bars: 10 pA, 5 ms. **C)** I-V relationship of AMPA-EPSC in the same cell as in **B)** (bottom) became linear after NASPM application (before: filled; after: open). Current amplitudes recorded at -70, 0 and +40 mV were normalized to the amplitude at -70 mV. **D)** NASPM application significantly increases the rectification index (RI) after NASPM application. *p<0.05 by paired t-test.

3.3.6 Sr-depression does not require CP-AMPARs

If synaptic lability at recently potentiated synapses is mediated by the removal of CP-AMPARs, we should expect that when CP-AMPARs are blocked, Sr-depression can no longer occur. To test this hypothesis, NASPM was bath applied to slices from SWE-treated animals, and Sr-depression was induced by post-synaptic depolarization. Because this antagonist is an open-channel blocker, care was taken to bath apply the drug with afferent stimulation for at least 15 minutes prior to pairing. In half the cells (4/9), a significant depolarization-induced reduction in Sr-EPSC amplitude was observed (Figure 14A,B), indicating that in these cells reduced current through CP-AMPARs was not required for depression. The magnitude of depression in cells that showed a significant depolarization-induced change in Sr-EPSC amplitude was identical to that which we characterized earlier, ~20% (Figure 14B and 8G, 16±3%, n=4). The cumulative distribution of Sr-EPSC amplitude was also shifted after the pairing protocol in NASPM (Figure 14C, $p<0.001$ by Kolmogorov-Smirnov test). Overall, these findings are incompatible with an obligatory role for CP-AMPARs, either for induction or expression, in Sr-depression.

We also evaluated the decay kinetics of the post-pairing Sr-EPSC. If fast-decay CP-AMPARs are removed by this pairing protocol, it was reasonable to hypothesize that there might be an increase in the decay time constant. However, the lack of significant change after NAPSM application suggested we might not be able to detect a small change in decay kinetics. A comparison of the baseline and post-pairing decay time constant of the Sr-EPSC revealed that the decay time constant was not slower after Sr-depression (3.86±0.09 vs. 3.27±0.16 for baseline vs. post-pairing window, n=8 cells, $p=0.52$ by Mann-Whitney U test). These data are inconsistent with the removal of fast-decay CP-AMPARs during Sr-depression.

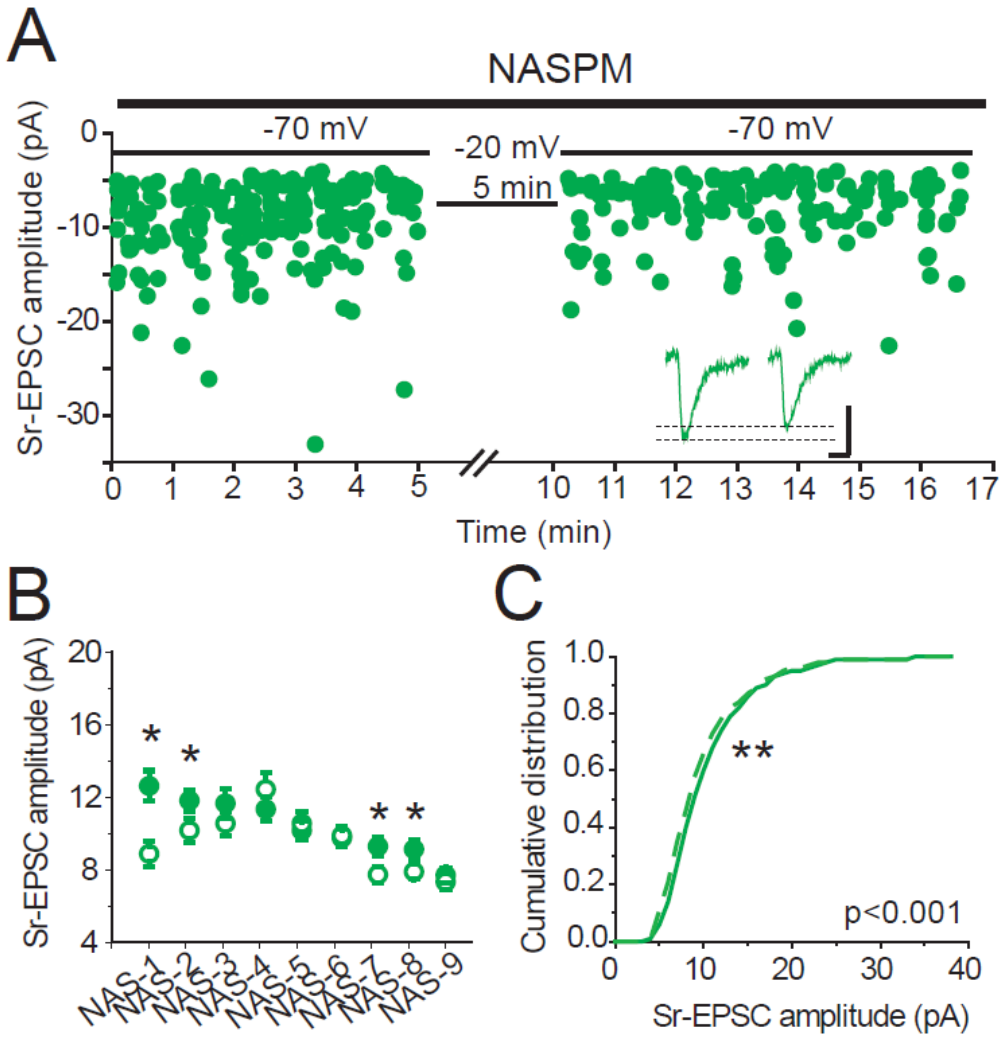


Figure 14. CP-AMPARs are not required for Sr-depression. A) Example scatter plot of a Sr-depression experiment in the presence of NASPM that still showed depression. Inset: average traces. Scale: 5 pA, 5 ms. **B)** Within-cell comparison of Sr-EPSC amplitude between pre- (filled circle) and post-depression induction (open circle) in the presence of NASPM. *p<0.05 by Mann-Whitney U test. **C)** Cumulative distribution histogram of Sr-EPSC amplitude before (solid line) and after (dotted line) pairing at -20 mV with presynaptic stimulation in NASPM (n=9 cells).

3.3.7 Rectification is not correlated with Sr-depression

If CP-AMPARs are important for Sr-depression, either for its induction, or because they are selectively removed, then cells with more rectifying AMPAR-EPSCs should show greater depression. This was not the case (Figure 15). The amount of Sr-depression was uncorrelated with the cell's RI, when RI measurements in a Ca^{++} based ACSF were made before Sr^{++} wash-in and depolarization (Figure 15A, p=0.53; rectifying cells, RI 0.45±0.03, magnitude of depression 21±4% vs non-rectifying cells, RI 0.70±0.05, magnitude of depression 23±3%, n=4 cells each). Cells that showed a linear I-V (Figure 15B, RI 0.59) or a rectifying I-V (Figure 15C, RI 0.53) showed similar depression.

In a subset of cells, the RI was determined in a Ca^{++} based ACSF both before and after Sr-depression. In these cells, we noted that the RI became more linear (Figure 15E, pre RI 0.45±0.03 vs post 0.66±0.02, n=4, $p<0.05$ by paired t-test), suggesting that when present, CP-AMPARs might be removed during depression. Thus, CP-AMPARs can be removed during, but their presence is not required for, Sr-depression.

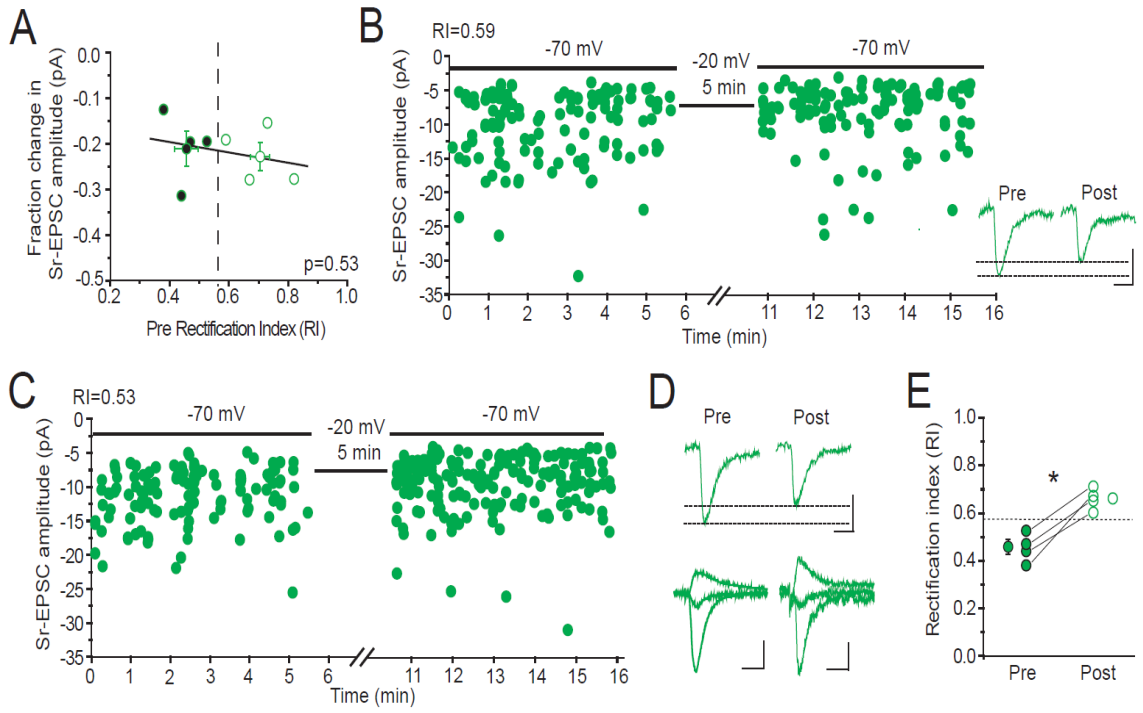


Figure 15. Sr-depression occurs irrespective of CP-AMPARs after 24 hr SWE. A) The RI of cells before the induction of Sr-depression is not correlated with the magnitude of depression in the same cells (n=8). Cells presented here all exhibited significant Sr-depression (n=8). Cells with rectifying (filled) and non-rectifying (open) AMPA-EPSCs all showed Sr-depression, the magnitude of which is indistinguishable between the two groups. **B)** Example Sr-depression experiment at a non-rectifying cell (RI 0.59) from a SWE-treated animal. Inset: average traces of Sr-EPSCs pre- and post-Sr-depression induction. Scale bar: 5pA, 5 ms. **C)** Example Sr-depression experiment at a rectifying cell (RI 0.53) from a SWE-treated animal. **D)** Rectification is absent after Sr-depression. Top: average Sr-EPSC pre- and post-Sr-depression from the same cell as in **C)**. Scale bars: 5 pA, 5 ms. Bottom: AMPA-EPSC at -70, 0 and +40 mV before (pre) and after (post) Sr-depression from the same cell. Scale bars: 50 pA, 10 ms (pre) and 10 pA, 10 ms (post). **E)** RI in rectifying cells significantly increases after Sr-depression (pre, filled; post, open). *p<0.05 by paired t-test.

3.3.8 Sr-depression is absent at older developmental ages

SWE-induced increased in the strength of layer 4-2/3 synapses can be observed at least until P14, although the contribution of CP-AMPARs to SWE-induced potentiation appears minimal at this time. The RI is identical for SWE at P14 compared to control cells (control RI 0.64±0.59 n=11 vs. SWE 0.59±0.13 n=4, see also (Wen and Barth, 2011), and NASPM showed a small effect on reducing the amplitude of the multiquantal EPSC (Figure 16A, control -8±12% n=6 vs. SWE -14±11%, n=4). At this age, Sr-depression in the spared whisker barrel column could not be induced in any cell (Figure 16C, 0/6 cells, p value range 0.49-0.70 baseline vs. post-pairing window by paired t-test).

The cumulative distribution of Sr-EPSC amplitudes were not different between pre- and post-pairing window (Figure 16D, p=0.936). The amplitude of SWE-induced synaptic strengthening was comparable between the two ages (Figure 16B, P13 11.62±0.48 pA, n=19 cells vs. P14 11.24±0.49 pA, n=8 cells, p=0.94 by Mann-Whitney U test; see also (Wen and Barth, 2011)), suggesting that pre-pairing response amplitude was not a factor in the induction of Sr-depression. These data suggest the presence of a critical period for Sr-depression which concludes at the end of the second postnatal week.

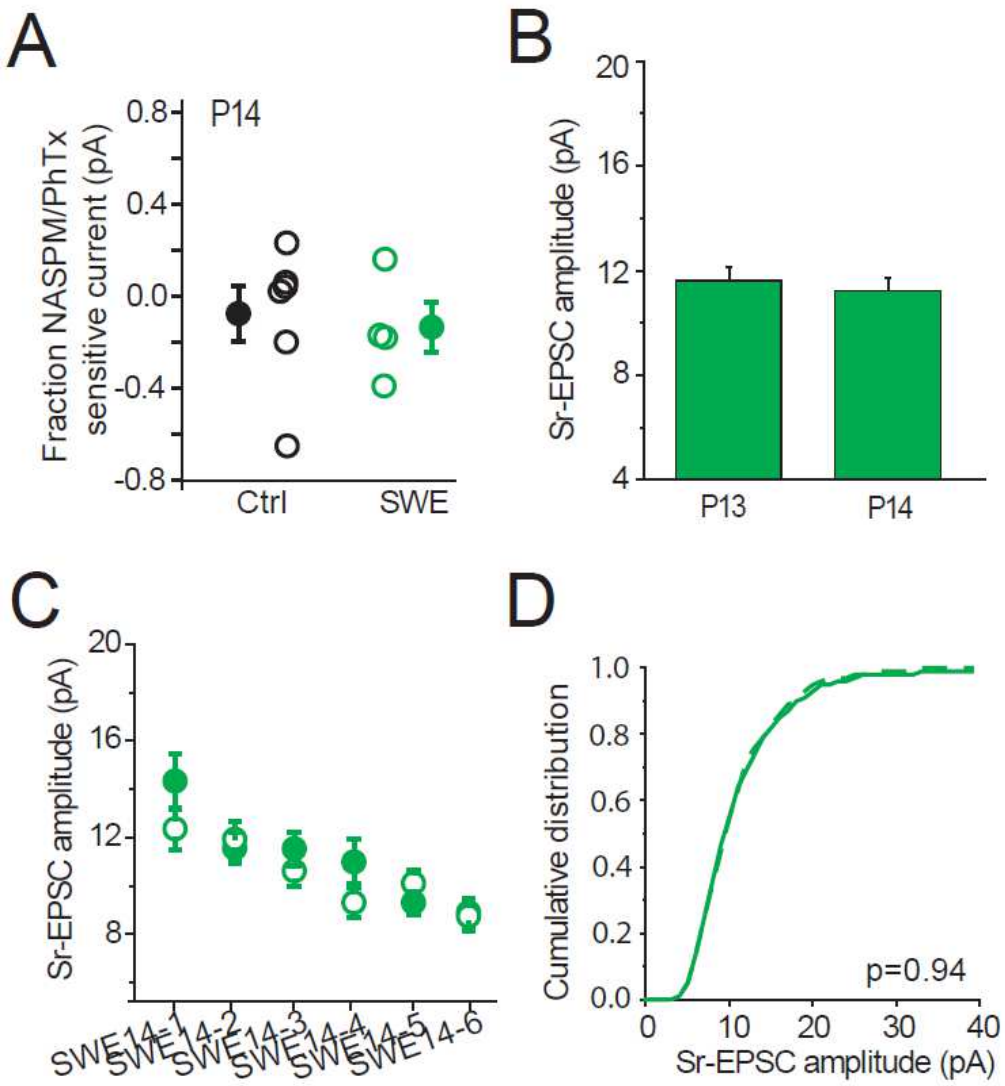


Figure 16. **Sr-depression was not observed at later developmental ages. A)** Fraction NASPM/PhTx sensitive current in P14 control and SWE-treated animals. **B)** Comparison of Sr-EPSC amplitude of the pre-pairing window between P13 and P14 SWE-treated animals. **C)** Within-cell comparison of Sr-EPSC amplitude between pre- and post-pairing window in all P14 SWE-treated cells. **D)** Cumulative distribution histogram of Sr-EPSC amplitude before (solid line) and after (dotted line) pairing at -20 mV with presynaptic stimulation (n=6 cells)

3.4 Discussion

CP-AMPARs have been observed at synapses across the CNS and can be mobilized in response to activity. Despite this, it has been controversial what the role of this special AMPAR subtype might be. Because the vast majority of AMPARs are CI-AMPARs, and CP-AMPARs appear to be tightly regulated, it has been tempting to speculate that these receptors might serve some special function. For example, the calcium-permeability of these receptors might allow activation of signal transduction cascades that normally require NMDARs in mediating synaptic plasticity (Burnashev et al., 1996; Mahanty and Sah, 1998; Biou et al., 2008; Wiltgen et al., 2010).

Studies presented here were designed to evaluate the specific hypothesis that the presence of newly-trafficked CP-AMPARs might confer a capacity for synaptic depression, possibly akin to the process of depotentiation that has been well-studied in other systems (O'Dell and Kandel, 1994; Kim et al., 2007). Previous work has shown that after SWE-initiated plasticity, potentiated layer 4-2/3 synapses exhibit a pronounced NMDAR-dependent depression (Clem *et al.*, 2008). Thus, although NMDARs are required to initiate potentiation at the onset of SWE, subsequent NMDAR activation triggers synaptic depression. We have developed a novel in vitro method, Sr-depression that recapitulates essential features of this depression, and tested the hypothesis that it proceeds via the activation and/or removal of CP-AMPARs. Our findings suggest that CP-AMPARs are not essential for synaptic depression at synapses that have been recently potentiated by sensory experience.

CP-AMPARs are not associated with Sr-depression at layer 4-2/3 synapses

Our conclusions are based upon the following findings from this study and others. First, blockade of CP-AMPARs by NASPM was not sufficient to abolish Sr-depression, indicating that reduced current via CP-AMPARS, either because of their synaptic removal or

decreased conductance, is not necessary for this phenomenon. Sr-depression could be induced in cells that express only CI-AMPARs, or express some CP-AMPARs, and the amount of Sr-depression was comparable between the two groups of cells. Layer 4-2/3 synapses are still labile, even when CP-AMPARs have been pharmacologically blocked. Second, although a subset of neurons from control animals showed rectifying AMPAR EPSCs (4/14 cells showed an RI <0.5) and substantial NASPM sensitivity (4/13 showed >15% EPSC block by NASPM; see also (Kumar *et al.*, 2002)), we never observed Sr-depression at layer 4-2/3 synapses from control animals. If the presence of CP-AMPARs was sufficient to confer a capacity for Sr-depression, we would expect to see this phenomenon at least occasionally in control tissue. Additionally, not all cells that express CP-AMPARs in SWE-treated animals exhibited Sr-depression (1/5 cells did not show Sr-depression). This dissociation between the absence of Sr-depression and the presence of CP-AMPARs was also observed at a slightly later developmental age (P14), where layer 4-2/3 inputs still display some NASPM sensitivity (in the current analysis, 3/4 cells show a >15% EPSC block by NASPM), but no Sr-depression.

The presence of Sr-depression during NASPM blockade in a subset of cells indicates not only that depression is not mediated by the removal of CP-AMPARs, but also that CP-AMPARs are not required to initiate Sr-depression. Because CP-AMPARs can flux some Ca^{++} (Burnashev *et al.*, 1996), it has been proposed that they might serve as a novel source for Ca^{++} entry to regulate plasticity under some conditions. However, we note that the conditions where Ca-entry via CP-AMPARs is required for the trafficking of AMPARs in excitatory neurons may be exceptional, such as in GluR2/B deficient animals (Biou *et al.*, 2008; Wiltgen *et al.*, 2010). Thus, at layer 4-2/3 synapses, CP-AMPARs may not be essential either for the induction or the expression of Sr-depression. Interestingly, although CP-AMPARs are not

required for Sr-depression, we observed that they could be removed during Sr-depression (after Sr-depression, the AMPAR-EPSC shifted from rectifying to linear in 4/4 cells), indicating that these receptors can be mobilized during this form of depression. NAPSM application modestly reduced the fraction of cells showing Sr-depression, from 80% of the cells to 50% of cells that showed a pairing induced reduction in Sr-EPSC amplitude, suggesting that CP-AMPARs might play some role in the initiation or expression of Sr-depression at a subset of synapses.

CP-AMPARs and potentiation

Are CP-AMPARs required for experience-dependent *potentiation* at neocortical synapses? Our previous studies showed that AMPARs became rectifying after LTP in vitro and also after SWE in vivo, suggesting that these receptors were acutely trafficked to and could be maintained at potentiated synapses (Clem *et al.*, 2008). However, SWE triggers plasticity at layer 2/3-2/3 synapses where CP-AMPARs are not detectable (Wen and Barth, 2011), and in PICK-1 knock-out animals, SWE still potentiates layer 4-2/3 inputs without adding CP-AMPARS (Clem *et al.*, 2010). Finally, we note that a capacity for further synaptic potentiation *in vitro,* after the onset of SWE-induced synaptic strengthening, does not require CP-AMPARs, since pharmacological blockade of CP-AMPARS does not impair LTP after the onset of SWE-induced strengthening (Clem *et al.*, 2008). Taken together, these data indicate that CP-AMPARs are not broadly required for synaptic potentiation at neocortical synapses.

Estimating the contribution of CP-AMPARs to the EPSC

As in other studies, both pharmacological and electrophysiological methods were used here to ascertain the presence of CP-AMPARs. Our results suggest that antagonists that have been used as specific blockers of CP-AMPARs may have some unanticipated effects on EPSCs. For example, in a number of cases we observed an increase in the multiquantal EPSC

amplitude after NAPSM application (an increase of 5-40% in approximately one third of all cells), suggesting that a NASPM-sensitive receptor might normally reduce presynaptic release probability. Since these experiments were carried out in the presence of D-APV, it is unlikely that NASPM block of presynaptic NMDARS that have been hypothesized to exist at this synapses (Bender et al., 2006b; Banerjee et al., 2009) are responsible for this effect. NASPM has been shown to block kainate receptors (Sun *et al.*, 2009), and presynaptic kainate receptors have been described at thalamocortical synapses in somatosensory cortex (Kidd *et al.*, 2002). Thus, we hypothesize that presynaptic kainate receptors may be present at layer 4-2/3 synapses. Although investigating the effects of polyamine antagonists on release probability was not a focus of the current work, further investigations into this effect may be of interest.

These NASPM effects complicate the interpretation of ours and others' results, since an increase in release probability would lead to an apparent increase in the amplitude of the post-synaptic EPSC and underestimation of the contribution of post-synaptic CP-AMPARs. However, our electrophysiological analysis showing rectification of the AMPAR-EPSC is consistent with the presence of these receptors at layer 4-2/3 synapses under some conditions.

Sr-depression: a new experimental approach to study synaptic plasticity in vitro

Our finding that we can induce depression of the quantal EPSC amplitude in a Sr-ACSF solution is provocative. This method offers the advantage of precisely evaluating how post-synaptic depolarization influences both the frequency and the amplitude of EPSCs at individual synaptic contacts onto a cell. Consistent with a post-synaptic locus for depression, we find EPSC amplitudes at layer 4-2/3 synapses are decreased without any change in event frequency. On average, a 20% reduction in Sr -EPSC amplitude was observed. The magnitude of the depression appears modest, but we note that a 20% reduction in Sr-EPSC amplitude

normalizes SWE-induced increases back to control levels. Although a role for presynaptic NMDARs in depression of layer 4-2/3 excitatory synapses has been proposed (Bender et al., 2006b; Banerjee et al., 2009), it is important to note that the effects characterized here are likely post-synaptic in origin.

The protocol developed here to elicit synaptic depression in the presence of Sr^{++} is novel, and we have used it to probe the mechanisms that underlie synaptic lability at recently potentiated synapses. How long does this synaptic depression persist after pairing? The post-pairing period analyzed here was admittedly short (5 min), and future experiments will be required to determine the duration of this effect. Although our data suggest that removal of CP-AMPARs can sometimes occur during Sr-depression, depression may also result from the removal of CI-AMPARs or activation of intracellular signaling cascades to reduce channel conductance. Thus, there may be several different mechanisms that underlie Sr-depression. Alternatively, Sr-depression might occur via a common mechanism involving GluR1, a subunit that could be found in both rectifying and non-rectifying AMPARs. In addition, we note that some forms of LTD might specifically target the removal of CP-AMPARs; there are likely to be diverse processes that regulate synaptic depression across the CNS. However, the relative simplicity of this assay should facilitate its use in other experimental preparations.

The mechanism by which Sr^{++} triggers vesicle fusion at the presynaptic terminal has been studied (Goda and Stevens, 1994; Xu-Friedman and Regehr, 1999), but a role for this ion in activating post-synaptic signaling cascades has not been evaluated. Because it is impossible to remove all Ca^{++} from our bath solution (even without addition of Ca^{++}, free Ca^{++} may be in the low micromolar range), we cannot determine whether Sr^{++} is acting on normally Ca^{++}-dependent signaling cascades, or whether residual Ca^{++} in the ACSF is sufficient to do

this. NMDARs are permeable to Sr^{++} (Mayer and Westbrook, 1987), and thus it is possible that depolarization leads to influx of this ion to activate post-synaptic signaling cascades for depression. A more detailed investigation into this phenomenon is warranted.

4. Ongoing sensory experience induces distinct phases of synaptic plasticity in barrel cortex

4.1 Introduction

Experience in vivo can induce synaptic strengthening by trafficking of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) (Shi et al., 1999; Takahashi et al., 2003; Rumpel et al., 2005; Bellone and Luscher, 2006; Clem and Barth, 2006; Matsuo et al., 2008; Clem and Huganir, 2010). Long-term potentiation (LTP)-like synaptic strengthening is associated with improved learning and blockade of molecules and pathways required for synaptic strengthening impairs learning and memory (Morris et al., 1986; Silva et al., 1992; Moser et al., 1998). Therefore experience-induced synaptic strengthening serves as a cellular substrate and read-out for learning and memory. Past decades of research on hippocampus-dependent spatial memory and amygdala-dependent emotional memory has established that new memories are labile shortly after their acquisition and are sensitive to disruption by a wide variety of manipulations before they are stabilized, a process known as "consolidation" (Nader et al., 2000; Nader and Einarsson, 2010). Furthermore, the "consolidated" memories can re-enter the labile state of heightened sensitivity to disruption during later memory retrieval or reactivation, a process known as "reconsolidation". In addition, procedural motor memories have displayed a similar labile phase during reactivation (Walker et al., 2003). These behavioral findings support the notion that memories are labile.

Consistent with a labile nature of memories, the maintenance of enhanced synaptic strength after LTP induction is unstable in vivo and in vitro. At the medial perforant path-dendate gyrus synapse, LTP induced by theta burst stimulation (TBS) protocol in awake, freely moving rats decays within 3-5 days (Villarreal et al., 2002). Sustained injection of NMDAR antagonist, CPP into the animals after LTP induction can block the decay of LTP

(Villarreal et al., 2002). Consistent with the effect of prolonged LTP, sustained CPP injection can enhance spatial memory retention (Villarreal et al., 2002). These findings indicate that activity-dependent increase in synaptic strength is labile and subject to NMDAR-mediated depression in vivo. The lability of experience-induced increase in synaptic strength has also been observed in vitro, where subsequent activity or experience following the initial LTP induction rapidly degrades synaptic strengthening (Barrionuevo et al., 1980; Xu et al., 1998; Zhou et al., 2003; Whitlock et al., 2006).

Layer 2/3 pyramidal cells can encode sensory information and drive behavior (Huber et al., 2008). Layer 4-2/3 excitatory synapses are the major pathway for propogation of sensory information within primary somatosensory cortex and these synapses provide the major excitatory inputs to layer 2/3 pyramidal cells. Pronounced experience-driven synaptic plasticity has been documented at layer 4-2/3 synapses, which is correlated with the plasticity of cortical responsiveness of layer 2/3 cells (Allen et al., 2003; Heynen et al., 2003; Clem and Barth, 2006; Benedetti et al., 2009). Thus the properties of experience-induced synaptic plasticity at layer 4-2/3 synapses are critical for shaping the firing reponse of layer 2/3 pyramidal cells and influencing the abilities of layer 2/3 cells to encode information and drive behavior. In this chapter of the thesis, we will investigate how ongoing sensory experience modifies the strength of layer 4-2/3 synapses in barrel cortex of young postnatal mice and the underlying molecular mechanisms for these time-dependent changes.

We have previously reported that unbalanced whisker experience modifies the strength of layer 4-2/3 excitatory synapses in barrel cortex (Clem and Barth, 2006; Clem et al., 2008). While the initiation of single whisker experience (SWE) where all but one whisker have been removed triggers synaptic potentiation of layer 4-2/3 synapses, subsequent experience induces

synaptic depression (Clem and Barth, 2006; Clem et al., 2008). However, the time scale at which this transition occurs as well as the underlying molecular mechanisms remain unknown. Here, in order to investigate how ongoing whisker experience changes layer 4-2/3 synaptic strength over time, we recorded synaptic strength from animals that underwent various duration of experience. We discovered that ongoing whisker experience triggered an early phase of plasticity where synaptic strength progressively increased, followed by a labile phase where synaptic strength weakened and a subsequent stabilization phase where synaptic strength did not change with additional experience.

The clearly delineated time course of synaptic plasticity allows us to dissect specific mechanisms involved in individual phases of plasticity. We demonstrated that although both the early and the labile phases of plasticity required NMDAR activation, postsynaptic NMDARs became less sensitive to the selective NR2B antagonist ifenprodil during the transition from the early to the labile phase. Taken together, these findings indicate that experience-dependent changes in excitatory synaptic strength in vivo occur in distinct phases and the functional switch of NR2 subunit composition during experience-dependent in vivo plasticity may serve as a molecular mechanism for modulating the direction of synaptic plasticity in primary sensory cortices.

4.2 Materials and Methods

Animals

There are two sensory paradigms used in this study, single whisker experience (SWE) vs. single row experience (SRE). In the SWE paradigm, bilateral whisker deprivation was performed where all but the D1 whisker on one side of an animal's snout was removed (Barth

et al., 2000). In the SRE paradigm, all whiskers were deprived bilaterally except a single set of D row whiskers on one side (Finnerty et al., 1999). Wild-type or heterozygous mice from a fosGFP (1-3 line, C57Bl6 background) transgenic line (fosGFP+/-, aged P12-P14) were used for experiments where SWE was induced. Non-transgenic C57Bl6 mice of the same age were used where SRE was induced. Animals were returned to their home cages for varied amount of time (0-72 hrs) before sacrifice for recording. Control animals were undeprived whisker-intact littermates of the deprived animals and because control animals did not undergo SWE, the data from these animals were taken as "0 hr" SRE-treated. Recordings from any barrel column in control (0 hr) animals were grouped because the barrel columns in these animals were considered equivalent. The barrel column representing the spared D1 whisker in SWE-treated animals was identified after at least 18 hr SWE by enhanced fosGFP expression and relative position to the hippocampus in acute brain slices. The spared D barrel in SRE-treated animals was identified as the fourth barrel from the lateral side of slices that contain 5 barrels (A to E, lateral to medial). Any slice that did not contain clear A-E barrels was discarded. There are usually one to two slices per brain containing 5 barrels.

Whole-cell recording

Animals were anesthetized with isoflurane and decapitated. Slices were prepared in two different ways according to the type of sensory experience (SWE vs. SRE) that animals underwent *in vivo*. Coronal slices with 350 um thickness for SWE-treated animals were vibratome sectioned in regular artificial cerebrospinal fluid (ACSF) at 2-6ºC composed of (mM): 119 NaCl, 2.5 KCl, 2.5 $CaCl_2$, 1.3 $MgSO_4$, 1 NaH_2PO_4, 26.2 $NaHCO_3$, 11 glucose and equilibrated with 95/5% O_2/CO_2. Slices from SRE-treated animals were prepared by an "across-row" protocol (Finnerty et al., 1999; Clem et al., 2010). The mouse brain was put in a

flat surface (no incline) and one cut was made at the posterior end of the brain along a plane that is 45 degree towards the midline. The hesmisphere contralateral to the spared whiskers was saved and the sectioning plane was mounted to the magnet plate of the vibratome. The cutting procedures and slice thickness were kept the same as those from SWE-treated animals. Slices were maintained and whole-cell recordings were performed at room temperature. Somata of lower layer 2/3 pyramidal neurons in barrel cortex were targeted for whole-cell recording with borosilicate glass electrodes with a resistance of 4-8 MOhm. The depth of neurons was kept consistent across all animals and conditions. Electrode internal solution was composed of (in mM): 130 cesium gluconate, 10 HEPES, 0.5 EGTA, 8 NaCl, 10 TEA-Cl, 4 Mg-ATP and 0.4 Na-GTP, 5 QX 314 at pH 7.25-7.30, 290-300 mOsm and contained trace amounts of Alexa-568. Pyramidal cell identity was confirmed after the recording session by pyramidal somata morphology and the presence of dendritic spines. Only cells with $R_{series} \leq$ 30 MΩ and $R_{input} \geq$ 200 MΩ, where changes in either measurement were less than 30% were included for analysis. Stimulation of presynaptic afferents was applied at 0.1 Hz by placing glass monopolar electrodes in the center of layer 4 barrels. Postsynaptic responses from layer 2/3 pyramidal neurons within the same barrel column were recorded. Electrophysiological data were acquired by Multiclamp 700A (Axon Instruments, Foster City, CA) and a National Instruments acquisition interface. The data were filtered at 3 kHz and digitized at 10 kHz and collected by Igor Pro 6.0 (Wavemetrics, Lake Oswego, Oregon). Extracellular simulation was controlled by a Master-8 (A.M.P.I, Israel) and a stimulus isolator Isoflex (A.M.P.I, Israel).

Evoked Sr–AMPA miniature EPSC measurement

To measure the amplitude of stimulus-evoked miniature AMPAR-EPSCs, Sr^{++} (3 mM) was substituted for Ca^{++} in regular ACSF to drive asynchronous glutamate release. D-APV (50

μM) and picrotoxin (Ptx, 50 μM) were included to pharmacologically isolate AMPAR-mediated EPSCs. Layer 2/3 pyramidal neurons were voltage-clamped at -70 mV. The evoked response has an initial synchronous component (~50 ms post the stimulus artifact) which was excluded in the analysis. Isolated, asynchronous events that occurred from 50-500 ms after the stimulus were manually selected and analyzed using Minianalysis software (Synaptosoft, Inc. Decatur, GA). The detection threshold for events was set at 2x RMS noise (usually around 4-5 pA) and data were filtered with a low-pass filter at 1 kHz. Approximately 100 randomly selected events were selected for each cell and then grouped to obtain the average trace or to measure the amplitude distribution for each condition. Comparisons were made between groups. Averaged traces for each experimental condition were obtained by grouping average traces from selected events for all cells.

NMDAR-mediated EPSC and ifenprodil sensitivity

Evoked NMDAR-mediated EPSC was isolated by including 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide hydrate (NBQX, 5 μM) and Ptx (50 μM) in the bath solution (regular ACSF). Layer 2/3 pyramidal cells were voltage-clamped at +40 mV. A single-exponential function was fitted to the average NMDA–EPSC trace from its peak to 200 ms after the stimulus onset and a decay constant τ was determined from the fit. Baseline recordings of NMDA currents lasted at least 10 min to make sure there is a stable baseline of 5-10 min. To assess the content of NR2B-containing NMDARs, ifenprodil (5 μM), a specific NR2B antagonist was infused to the recording chamber locally while recordings were continued. Because the action of ifenprodil was poorly reversible (Kumar and Huguenard, 2003), we expected no recovery of the currents after drug application which was observed in all of our experiments. The average baseline response was

obtained by averaging 20-30 sweeps right before the drug was added. The average post-drug response was obtained by averaging 20-30 sweeps towards the end of recording after the response stabilized.

Measurement of non-postsynaptic NMDARs

Evoked AMPAR-mediated EPSC (AMPA-EPSC) was isolated by including Ptx (50 μM) in the bath solution containing 4 mM Ca^{++} and 4 mM Mg^{++} to block polysynaptic activity. Layer 2/3 pyramidal cells were voltage-clamped at -80 mV while postsynaptic NMDARs were blocked by non-competitive open-channel blocker of NMDARs, (+)-5-methyl-10,11-dihydro-5H-dibenzo [a,d] cyclohepten-5,10-imine maleate, MK801 (1 mM) included in the internal solution (Sjostrom et al., 2003; Bender et al., 2006b; Corlew et al., 2007; Brasier and Feldman, 2008). Baseline recordings lasted about 10 min to allow efficient blockade of postsynaptic NMDARs by MK801. Two pulses with inter-pulse interval of 33 ms (30 Hz) (Brasier and Feldman, 2008) were administered at a frequency of 0.1 Hz for recordings of paired pulse ratio of AMPA-EPSCs. Cells with a latency of 2-5 ms for the 1^{st} EPSC and that showed clear monosynaptic component EPSC were included for analysis. Baseline responses and post-drug responses were obtained by averaging 20 sweeps right before drug application or 5-10 min after drug application when responses were normally stabilized. Paired pulse ratio (PPR) was expressed as the ratio of the 2^{nd} EPSC amplitude and the 1^{st} EPSC amplitude of the average traces (2^{nd} EPSC/1^{st} EPSC).

Timed injection of NMDAR antagonist

NMDAR antagonist, CPP (10 mg/kg body weight) was injected intraperitoneally at different time points after the onset of SWE and whole-cell recordings were performed on

injected animals at 24, 48 or 72 hours. Injections were administered once and the animals were put back to their home cages until sacrifice.

Statistics

For all non-pairwise comparisons, a non-parametric Mann Whitney U test (two-tailed) was used. Cumulative distributions of event amplitude for grouped cells between conditions were compared using Kolmogorov-Smirnov Test (KS test). A p value less than 0.05 was considered statistically significant.

4.3 Results

4.3.1 Altered whisker experience induces an early phase of increase in Sr-EPSC amplitude

Brief exposure to new experience *in vivo* leads to rapid increase in synaptic strength in many brain regions. Many studies have shown that the strength of recently potentiated synapses is unstable and subject to activity-dependent depression (Barrionuevo et al., 1980; Zhou et al., 2003; Whitlock et al., 2006; Wen and Barth, 2012). Previously, our laboratory has demonstrated that 24 hr SWE induced an initial increase in synaptic strength of layer 4-2/3 excitatory synapses and later switched to weaken it (Clem and Barth, 2006; Clem et al., 2008; Wen and Barth, 2011). However, the time scale for the trainsition from synaptic potentiation to depression is unknown. In order to investigate the time course of experience-dependent changes in synaptic strength, we recorded miniature AMPAR- EPSCs in Sr^{++}-replaced ACSF (Sr-EPSC) (Goda and Stevens, 1994; Abdul-Ghani et al., 1996; Xu-Friedman and Regehr, 1999) from animals that undergo different lengths of whisker experience. The amplitude of

Sr-EPSCs recorded at each time point represents the experience-induced alteration in synaptic strength as a result of cumulative prior experience.

Selective up-regulation of fosGFP in the spared barrel column of fosGFP transgenic mice after SWE has enabled us to identify and study synaptic changes in the brain circuits affected by experience. Despite the fact that we can identify the spared D1 barrel after 24 hrs of SWE, it has proven difficult for unequivocal identification before 12 hrs of SWE, a result that might be caused by insufficient activation of the spared whisker for less than 12 hrs. Therefore, we used SRE where all whiskers except a single D row are removed bilaterally. Using a para-sagittal slicing protocol (Fig. 17A), we could identify the spared D barrel from slices with an orderly array of A-E barrel (Fig. 17B, right). It has been shown that SRE can also increase layer 4-2/3 synaptic strength (Clem et al., 2010). We compared Sr-EPSCs from animals that underwent SWE vs. SRE to see if both paradigms are similar. In undeprived control animals (0 hr SRE), the Sr-EPSC amplitude was not different between SWE and SRE (Fig. 17C,D and Fig. 17E SWE 0 hr, 9.9±0.80 pA, n=9 cells vs. SRE 0 hr 9.2±0.37 pA, n=14 cells, p=0.30), suggesting that there is no bias in the population of synapses under investigation that might be introduced by different slicing protocols. Similar to SWE, twenty-four hours of SRE induced an increase in the Sr-EPSC amplitude (Fig. 17C,D, 0 hr vs. 24 hr SRE p=0.01) and the magnitude of synaptic potentiation for SWE tended to be slightly higher than SRE (Fig.17E, SWE 24 hr 11.9±-0.43 pA n=15 cells vs SRE 24 hr 10.7±0.40 pA, n=12 cells, p=0.06). These initial comparisons suggest that SWE and SRE are similar in terms of the capacity to increase synaptic strength in vivo and that SRE offers accessibility to the early time points of sensory experience and could be used as a tool to study the time course of synaptic change

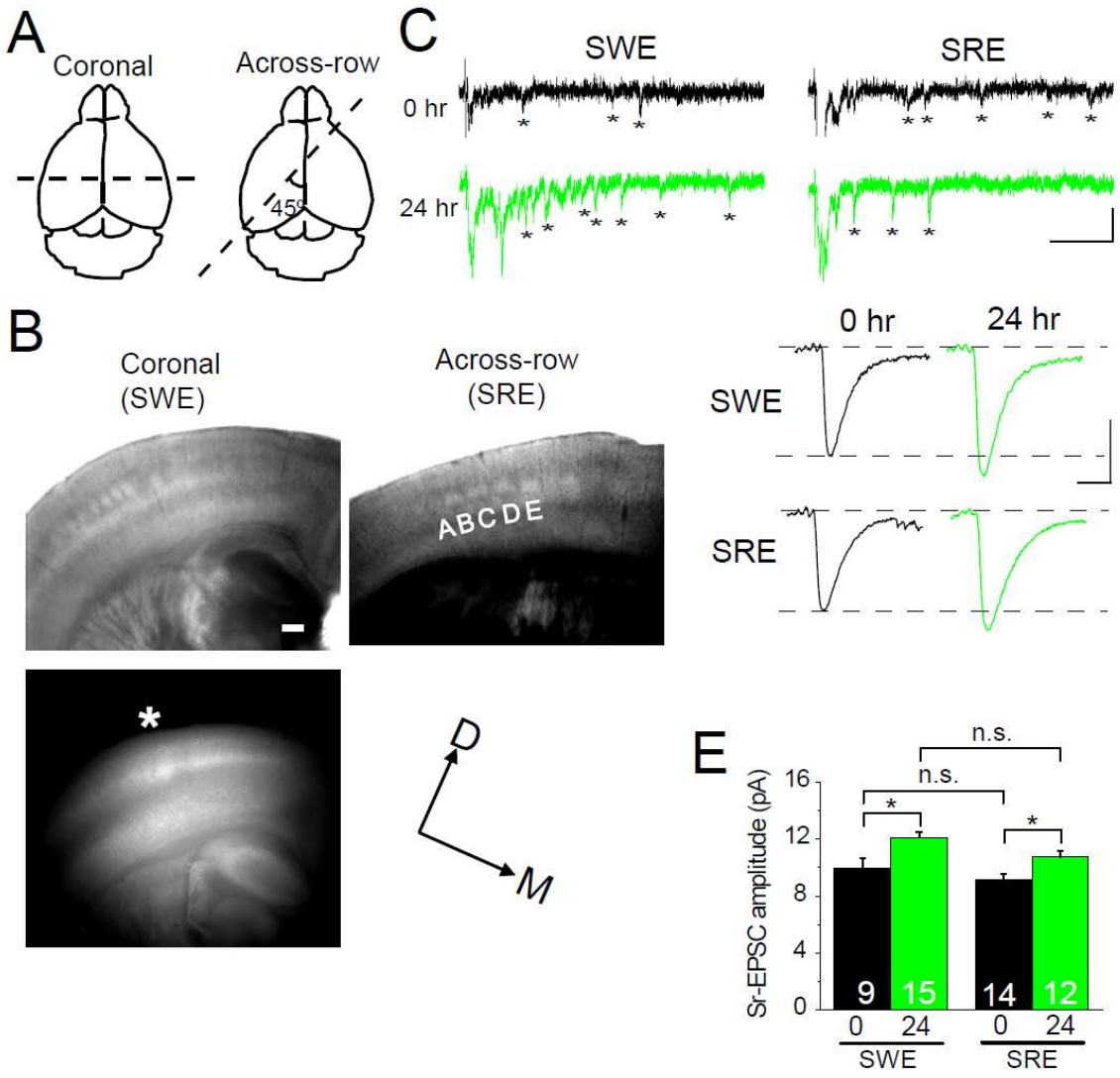


Figure 17. SRE is similar to SWE. A) Schematic of two different slicing procedures, coronal vs. across-row. **B)** Images of slices that contain layer 4 barrels using coronal and across-row slice preparations. Top: bright field images. Scale bar: 300 um. Bottom: green fluorescent image of a coronal slice that contains the spared D1 barrel (*) from fosGFP animals after 24 hr SWE. D: dorsal. M: medial. Slices from SWE-treated animals were prepared by coronal dissection and slices from SRE-treated animals were prepared by across-row dissection. **C)** Example traces of layer 4-evoked Sr-EPSCs in layer 2/3 pyramidal cells recorded from animals that underwent 0 and 24 hr of SWE vs. SRE, in coronal and across-row slices, respectively. **D)** Average traces of Sr-EPSCs from 0 and 24 hr of SWE vs. SRE. Black: 0 hr; green: 24 hr. Scale bars: 5 pA, 5 ms. **E)** Summary of Sr-EPSC amplitude for 0 and 24 hrs of SWE and SRE.

4.3.2 SRE induces an early phase of increase in Sr-EPSC amplitude

In ordert to investigate the time course of SRE-induced synaptic plasticity at layer 4-2/3 synapses, we first recorded Sr-EPSCs from 6 and 12 hrs after the onset of SRE. Compared with that in the undeprived control animals (0 hr), the Sr-EPSC amplitude at 6 and 12 hrs significantly increased in a progressive manner (Fig. 18B,C, 0 hr 9.4±0.34 pA, n=13 cells; 6 hr 11.1±0.51 pA, n=8 cells; 12 hr 13.2±0.46 pA, n=10 cells, $p<0.0001$ by one-way ANOVA followed by Mann Whitney U test 0 vs 6 hr, $p=0.006$; 6 vs. 12 hr, $p=0.01$). Synaptic strength increased by about 40% from 0 hr to 12 hr and the rate of synaptic strengthening is relatively constant between 0-6 hr and 6-12 hr (~0.3 pA/hr). Consistently, cumulative distribution of the event amplitude also exhibited a progressive increase from 0 hr to 12 hr (Fig. 18D, 0 vs. 6 hr, $p<0.001$; 6 vs. 12 hr, $p<0.001$ by KS test). These data show that during the first 12 hrs of SRE, synaptic strength progressively increases at layer 4-2/3 excitatory synapses.

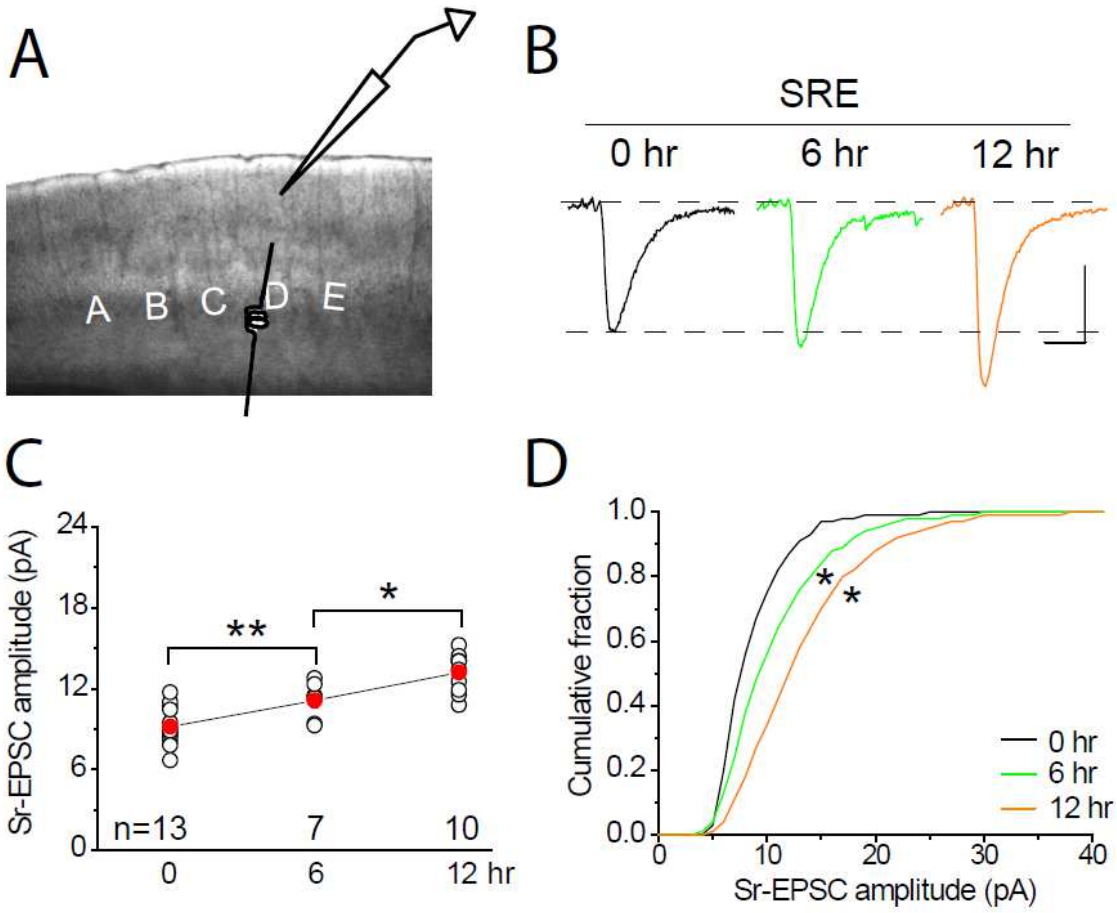


Figure 18. The early phase of plasticity: progressive increase in Sr-EPSC amplitude. A) Schematic of a "across-row" slice containing A-E barrels with electrode configurations. **B)** Average traces of Sr-EPSCs from animals that undergo 0, 6 and 12 hrs of SRE. Scale bars: 5 pA, 5 ms. **C)** Scatter plot of Sr-EPSC amplitude at 0, 6 and 12 hrs of SRE. **D)** Cumulative histograms of Sr-EPSC amplitude from animals that undergo 0, 6 and 12 hrs of SRE.

4.3.3 A labile phase of decrease in Sr-EPSC amplitude

We then asked if additional experience continues to strengthen synapses. Interestingly, the Sr-EPSC amplitude after 12 hr of SRE did not continue to increase, instead, it gradually decreased. The Sr-EPSCs recorded at 24 hr was ~3 pA smaller, compared to 12 hr (Fig. 19A,B, 18 hr, 12.3±0.70 pA, n=10 cells and 24 hr, 10.7±0.40 pA, n=12 cells, p=0.003 by one-way Anova followed by Mann-Whitney U test, 12 vs. 24 hr, p=0.16, 18 vs. 24 hr p=0.04). This

decrease in synaptic strength was also evident from the cumulative distribution (Fig. 19C, 12 vs. 18 hr, $p<0.001$; 18 vs. 24 hr, $p<0.001$ by KS test), however, the amplitude at 24 hr was still higher compared to 0 hr (Fig. 19C, 0 vs. 24 hr, $p<0.001$ by KS test). The rate of synaptic weakening between 12 and 24 hr was ~0.2 pA/hr, slightly smaller than that of synaptic strengthening between 0 and 12 hr, resulting in a net synaptic strengthening at 24 hr. Thus we refer to the period between 12 and 24 hr where the Sr-EPSC amplitude decreases as the "labile" phase.

Since the spared barrel could be identified in SWE-treated fosGFP mice at 18 hr after the experience onset, we wanted to see if SWE triggers a similar time course of synaptic depression. Consistent with the results from SRE-treated animals at 18 hr, the Sr-EPSC amplitude was significantly higher at 18 hr compared with 24 hr in SWE-treated animals (Fig. 19D). We normalized the Sr-EPSC amplitude at 0, 18 and 24 hr to the mean value at 0 hr for SWE and SRE respectively, and compared the relative change induced by the two sensory paradigms. Our data show that there was no significant difference between the magnitude of synaptic changes at 18 hr or 24 hr (Fig. 19D, 18 hr SWE vs. SRE, $p=0.57$; 24 hr SWE vs. SRE $p=0.50$). Both experience paradigms showed an early potentiation phase (compare 18 hr and 0 hr) and a labile phase (compare 18 and 24 hr). These data indicate that these phase-specific changes in synaptic strength are a general phenomenon of experience-dependent synaptic plasticity in barrel cortex, independent of the type of sensory paradigm being used.

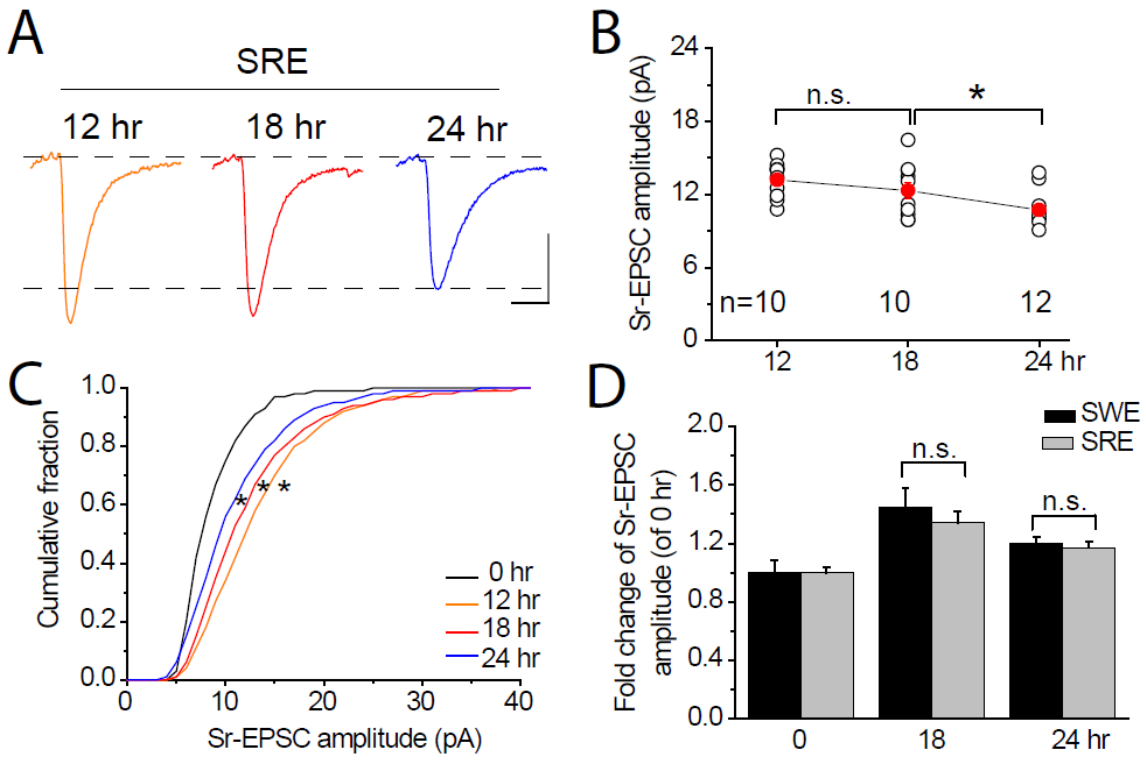


Figure 19. The labile phase: a decrease in Sr-EPSC amplitude. A) Average traces of Sr-EPSCs from animals that undergo 12, 18 and 24 hrs of SRE. Scale bars: 5 pA, 5 ms. **B)** Summary of Sr-EPSC amplitude at 12, 18 and 24 hr. **C)** Cumulative histograms of Sr-EPSC amplitude at 0, 12, 18 and 24 hr of SRE. **D)** Comparison of Sr-EPSC amplitude between SWE (black) and SRE (gray) in relative fractions normalized to the average 0 hr values for SWE and SRE.

4.3.4 The stabilization phase of no change in Sr-EPSC amplitude

To test if synaptic strength continues to decrease, we measured Sr-EPSC amplitude in 48 hr SRE-treated animals. The average amplitude of Sr-EPSCs at 48 hr was not different from that at 24 hr (Fig. 20A,B, 24 hr SRE 10.7±0.4 pA vs. 48 hr SRE 10.9±0.9 pA, p=0.82). Neither did the cumulative distribution of the event amplitude reveal any difference (Fig. 20C, p=0.61 by KS test). Thus, we find that after a brief labile phase following the initial synaptic potentiation, synaptic strength appears to be stabilized at layer 4-2/3 excitatory synapses. We refer to this period where continued experience does not further change the Sr-EPSC amplitude as the "stabilization" phase.

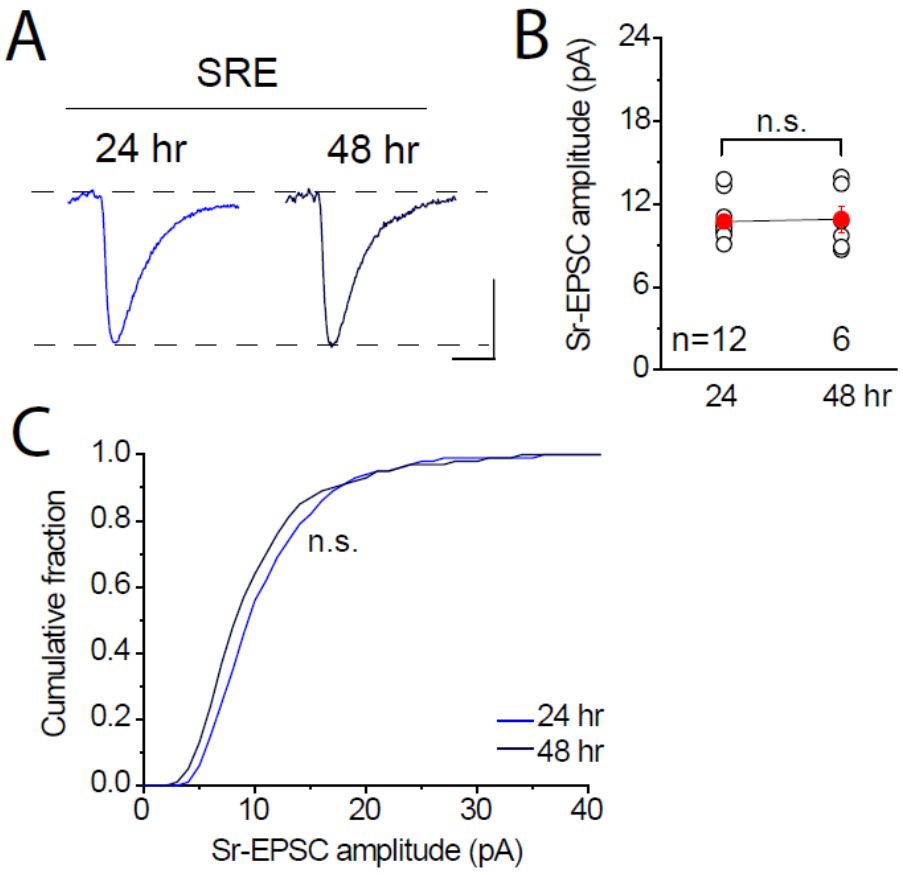


Figure 20. The stabilization phase. A) Average traces of Sr-EPSCs from animals that undergo 24 and 48 hr of SRE. Scale bars: 5 pA, 5 ms. **B)** Scatter plot of Sr-EPSC amplitude at 24 and 48 hr. **C)** Cumulative histogram of Sr-EPSC amplitude at 24 and 48 hr.

4.3.5 Phase-specific transition of NMDAR function in experience-dependent synaptic plasticity

By whole-cell recordings of Sr-EPSCs at layer 4-2/3 excitatory synapses, we identified three distinct phases of experience-dependent changes in synaptic strength. The discovery of these discrete phases constrains the temporal window to studying specific molecular mechanisms responsible for plasticity at individual phases as well as for metaplasticity that occurs during phase transitions. Previously our laboratory has demonstrated that the initiation of SWE-induced synaptic strengthening requires NMDARs, however, continued activation of NMDARs during ongoing whisker activity starts to weaken synaptic strength (Clem et al.,

2008). Therefore, ongoing whisker experience triggers NMDAR-dependent metaplasticity where NMDARs first mediate synaptic potentiation, then switch to mediate synaptic depression. The potentiation-labile-stabilization transitioning of synaptic strength suggests that NMDAR-dependent metaplasticity could underlie the transition of these different phases. To further test this hypothesis, we performed *in vivo* injection of the NMDAR antagonist, CPP (10 mg/kg body weight) in animals that undergo different duration of experience and recorded Sr-EPSCs from CPP-treated animals. Since SWE and SRE both induce an early potentiation phase followed by a labile phase and the timing of these phases are similar (Fig. 3D), we used SWE in the CPP injection experiments.

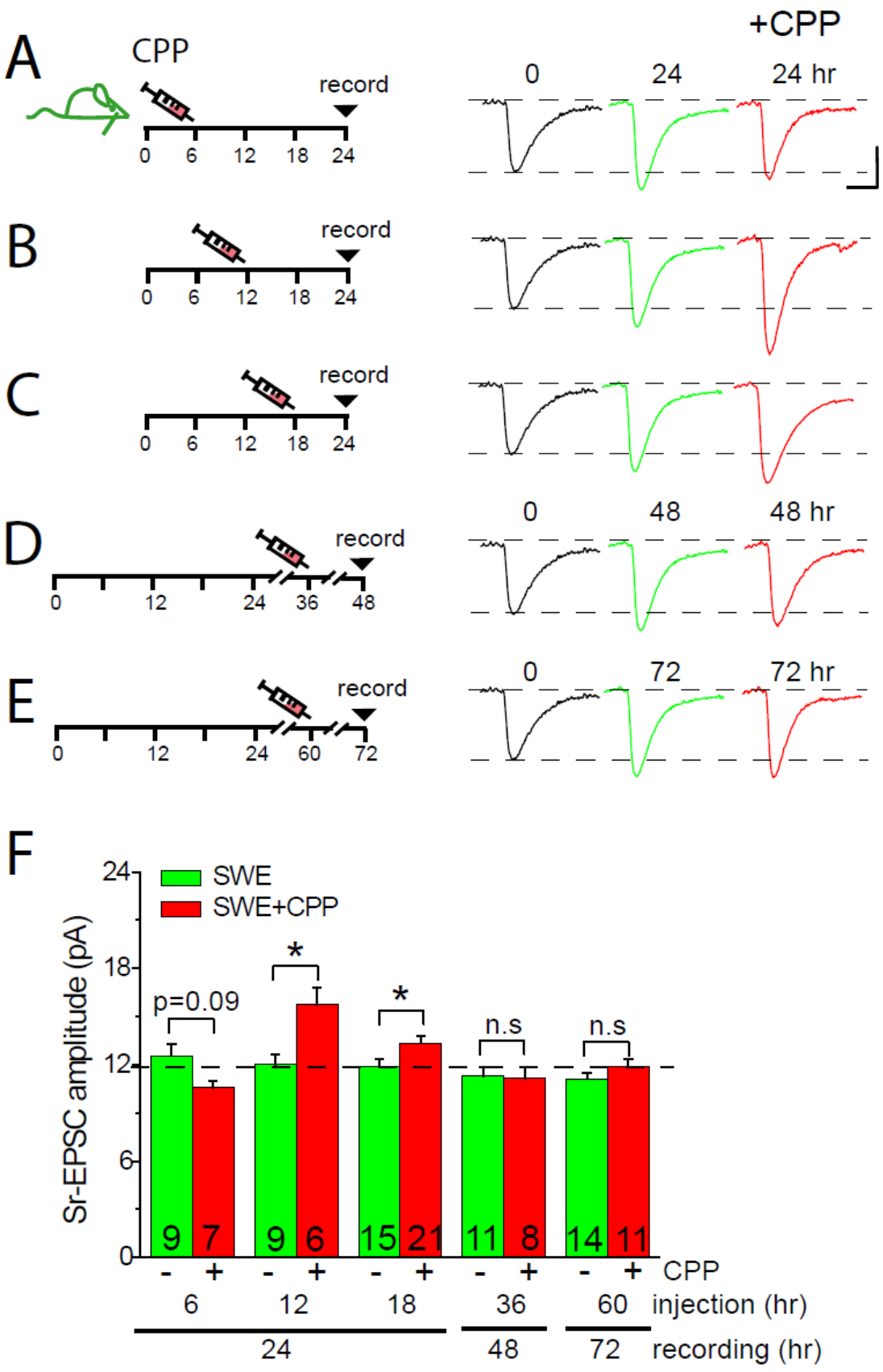
SWE-treated mice were injected once with CPP at 6, 12, 18 hr respectively and later assayed for Sr-EPSCs at 24 hrs and the results from SWE-treated animals with CPP injection (Fig. 21A-C, red traces) and without (Fig. 21A-C, green traces) were compared. Injections made at 6 hrs showed almost complete blockade of synaptic strengthening (Fig. 21A and Fig. 21F, 6 hr SWE-CPP 12.5±0.76 pA n=9 cells vs. SWE+CPP 10.6±0.37 pA n=7 cells, p=0.09). This result was consistent with our previous finding that CPP injection at 0 hr completely blocked synaptic strengthening when measured at 24 hr (Clem et al., 2008). These data indicate that during the early phase of plasticity NMDARs are required for synaptic potentiation.
In contrast, the Sr-EPSC amplitude from CPP injection made at 12 hr and assayed at 24 hr was increased by ~4 pA, compared with non-injected animals (Fig. 21B and 21F, 12 hr SWE-CPP 12.0±0.63 pA n=9 cells vs. SWE+CPP 15.72±1.05 pA n=6 cells, p=0.03). Moreover, CPP injections made at 18 hr increased the Sr-EPSC amplitude by 1.5 pA (Fig. 21C and 21F, 18 hr SWE-CPP, 11.9±0.43 pA, n=15 cells vs. SWE+CPP 13.3±0.48 pA, n=21 cells, p=0.03). Together, these data show that during the labile phase NMDARs suppress synaptic strength

and that the switch in NMDAR function is tightly correlated with the transition from the early to the labile phase.

We then performed CPP injection during the stabilization phase. First, we recorded Sr-EPSCs at 72 hr and found that the Sr-EPSC amplitude recorded at 72 hr SWE was similar to that at 24 hr and 48 hr (Fig. 21C-E and 21F, green bars; 24 hr SWE 11.9±0.43 pA n=15 cells, 48 hr SWE 11.3±0.56 pA n=11 cells and 72 hr SWE 11.1±0.39 pA n=14 cells, one-way ANOVA p=0.44). This suggests that the stabilization phase starts at 24 hr and lasts at least till 72 hr during SWE. Secondly, to investigate the role of NMDARs during the stabilization phase, we injected CPP at 36 hr and recorded Sr-EPSCs at 48 hr during the early half (24-48 hr) of the stabilization phase. The Sr-EPSC amplitude did not increase or decrease after CPP injection, compared with non-injected animals (Fig. 21D,F, 36 hr SWE-CPP 11.3±0.56 pA, n=11 cells vs. SWE+CPP 11.1±0.73 pA, n=8 cells p=0.84). We then asked if the lack of NMDAR contribution to plasticity lasts through the second half of the stabilization phase (48-72 hr). CPP was injected at 60 hr and the Sr-EPSC amplitude was measured at 72 hr. Consistent with a lack of NMDAR involvement, CPP injection did not change synaptic strength during the second half of the stabilization phase (Fig. 21E,F, 60 hr SWE-CPP 11.1±0.39 pA n=14 cells vs. SWE+CPP 11.8±0.50 pA, n=11 cells, p=0.43). These results indicate that NMDARs are not involved during the stabilization phase.

Taken together, our data demonstrate that both the early and the labile phases of synaptic plasticity require activation of NMDARs, but NMDARs switch their function from mediating synaptic potentiation during the early phase to depression during the labile phase. Furthermore, NMDAR-dependent synaptic depression is transient and the stabilization phase no longer requires NMDAR activation. The tight temporal correlation between the effects of

NMDAR blockade on and experience-induced alteration in synaptic strength strongly argues for an NMDAR-dependent metaplasticity mechanism that underlies the phase transition of synaptic plasticity at layer 4-2/3 synapses.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F
SWE
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Sr-EPSC amplitude (pA)
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18
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- + - + - + - + - +
CPP
6 12 18 36 60
injection (hr)
24 48 72
recording (hr)

Figure 21. Phase-specific transition in NMDAR function. A) Injection of NMDAR antagonist CPP at 6 hr SWE reduces Sr-EPSC amplitude to the 0 hr level. Black: 0 hr; green: 24 hr SWE without CPP injection; red: 24 hr SWE with CPP injection at 6 hr. Scale bars: 5 pA, 5ms. **B)** Injection of CPP at 12 hr further increases Sr-EPSC amplitude. **C)** Injection of CPP at 18 hr further increases Sr-ESPC amplitude. **D)** Injection of CPP at 36 hr does not change Sr-EPSC amplitude. Black: 0 hr; green: 48 hr SWE without CPP injection; red: 48 hr SWE with CPP injection at 36 hr. **E)** Injection of CPP at 60 hr does not change Sr-EPSC amplitude. Black: 0 hr; green: 72 hr SWE without CPP injection; red: 72 hr SWE with CPP injection at 60 hr. **F)** Summary of Sr-EPSC amplitude with and without CPP injection at various injection and recording times.

4.3.6 NR2B content decreases during the labile phase

One parsimonious hypothesis for the functional switch of NMDARs from mediating synaptic potentiation to depression is a change in channel properties of NMDARs induced by ongoing sensory experience. Visual experience has been shown to cause bidirectional switch in NR2 subunit composition between NR2A and NR2B, leading to changes in cortical NR2 expression, NMDA current decay kinetics and sensitivity to selective NR2B antagonist ifenprodil (Quinlan et al., 1999a; Quinlan et al., 1999b; Philpot et al., 2001). The experience-driven alteration in NR2B and 2A subunit composition has resulted in a shift in the capacity to induce LTP vs. LTD (Philpot et al., 2003), which has been proposed as a molecular basis for experience-dependent plasticity in visual cortex. In order to examine if changes in NR2 subunit composition occur after whisker experience in layer 2/3 pyramidal neurons, we first investigated NR2B sensitive NMDA currents using selective NR2B antagonist, ifenprodil. We measured NMDAR-mediated EPSCs (NMDA-EPSCs) by voltage-clamping layer 2/3 cells at +40 mV in the presence of GABA receptor antagonist picrotoxin (Ptx, 50 μM) and AMPAR antagonist NBQX (5 μM). Comparison of the average traces of NMDA-EPSCs showed no difference in shapes of traces amongst 0, 6 and 18 hr SRE-treated animals (Fig. 22A). The decay time constants derived from fitting a single-exponential function yielded no significant change in the decay kinetics (Fig. 22B, 0 hr, τ=82.3±4.44 ms, n=10 cells; 6 hr, τ=75.4±2.48 ms,

n=11 cells; 18 hr τ=76.0±2.82 ms, n=14 cells, p=0.30 by one-way ANOVA). However, while the fraction of NMDA-EPSC blocked by ifenprodil at 0, 6 and 24 hr was similar, it was significantly smaller at 18 hr (Fig. 22C,D, 0 hr 0.60±0.08, n=5 cells; 6 hr 0.66±0.08, n=6 cells; 18 hr 0.41±0.07, n=5 cells, 24 hr 0.57±0.08, n=6 cells; 6 hr vs. 18 hr p=0.03 by Mann-Whitney U test). Taken together, the NMDA-EPSC decay kinetics and ifenprodil sensitivity data suggest that although decay time constants remain the same between the early and the labile phase, ifenprodil-sensitive NMDA currents is significantly reduced during the labile phase.

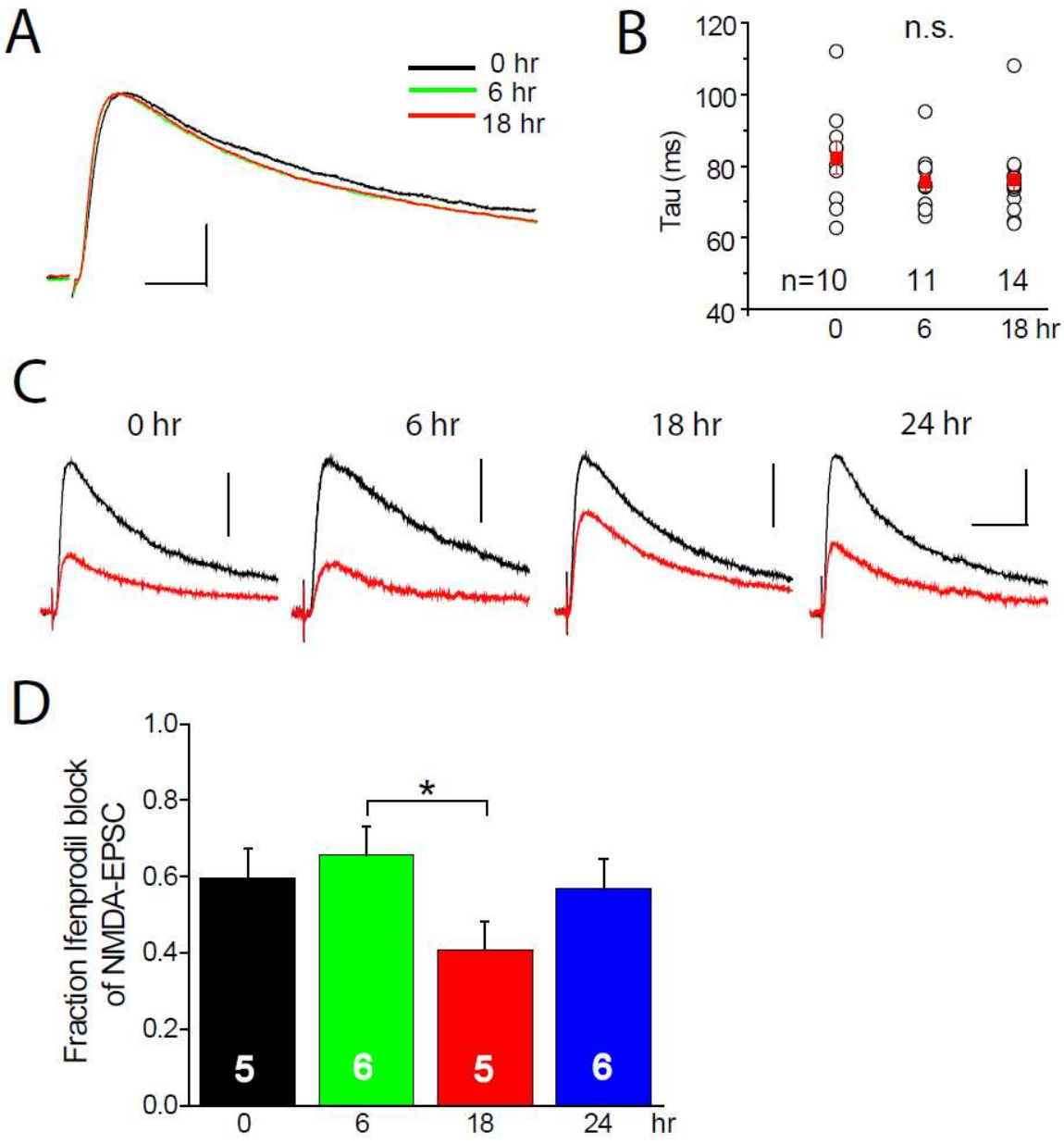

Figure 22. Ifenprodil sensitivity of NMDA currents decreases during the labile phase. A) Comparison of peak-scaled evoked NMDA-EPSCs recorded at +40 mV at layer 4-2/3 excitatory synapses at 0, 6 and 18 hr after the onset of SRE. Scale bars: 50 pA, 25 ms. Black: 0 hr; green: 6 hr; red: 18 hr. **B)** Comparison of single exponential decay kinetics (tau) among 0, 6 and 18 hr after the onset of SRE. **C)** Example traces of peak-scaled NMDA-EPSCs recorded before (black) and after ifenprodil application (red) at 0, 6, 18 and 24 hr after the onset of SRE. Scale bars: 50 pA, 50 ms. **D)** Summary of the fractional block of NMDA-EPSC by ifenprodil at 0, 6, 18 and 24 hr after the onset of SRE.

4.3.7 Up-regulation of non-postsynaptic NR2B-containing NMDARs

Despite the observed reduction in the fractional blockade of NMDA currents by ifenprodil, the origin or synaptic location of such a change is unclear. Non-postsynaptic NR2B-containing NMDARs have been found at layer 4-2/3 excitatory synapses in barrel cortex that promote spontaneous and evoked glutamate release (Bender et al., 2006; Brasier and Feldman, 2008; Rodriguez-Moreno and Paulsen, 2008). The reduction in the ifenprodil-sensitive NMDA currents could be due to a decrease in evoked presynaptic release by blocking non-postsynaptic, NR2B-containing NMDARs. Although previous studies suggest that these functional non-postsynaptic NMDARs may be located presynaptically in the axon terminals (Brasier and Feldman, 2008), no direct evidence has proven that this is the case. To be accurate with the terminology, we will refer to these receptors with a function to promote presynaptic release as “non-postsynaptic” NMDARs.

In order to investigate if a decrease in presynaptic release probability contributes to the reduction in NMDAR-mediated synaptic transmission after ifenprodil application, we recorded AMPA-EPSCs at layer 4-2/3 synapses by hyperpolarizing layer 2/3 cells to -80 mV and blocking postsynaptic NMDARs with intracellular MK-801 (1 mM), an open-channel blocker of NMDARs. Hyperpolarization and intracellular MK801 have proven successful in blocking almost all postsynaptic NMDARs (Berretta and Jones, 1996; Sjostrom et al., 2003; Bender et al., 2006; Yang et al., 2006; Corlew et al., 2007; Brasier and Feldman, 2008; Rodriguez-Moreno and Paulsen, 2008). Since postsynaptic NMDARs are blocked under these conditions, any change in AMPA-EPSC amplitude after ifenprodil application would be attributed by a change in presynaptic release probability. In particular, a decrease in AMPA-EPSC amplitude would indicate a decrease in presynaptic release probability,

presumably due to ifenprodil blockade of non-postsynaptic NR2B-containing NMDARs that normally enhance release probability.

Non-postsynaptic NMDARs in layer 4-2/3 synapses were detected in previous studies at the physiological temperature or room temperature in the presence of TBOA, a blocker of glutamate transporter to increase ambient glutamate concentration (Bender et al., 2006b; Brasier and Feldman, 2008). However, our experiments were done at room temperature without adding TBOA, in order to keep the experimental conditions consistent with those under which we saw a reduction in NMDA currents by ifenprodil application. Two pulses (ISI: 33 ms, 30 Hz) (Brasier and Feldman, 2008) were given at a frequency of 0.1 Hz, the regular stimulation frequency used to elicit evoked synaptic currents. AMPA-EPSCs in response to the paired-pulse were obtained and the amplitude of the 1st EPSC was collected over time continuously. Since the action of ifenprodil on NR2B-containing NMDARs is poorly reversible (Kumar and Huguenard, 2003), ifenprodil (5 μM) was infused locally to the recording chamber after 5-10 min of a stable baseline was achieved. We saw no obvious recovery of the AMPA-EPSC amplitude over the 5-20 min during the post-ifenprodil session (Fig. 23A). Interestingly, we discovered a robust decrease in the amplitude of the 1st AMPA-EPSC at 6 hr by ~40%, compared with 0 hr which showed a minimal blockade by ifenprodil (Fig. 23A, 0 hr vs. 6 hr; Fig. 23C, 0 hr, 0.12±0.13 n=6 cells vs. 6 hr, -0.39±0.07, n=5 cells, p=0.004). We further examined 18 hr, finding that the fractional reduction in the 1st EPSC amplitude at 18 hr was similar to that at 6 hr and was still significantly greater than 0 hr (Fig. 23A,C, 18 hr -0.33±0.11, n=6 cells, 6 vs. 18 hr p=0.42; 18 hr vs. 0 hr, p=0.03). The fractional blockade by ifenprodil at 24 hr appears to return to the 0 hr level (Fig. 23A,C, 24 hr -0.14±0.09 n=6 cells, 0 hr vs. 24 hr p=0.13). It is also important to note that some cells in the 0

hr and 24 hr conditions showed an increase in the 1st EPSC amplitude, whereas all the cells in the 6 and 18 hr conditions showed a decrease after ifenprodil application (Fig. 23C), suggestive of non-postsynaptic NMDARs during the early and labile phases. However, a recent study performed in the basolateral amygdala has shown off-target effects of ifenprodil on presynaptic release probability by partially blocking presynaptic P/Q-type Ca^{++} channels (Delaney et al., 2011). Considering there is no evidence of such non-specific effects of ifenprodil in the neocortex, ifenprodil-induced decrease in AMPA-EPSC amplitude is more consistent with blockade of non-postsynaptic NR2B-containing NMDARs that have been reported at theses synapses. Nonetheless, a more convincing conclusion would be reached by using a different NMDAR angtagonist such as D-APV which has no reported off-target effects on presynaptic Ca^{++} channels to see if the same results can be obtained as with ifenprodil, an observation consistent with the contribution of non-presynaptic NR2B-containing NMDARs.

Taken together, these results suggest experience-induced up-regulation of non-postsynaptic NR2B-containing NMDARs that normally promote evoked glutamate release during the early and labile phases, a finding consistent with previous work of non-postsynaptic NMDARs at layer 4-2/3 synapses (Bender et al., 2006; Brasier and Feldman, 2008; Rodriguez-Moreno and Paulsen, 2008).

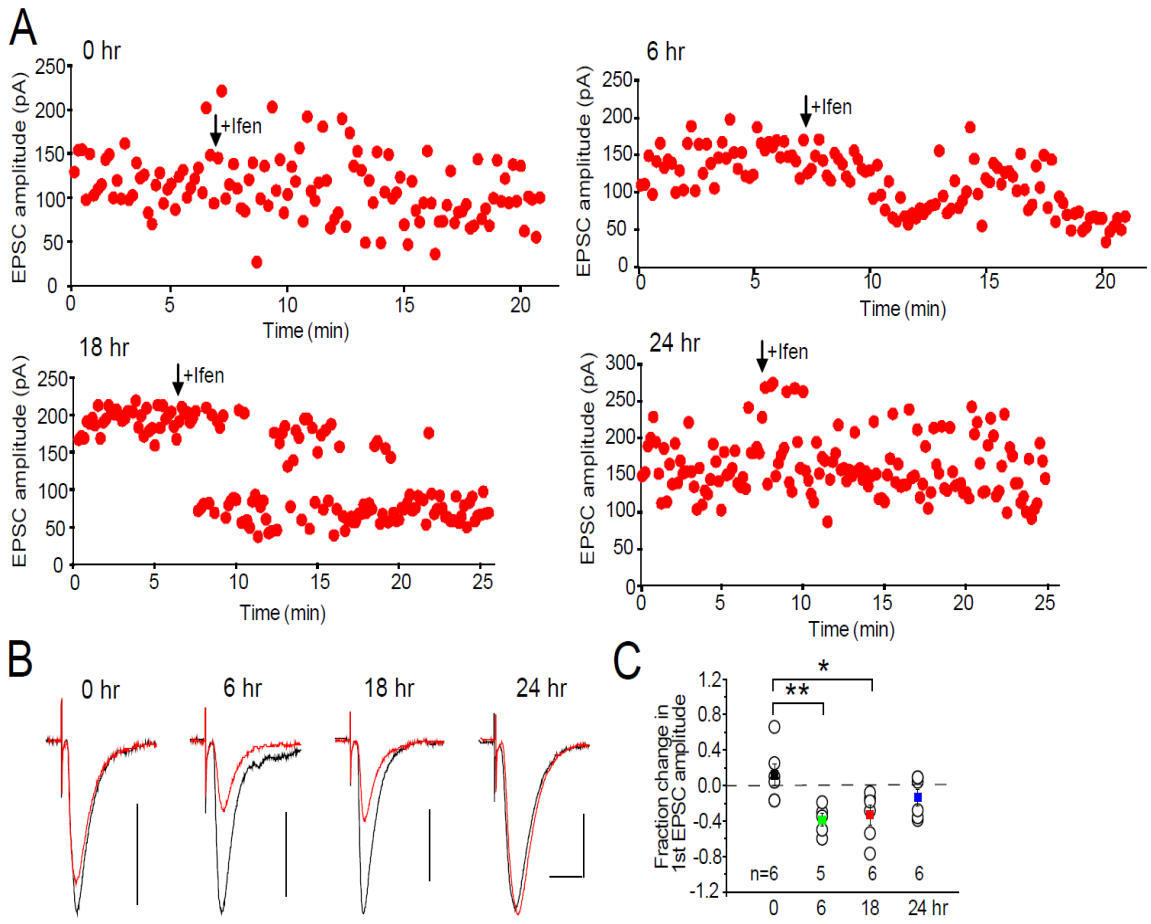

Figure 23. Ifenprodil decreases AMPAR-mediated EPSC amplitude during the early and labile phases. A) Example experiments of the effect of ifenprodil on evoked AMPA-EPSC amplitude with postsynaptic NMDARs being blocked by MK801, at 0, 6, 18 and 24 hr after the onset of SRE. The peak amplitude of evoked AMPAR-mediated EPSC was plotted over time. The arrow indicates the time when ifenprodil (5 um) was added into the recording chamber. **B)** Example traces of peak-scaled AMPA-EPSC (1st EPSC taken from the paired pulse measurement) before (black) and 5-10 min after the onset of ifenprodil application (red). Scale bars: 50 pA, 10 ms. **C)** Summary of fractional changes in the 1st EPSC amplitude after ifenprodil application at 0, 6, 18 and 24 hr after the onset of SRE.

However, a reduction in the 1st EPSC amplitude is only one criterion for a decrease in presynaptic release probability, since effects that are not caused by changes in presynaptic release machinery might induce a change in postsynaptic response, for example a non-specific run-down of the AMPA-EPSC. Therefore, it is necessary to use a second criterion to identify cells that express functional non-postsynaptic NMDARs that normally facilitate glutamate release. We used the paired pulse ratio (PPR) (2nd EPSC/1st EPSC) as the second criterion. Paired pulse ratio is inversely correlated with presynaptic release probability in that an increase in PPR suggests a decrease in release probability and a decrease in PPR suggests an increase in release probability. Therefore, cells expressing non-postsynaptic NR2B-containing NMDARs that promote presynaptic release would display both a decrease in the 1st EPSC amplitude and an increase in PPR, after ifenprodil application. We compared the baseline and post-ifenprodil PPR for each time point (Fig. 24A, right column). Although at none of the time points did the baseline PPR significantly differ from the post-ifenprodil PPR (Fig. 24A), it is interesting to note that a higher fraction of cells at 6 hr (3 out of 5 cells) and 18 hr (4 out of 6 cells) satisfied both criteria (decrease in 1st EPSC and increase in PPR), compared with 0 hr (1 out of 6 cells) and 24 hr (1 out of 6 cells), respectively. However, the small sample size ($n \leq 6$ cells) might prevent us from seeing a significant change in the PPR after ifen application that will suggest a change in presynaptic release probability. We then grouped the cells from 0 and 24 hr where there was little evidence for presynaptic NMDARs, 6 and 18 hr where presynaptic NMDARs were clearly present and plotted the fractional change in the 1st EPSC amplitude vs. the fractional change in PPR. By grouping the cells, we find that 2 out of 12 cells in the 0-24 hr group showed both an increase in PPR and a reduction in the 1st EPSC amplitude after ifenprodil application (top left quadrant, Fig. 24B); in contrast, 7 out of 11 cells in the 6-18 hr

group showed both changes (top left quadrant, Fig. 24C). Moreover, we find that all cells (11/11, Fig. 24C) in the 6-18 hr group displayed a reduction in the 1st EPSC amplitude, compared to a small fraction (5/12) in the 0-24 hr group (Fig. 24B). The fractional change in the 1st EPSC amplitude in the 6-18 hr group tended to correlate with the fraction of change in PPR given the small sample size (red line, p=0.15, Fig. 24C), however, there was no obvious relationship between the two measures in the 0-24 hr group (p=0.77, Fig. 24B).

Taken together, the combined data of kPPR and the 1st AMPA-EPSC amplitude together provide reliable measurements of functional non-postsynaptic NR2B-containing NMDARs at layer 4-2/3 synapses. At room temperature and normal simulation frequency (0.1 Hz) without artificially increasing ambient glutamate concentration, we were able to detect non-postsynaptic NR2B-containing NMDARs that promote evoked glutamate release. We find that ongoing experience drives an up-regulation of functional non-postsynaptic NR2B-containing NMDARs during the early synaptic potentiation phase (6 hr) and that expression of these receptors persists throughout the labile phase (18 hr) before returning to the control level.

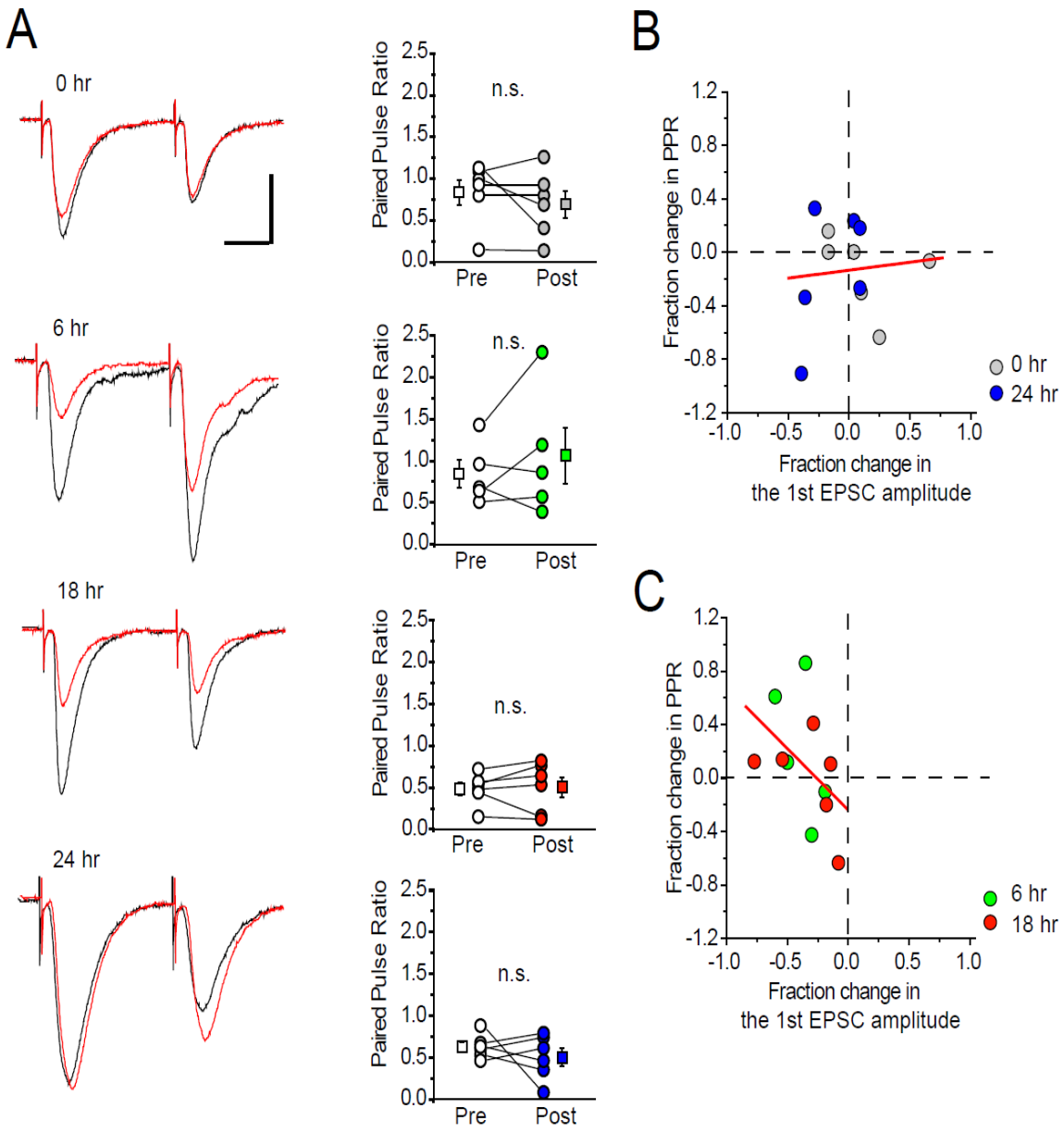

Figure 24. Non-postsynaptic NMDARs are regulated during the early and labile phases. A) Changes in paired pulse ratio (PPR) at different time points after ifenprodil application. Left: example traces of the baseline (black) and post-ifenprodil (red) of AMPA-EPSC at 0, 6, 18 and 24 hr after the onset of SRE. Right: PPR before (pre) and after (post) ifenprodil application for corresponding time point shown on the left. **B)** Fractional change in PPR as a function of fractional change in the 1st AMPA-EPSC amplitude from all cells at 0 hr (gray) and 24 hr (blue) after the onset of SRE. Linear regression was performed on theses cells. p=0.77, n=12 cells. **C)** Fractional change in PPR as a function of fractional change in the 1st AMPA-EPSC amplitude for all cells in 6 (green) and 18 hr (red) after the onset of SRE. p=0.15 for linear regression, n=11 cells.

4.3.8 Synaptic mechanisms that regulate distinct phases of experience-dependent synaptic plasticity in vivo

The changes we observed in NMDAR properties allow us to make some predictions about molecular mechanisms that underlie the transition between different phases of synaptic plasticity. Based on the ifenprodil results (Fig. 22 and 23), ifenprodil-induced alteration in the NMDA-EPSC amplitude can be parsed into two components, one being a change of NR2B-mediated postsynaptic reponse and the other being a change in presynaptic release probability mediated by non-postsynaptic NR2B-containing NMDARs. In comparison, ifenprodil-induced alteration in the AMPA-EPSC amplitude with postsynaptic NMDARs blocked has only one component, i.e., a change in presynaptic release probability mediated by non-postsynaptic NR2B-containing NMDARs (Fig. 23 and 24).

By plotting the fractional blockade of the NMDA-EPSC amplitude (Fig. 25A, solid bars) and that of the AMPA-EPSC amplitude (Fig. 25A, lined bars) together, we were able to see distinct patterns of post- and non-post- (solid) vs. non-postsynaptic (lined) ifenprodil sensitivity during individual phases of plasticity. These data allow us to dissect changes in NR2B-mediated response attributed by the postsynaptic component vs. the non-postsynaptic component during different phases of plasticity. At 0 hr and 24 hr, the majority of ifenprodil sensitivity is composed of postsynaptic component since the non-postsynaptic component was negligible under both conditions (0 hr pre+post -0.6, pre 0.1; 24 hr pre+post -0.6, pre -0.1). Interestingly, a similar level of ifenprodil sensitivity attributed by the non-postsynaptic component was observed between 6 and 18 hr (Fig. 25A, 6 vs. 18 hr, -0.39 vs. -0.33), which was significantly increased compared to 0 hr and 24 hr, suggesting an increase in non-postsynaptic NR2B-containing NMDARs. There was no significant difference between the the fractional blockade of the NMDA-EPSC amplitude (40%, post- + non-post) and that of the

AMPA-EPSC amplitude (33%, non-post) at 18 hr (Fig. 25A), suggesting a lack of NR2B-mediated response from postsynaptic cells. In contrast, at 6 hr, the fractional blockade of the NMDA-EPSC amplitude (60%, post- + non-post) is significantly higher than that of the AMPA-EPSC amplitude (39%, non-post), suggesting that part of the ifenprodil sensitivity of NMDA currents comes from postsynaptic NR2B-containing NMDARs. Therefore, these results are consistent with a subunit switch from NR2B to NR2A in postsynaptic layer 2/3 cells from the early phase (6 hr) to the labile phase (18 hr).

Overall, the analysis of ifenprodil sensitivity of NMDA-EPSCs and AMPA-EPSCs during individual phase of in vivo plasticity reveals some interesting results of changes in postsynaptic NMDAR composition (Fig. 25B). First, there is a decrease in postsynaptic NR2B-mediated currents during the early phase (0-6 hr). Considering that ifenprodil sensitivity attributed by postsynaptic NMDARs decreases from 0 hr to 6 hr (from explaining most of to part of total ifenprodil sensitivity, 60%), one possibility for this reduction is a change in the expression of postsynaptic NR2B at layer 4-2/3 synapses. An alternative possibility is an increase in NR1/NR2A/NR2B trihetermers and a decrease in NR1/NR2B dihetermers in postsynaptic membranes, without a change in the overall expression level of NR2B subunit. Indeed, some studies have shown that a substrantial population of NMDARs in the forbrain may exist in the form of NR1/NR2A/NR2B trihetermers. It has been demonstrated that the efficacy of ifenprodil measured by the percent block of currents at saturating antagonist concentration, is 4-5 times smaller for NR1/NR2A/NR2B trihetermers compared with NR1/NR2B dihetermers, although the affinity of both receptor types to ifenprodil is similar (Hatton and Paoletti, 2005). Second, during the transition from the early phase to the labile phase (6-18 hr), there is a nearly complete loss of postsynaptic NR2B-mediated currents that

coincides with NMDAR-dependent synaptic depression at this phase. Third, postsynaptic NR2B-mediated responses recover at the stabilization phase (24 hr). These findings of alteration in postsynaptic NMDAR composition that coincides with the transition between distcint phases may provide a molecular mechanism for ongoing experience-induced metaplasticity at layer 4-2/3 synapses.

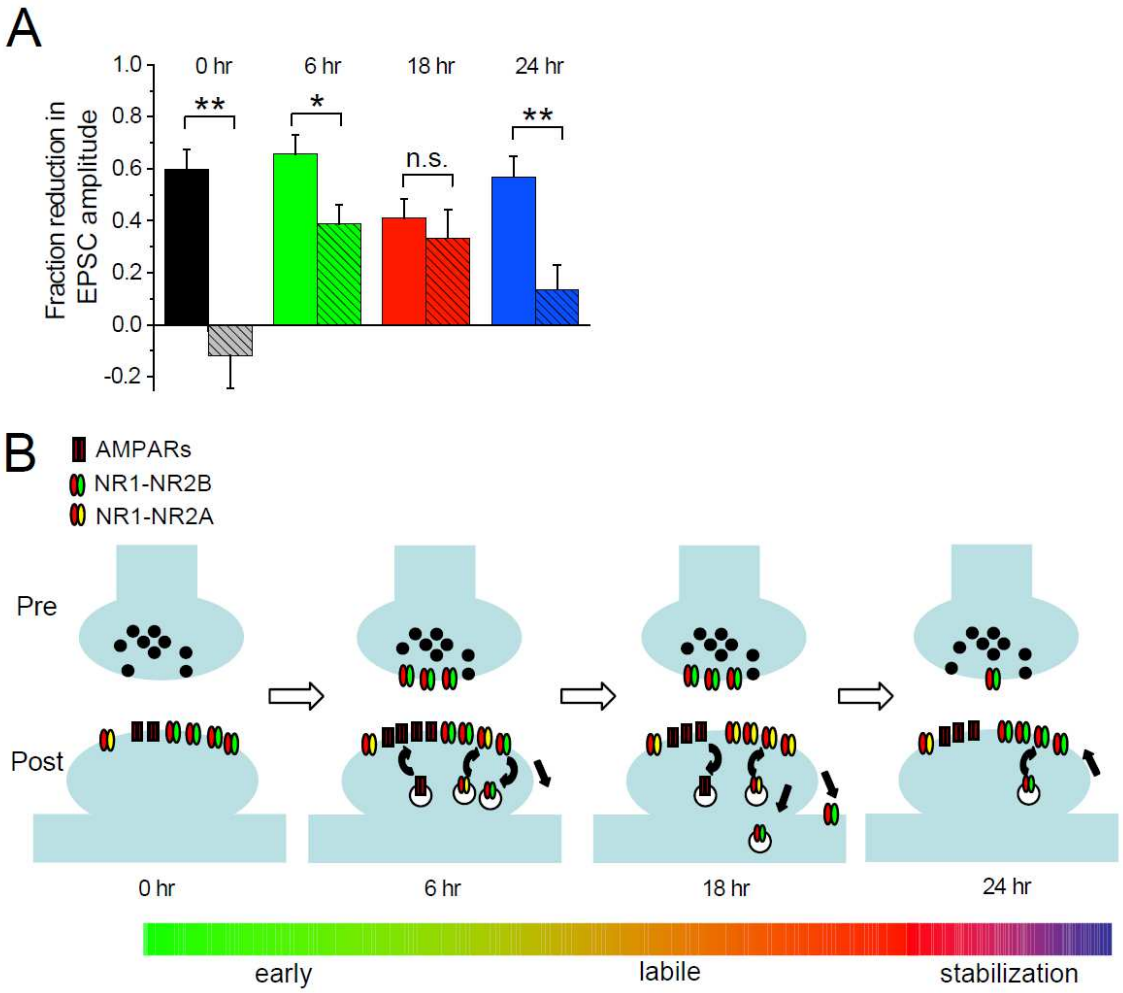

Figure 25. Synaptic mechanisms regulating the distinct phases of experience-dependent synaptic plasticity *in vivo*. A) Comparison of the fractional change in NMDA-EPSC amplitude (solid, as in **Fig. 22D**) and in the AMPA-EPSC amplitude (lined, as in **Fig. 23C**) by ifenprodil application. Solid: ifenprodil block of NMDA-EPSC; lined: ifenprodil block of AMPA-EPSC. **B)** A model of synaptic mechanisms for the distinct phases of experience-dependent changes in synaptic strength.

4.3.9 The role of mGluR5 during the labile and stabilization phases

Previously, our laboratory has demonstrated that group I metabotropic glutamate receptors (mGluRs) mediate synaptic potentiation opposing NMDAR-dependent depression during ongoing SWE (Clem et al., 2008). However, what subtype of mGluRs is responsible for this potentiation is unclear. Group I mGluRs consist of two subtypes, mGluR1 and mGluR5; mGluR5 is highly expressed in the superficial layers of barrel cortex and is implicated in different forms of synaptic plasticity. Thus we hypothesize that mGluR5 might be required for synaptic potentiation that opposes NMDAR-dependent synaptic depression during the labile phase.

In order to examine this hypothesis, we injected specific mGluR5 antagonist, MPEP (10 mg/kg body weight) into SWE-treated animals at 18 hrs after the SWE onset, the same time point at which the wide-spectrum mGluR antagonist AIDA was used and shown to block all synaptic strengthening in our previous study (Clem et al., 2008). The Sr-EPSCs measured at 24 hr showed that MPEP completely blocked SWE-induced synaptic strengthening, reducing it to the control level (Fig. 26, SWE+MPEP: 9.94±0.65 pA vs. SWE: 12.72±0.45 pA, $p=0.006$), a result similar to that with AIDA injection (Clem et al., 2008). These data strongly indicate that mGluR5 is responsible for maintaing synaptic strength after NMDAR-dependent synaptic depression at 24 hr in vivo.

Also, since NMDARs are not involved during the stabilization phase (Fig. 21), we ask what the requirement for the maintenance of synaptic strength is. We discovered that MPEP injection during the stabilization phase completely blocked the maintenance of the Sr-EPSC amplitude, reducing it to the 0 hr level (Fig. 26, MPEP 9.40±0.45 pA, vs. 0 hr control 9.30±0.34 pA, $p=0.76$). Taken together, these data indicate that mGluR5 is responsible for

synaptic potentiation that opposes NMDAR-dependent depression during the labile phase as well as for the maintenance of synaptic strength during the stabilization phase.

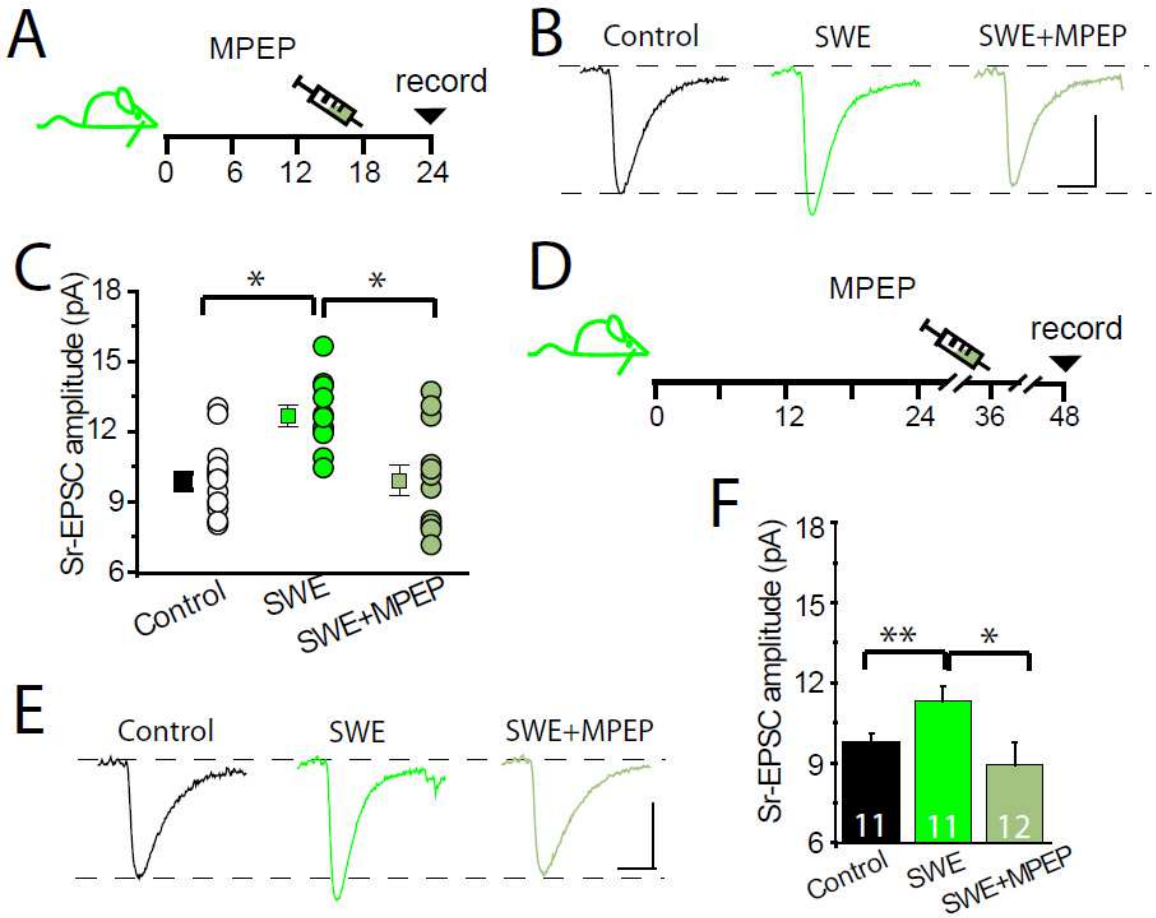

Figure 26. mGluR5 is required during the labile phase and the stabilization phase. A) Schematic of MPEP injection paradigm during the labile phase, at 18 hr. **B)** Average Sr-EPSC traces for control, 24 hr SWE and 24 hr SWE+MPEP (injected at 18 hr). Scale bars: 5 pA, 5 ms. **C)** Scatter plots of Sr-EPSC amplitude for the conditions as in **B)**. **D)** Schematic of MPEP injection paradigm during the stabilization phase, at 36 hr. **E)** Average Sr-EPSC traces for control, 48 hr SWE and 48 hr SWE+MPEP (injected at 36 hr). Scale bars: 5 pA, 5 ms. **F)** Summary of Sr-EPSC amplitude for the control, 48 hr SWE and 48 hr SWE+MPEP (injected at 36 hr).

4.4 Discussion

Excitatory synaptic strength increases during novel experience in many brain regions, including primary sensory cortex. However, it remains unclear how cumulative experience progressively increases and maintains synaptic strength. We took advantage of animals that undergo different durations of ongoing whisker experience and electrophysiologically recorded synaptic strength at individual synaptic contacts of layer 4-2/3 excitatory synapses in barrel cortex. We find that after the initial 12 hr (0-12 hr) where synaptic strength increases, subsequent whisker experience decreases (12-24 hr) and then stabilizes synaptic strength (24-72 hr). These data indicate that ongoing sensory experience does not progressively increase synaptic strength but rather modifies synaptic strength in discrete steps, resulting in a potentiation-labile-stabilization sequence. By blocking NMDARs *in vivo* via antagonist injection, we discovered that NMDARs play a critical role in controlling the direction of synaptic plasticity, in that they mediate synaptic potentiation during the early phase and then switch to mediate depression during the labile phase, but they are not required during the stabilization phase. We further found that ongoing whisker experience induces changes in NR2 subunit composition of NMDARs. There is a rapid up-regulation of NR2B-containing non-postsynaptic NMDARs that promote evoked release probability during the early phase and an almost complete loss of postsynaptic NR2B-containing receptors during the labile phase. These findings suggest experience-dependent regulation of NR2B as a putative molecular mechanism that may underlie the phase-specific plasticity induced by ongoing whisker experience.

Is experience-dependent synaptic strengthening persistent?

It is widely accepted that experience-dependent synaptic plasticity serves as a physiological substrate for memory formation. Various learning paradigms have been shown to enhance synaptic strength in relevant neural circuits, associated with changes in learned behavior in animals. The minimal stimulus required to change synaptic strength can be as little as a single trial of behavioral training and synaptic modification can happen as fast as 30 min to a few hours after training (Whitlock et al., 2006; Clarke et al., 2010). However, whether activity-induced synaptic changes are stable is unclear. Studies in vitro have shown that specific pattern of activity or sensory experience could reverse activity-induced changes in synaptic strength (LTP and LTD), normalizing it to the control level (Zhou et al., 2003). Consistently, the initial experience-induced increase in synaptic strength has also been shown to be labile *in vivo*, subject to depression or depotentiation (Sacchetti et al., 2001; Clarke et al., 2010). In some form of memories, synaptic weakening has proven to be an essential step before memories could be consolidated (Ge et al., 2010).

Synaptic strengthening within primary sensory cortices upon repeated representation of a sensory stimulus has been observed, accompanying heightened sensory acuity specific to the trained stimulus, suggesting that experience-induced changes in synaptic strength may serve as a mechanism for sensory information storage within primary sensory cortices (Cooke and Bear, 2010). It has been shown that following a brief daily stimulus presentation, the amplitude of visually evoked potential (VEP) recorded in layer 4 of primary visual cortex keeps increasing for 4 days and then stabilizes (Cooke and Bear, 2010). Work from our laboratory has shown that 24 hr SWE leads to an increase in the firing rate of layer 2/3 neurons and a concomitant increase in synaptic strength of layer 4-2/3 excitatory synapses in the spared barrel

representation of barrel cortex (Clem and Barth, 2006; Glazewski et al., 2007; Benedetti et al., 2009; Wen and Barth, 2011). Since experience-dependent synaptic strengthening could in part account for the increase in neuronal firing of layer 2/3 neurons and thus behavioral output, it is important to know if these changes are stable over time.

Here, we investigated the time course of experience-dependent changes in synaptic strength at layer 4-2/3 synapses during ongoing whisker experience *in vivo*. The use of SRE and the para-sagittal slicing protocol provide the advantages of studying the early time points without relying on the fosGFP transgenic animals. We found that SRE and SWE alike do not increase synaptic strength monotonically but rather modify synaptic strength in at least three distinct steps, i.e., synaptic potentiation followed by synaptic weakening and stabilization. The results from this study confirm our previous work on NMDAR-dependent synaptic weakening but also provide a time scale at which this synaptic weakening occurs. The current study strongly indicates a predominant NMDAR-dependent synaptic depression that decreases synaptic strength during the labile period, consistent with an overall LTD manifested in pairing induced plasticity in vitro (Clem et al., 2008). In any case, the distinct phases of experience-dependent synaptic plasticity discovered here would provide important insights to understanding how ongoing sensory experience modulates synaptic properties and neural circuit function that underlie storage of sensory information in primary sensory cortices. Finally, it would be interesting to know how long-lasting the stabilization phase is, that is, if a longer period of experience (beyond 3 d) maintains or renormalizes the synaptic gain to the control level. Interestingly, our preliminary data from 5-day deprived mice seem to suggest a loss of synaptic gain at 5 d of SWE. The answer to the duration of the stabilization phase

would provide useful information about the temporal constraints of information storage within primary sensory cortices.

Experience-dependent synaptic weakening

Our finding that the initially potentiated synaptic strength decreases during a labile phase is consistent with depotentiation or LTD where LTP-induced synaptic strengthening could be reversed by subsequent activity or experience. Studies show that depotentiation might occur via a heterosynaptic mechanism mediated by a general increase in network activity. However, our data on cross-columnar layer 2/3-2/3 synapses made onto the same postsynaptic cell as layer 4-2/3 synapses, showed that NMDAR-dependent synaptic weakening might not occur at these synapses which are equally potentiated by sensory experience (Appendix C). These results suggest that instead of using a heterosynaptic mechanism that might potentially cause synaptic depression at all potentiated synapses within the cell, NMDAR-dependent synaptic weakening in barrel cortex might use a synapse-specific mechanism.

Additionally, consistent with the labile phase of experience-induced synaptic potentiation, we observed that potentiated layer 4-2/3 synapses were subject to depression upon brief postsynaptic depolarization in a Sr^{++}-based solution in vitro (Sr-depression) (Wen and Barth, 2012). We further found that trafficking of CP-AMPARs, which has been associated with synaptic plasticity in some conditions, was not sufficient or necessary for activity-induced synaptic depression in barrel cortex (Wen and Barth, 2012). Despite the fact that the postsynaptic nature of Sr-EPSC measurements indicates that the mechanisms involved in synaptic weakening in vitro and in vivo are likely to be postsynaptic, we could not rule out a presynaptic mechanism because of a reported role of non-postsynaptic, presumably presynaptic

NMDARs in timing-dependent LTD and deprivation-induced synaptic depression at layer 4-2/3 synapses (Bender et al., 2006a; Bender et al., 2006b; Rodriguez-Moreno and Paulsen, 2008; Banerjee et al., 2009) in barrel cortex and our own observation of non-postsynaptic NMDARs in this study. It is possible that both presynaptic and postsynaptic mechanisms could act in concert to regulate neuronal firing in barrel cortex. Future experiments to differentiate the pre- vs. postsynaptic contribution will help to explain the complete mechanisms of experience-dependent synaptic plasticity.

Postsynaptic NR2 subunit switch and synaptic metaplasticity

It is well established that NMDARs are required for neural circuit maturation, different forms of Hebbian plasticity and learning and memory. The functional diversity of NR2 subunit of NMDARs has caused tremendous interest as a candidate mechanism for synaptic plasticity. NMDAR channel properties and function are largely determined by the subtypes of NR2 subunit. NR2A and NR2B are the two predominant NR2 subtypes in the forebrain and most of the work in synaptic plasticity has been focused on these two NR2 subtypes. Although NR2B is the predominant synaptic NR2 subtype during early postnatal life in hippocampus and neocortex, NR2A-containing NMDARs become more abundant later in the development (Carmignoto and Vicini, 1992; Monyer et al., 1994; Sheng et al., 1994; Barth and Malenka, 2001). The NR2B to NR2A subunit switch has proven to be experience-dependent. Dark rearing from birth increases the expression of synaptic NR2B, maintaining synapses at the "young" state; subsequent visual experience, however, rapidly enhances NR2A expression (Quinlan et al., 1999a; Quinlan et al., 1999b; Philpot et al., 2001). Replacement of NR2B by NR2A could be acutely triggered by glutamate binding to receptors (Barria and Malinow, 2002)

or LTP induction protocol in hippocampal slices (Bellone and Nicoll, 2007). This NR2 subunit switch happens very quickly, within hours after activity which is similar to trafficking of AMPARs (Bellone and Nicoll, 2007). The long cytoplasmic tails of NR2B and 2A subunits contain PDZ and AP2 domains bearing key sites for interaction with various intracellular scaffolding proteins and protein kinases, providing an important mechanism for subunit-dependent localization of NMDARs with synapses (Prybylowski et al., 2005). Based on the unique properties of NR2B vs. NR2A, a series of cellular mechanisms have been proposed to regulate experience-dependent NR2B to 2A switch, for example, through exchange of synaptic with the intracellular pool of NMDARs (Barria and Malinow, 2002), lateral diffusion of synaptic- vs. extrasynaptic NMDARs (Tovar and Westbrook, 2002; Groc et al., 2006) and mGluR5-dependent pathways (Matta et al., 2011).

Although association of a specific NR2 subunit with one type of synaptic plasticity has been suggested (Liu et al., 2004; Massey et al., 2004), the precise roles of NR2A and NR2B have been largely controversial (Bartlett et al., 2007; Morishita et al., 2007). Indeed, in contrast to an obligatory requirement of NR2B for LTD proposed in some studies, NR2B has been more often associated with LTP in various brain regions (Tang et al., 1999; Cui et al., 2011; Gagolewicz and Dringenberg, 2011; Shen et al., 2011). Recent evidence suggests that unlike the scenario where a specific NR2 subunit is linked to one type of synaptic plasticity, the balance between NR2A and NR2B subunits is critical in determining the thresholds of LTP and LTD (Yashiro and Philpot, 2008). The ratio of NR2B and NR2A in primary visual cortex has been implicated in experience-dependent plasticity of visually evoked responses (Philpot et al., 2001; Philpot et al., 2003; Philpot et al., 2007), i.e. a high ratio of NR2B/2A leads to a lower

threshold for LTP, consistent with the hypothesis of shifting thresholds as proposed in the BCM theory.

We discovered in the present study that ongoing whisker experience triggers an initial synaptic potentiation phase followed by a labile phase of synaptic weakening, which was associated with a postsynaptic change from NR2B-containing to predominant NR2A-containing NMDARs at layer 4-2/3 synapses. Although further pharmacological experiments need to test if NR2B is being replaced by NR2A during the labile phase, the reduction in postsynaptic NR2B content is consistent with a subunit switch from NR2B to NR2A during light experience (Philpot et al., 2003; Philpot et al., 2007) and during extended monocular deprivation in primary visual cortex (Chen and Bear, 2007) that are associated with more synaptic depression as determined by the BCM theory. However, depotentiation was associated with NR2 subunit switch from NR2A to 2B in neonatal hippocampal slices (Bellone and Nicoll, 2007), suggesting that different plasticity mechanisms might exist that depend on age and brain region. Taken together, although our data suggest that experience-dependent plasticity of NR2 subunit composition might serve as a regulatory mechanism for metaplasticity in vivo, the correlative nature of our current findings prevent a conclusion to be drawn about a causal relationship between NR2B/2A ratio and the direction of synaptic plasticity. Future experiments using transgenic animals which overexpress NR2B or NR2A knock-out animals will be critical to understanding the molecular mechanisms for experience-dependent metaplasticity in barrel cortex.

Non-postsynaptic NR2B-containing NMDARs and synaptic plasticity

Recent evidence has shown that non-postsynaptic, presumably presynaptic NMDARs are involved in regulation of presynaptic release probability and synaptic plasticity in primary sensory cortices and other brain areas (Corlew et al., 2008). Specifically, non-postsynaptic NR2B-containing NMDARs have been shown to promote spontaneous and stimulus-evoked presynaptic release probability, and spike timing-dependent LTD (STDD) in the hippocampus and primary sensory cortices (Bender et al., 2006a; Bender et al., 2006b; Corlew et al., 2007; Brasier and Feldman, 2008; Corlew et al., 2008; Rodriguez-Moreno and Paulsen, 2008; Banerjee et al., 2009). Our analysis of experience-dependent changes in synaptic properties reveals an up-regulation of non-postsynaptic NR2B-containing NMDARs that act to promote layer 4 evoked presynaptic release probability, consistent with the reported presence of non-postsynaptic NR2B-containing NMDARs at the same synapses in barrel cortex (Bender et al., 2006a; Bender et al., 2006b; Brasier and Feldman, 2008; Rodriguez-Moreno and Paulsen, 2008; Banerjee et al., 2009). Distinct from previous work where non-postsynaptic NMDARs were detected in the presence of TBOA, the antagonist of glutamate transporter (Bender et al., 2006b; Brasier and Feldman, 2008), our study detected functional these NMDARs at basal condition (0.1 Hz) at 6 hr of SRE but not at 0 hr without increasing ambient glutamate concentration, consistent with an increase in non-postsynaptic NMDAR function during the early potentiation phase. This result is surprising because non-postsynaptic NMDARs have been implicated in STDD but not STDP in many brain regions. What is the physiological function of these non-postsynaptic NMDARs during the synaptic potentiation phase? One speculation is that since these receptors promote presynaptic release probability, they can increase the firing of postsynaptic cells and thus faciliate LTP. Thus, postsynaptic and

non-postsynaptic NMDARs might work in a concerted way to mediate LTP during the early phase.

Additionally, we observed a few cells in the control and SRE-treated animals that show a decrease in the AMPA-EPSC amplitude as well as a decrease instead of an increase in the PPR after ifenperodil application, suggesting off-target effects of ifenprodil. Since off-target effects of ifenprodil have been reported, for example, via blocking of some presynaptic Ca^{++} channels (Delaney et al., 2011), we compared the fraction of cells which showed consistent changes in the AMPA-EPSC amplitude and PPR that will predict a decrease in presynaptic release probability after ifenprodil application (Fig. 24). The increase in the fraction of cells that show both a decrease in the 1^{st} AMPA-EPSC amplitude and an increase in PPR at 6 and 18 hr (7/11 cells) compared to 0 and 24 hr (2/12 cells), is consistent with an up-regulation of non-postsynaptic NR2B-containing NMDARs during the early and labile phases. Future experiments will need to address the role of non-post vs. postsynaptic NMDARs in mediating synaptic plasticity at different phases.

The role of mGluR5 during phases of plasticity

Our previous results have demonstrated that the canonical pairing protocol (2 Hz stimulation with 0 mV postsynaptic depolarization) that induces LTP in the control animals induces a slight LTD in 24 hr SWE-treated animals in vitro (Clem et al., 2008). Blockade of NMDARs or mGluRs alone results in LTP or LTD, whereas blockade of both receptors results in no plasticity in 24 hr SWE-treated animals (Clem et al., 2008). These results suggest additive interaction between predominant NMDAR-mediated synaptic depression and mGluR-mediated synaptic potentiation. Consistently, during the labile phase of in vivo

plasticity (12-24 hr), an overall decrease in synaptic strength (24 hr compared to 12 hr) is manifested, predominated by NMDAR-dependent LTD; however, mGluR5 is shown to be required for the remaining synaptic gain as MPEP injection abolishes the synaptic gain to the control level. These in vivo results suggest that the remaining synaptic gain at the end of the labile phase (24 hr compared to 0 hr) is not merely a result of NMDAR-mediated synaptic depression during the labile phase with a slower rate than the rate of synaptic potentiation during the early phase, but a result of competition between active mGluR5-mediated synaptic potentiation and NMDAR-mediated synaptic depression.

Previously, our laboratory has shown that group I mGluRs are required for synaptic potentiation that opposes NMDAR-dependent synaptic depression during SWE (Clem et al., 2008). However, since group I mGluRs consist of both mGluR1 and mGluR5, the exact mGluR subtype involved in this synaptic potentiation is unknown. Here, we injected mGluR5 specific antagonist MPEP into SWE-treated animals at 18 hr after the onset of SWE and found that this manipulation alone abolished all synaptic gain and normalized synaptic strength to the control level (Fig. 26A-C). These data strongly indicate that mGluR5 is the sole mGluR subtype that is responsible for synaptic potentiation during the labile phase. Despite of a lack of detailed investigation of the cellular processes required for mGluR5-mediated synaptic potentiation, data from LTP experiments in vitro suggest that at least part of the mechanism is postsynaptic as mGluR-mediated LTP requires postsynaptic increase in Ca^{++} (Clem et al., 2008).

Furthermore, although the seemingly additive interaction between mGluR5 and NMDAR-mediated synaptic plasticity might suggest non-overlapping intracellular pathways involved in each type of plasticity during the labile phase (12-24 hr), an open question remains

as to if mGluR5 is required during the early synaptic potentiation phase (0-12 hr). Evidence has shown that the localization of group I mGluRs is close to NMDARs in postsynaptic membranes and that mGluR5 activation can facilitate NMDAR-mediated currents (Awad et al., 2000; Pisani et al., 2001) and amplify the responses of downstream effectors (Mao and Wang, 2002). The antagonist of mGluR5, MPEP can augment locomotor hyperactivity induced by NMDAR antagonist, phencyclidine (PCP); however, MPEP administered alone did not affect locomotor activity (Henry et al., 2002). In addition, blockade of mGluR5 has been shown to augment the cognitive deficits in PCP-treated animals (Campbell et al., 2004). These results indicate that mGluR5 might interact with NMDARs themselves and/or NMDAR-mediated downstream signaling cascades during synaptic plasticity and faciliate synaptic potentiation. Moreover, a recent study has suggested a critical role of mGluR5 in experience-dependent switch of NR2 subunit composition from NR2B to 2A when induced in vitro, further suggesting cross-talk between mGluR5- and NMDAR-mediated signaling (Matta et al., 2011).

Therefore, it is important to investigate the role of group I mGluRs, especially mGluR5 during the early phase of synaptic plasticity at layer 4-2/3 synapses as mGluR5 might play an instrumental role in initiating NMDAR-dependent synaptic potentiation. Experiments to test the role of mGluR5 during the early phase would involve injection of MPEP at the onset of experience (0 hr) and recording synaptic strength at a later time within the early phase, for example, 6 hr or 12 hr. A result of a partial decrease or complete abolishement of synaptic strengthening will suggest a required role of mGluR5 for the initiation of synaptic strengthening. Because NMDAR blockade during the initial phase completely blocks synaptic strengthening (Clem et al., 2008) (Fig. 21), partial or complete blockade of synaptic

strengthening by mGluR5 would also suggest an interaction between NMDAR- and mGluR5-mediated signaling, both of which are required for the initial synaptic potentiation.

Finally, previous work studying the maintenance of experience-induced changes in synaptic strength has identified a critical role of some important molecules, for example, protein kinase Mzeta (PKMζ) (Pastalkova et al., 2006; Shema et al., 2007) and CaMKII (Lisman et al., 2012). However, none of the previous studies has reported a role of mGluR5 in maintaining synaptic strength. Thus, the novel discovery of mGluR5 being required for synaptic maintenance provides a new mechanism for maintenance of synaptic strength and memory storage in primary sensory cortices. A mechanistic investigation of the novel role of mGluR5 to maintain the synaptic gain during the stabilization phase is necessary because the mechanism can involve different signaling cascades from those engaged during the labile phase or the early phase. Therefore the change in mGluR5 function from the early phases to the late phase of plasticity might add another layer of metaplastic control to the existing NMDAR-dependent metaplastic mechanism and both mechanisms might act in concert to regulate the overall outcome of synaptic plasticity during ongoing experience.

5. Final Discussion

5.1 Experience-dependent synaptic strengthening

Plastic changes in the brain can be induced by many types of experience. Experience-dependent neural plasticity has been widely considered an important mechanism for information storage and adaptive behavior in health and disease. A major goal in neuroscience is to understand cellular and molecular mechanisms involved in experience-dependent neural plasticity underlying adaptive behavior. Experience-dependent plasticity has been well documented in the neocortex, an area that performs critical sensory, perceptual and cognitive function. Primary sensory neocortex, in particular, shows strong effects of experience on neural circuits. Examples from primary visual and barrel cortex have shown that change in neuronal firing rates following sensory manipulation is correlated with change in synaptic strength in neocortical circuits and they seem to share common signaling pathways based on the occlusion studies (Feldman, 2009; Smith et al., 2009). As a result, experience-dependent synaptic plasticity has been considered the cellular and synaptic mechanism for experience-induced changes in receptive field plasticity and sensory representation in neocortical circuits.

Rodent barrel cortex provides an excellent system to study experience-dependent changes in map plasticity, sensory coding and underlying molecular mechanisms (Feldman, 2009) because of the presence of an orderly map of facial whiskers and the accessibility to different types of manipulation of sensory activity. Trimming or plucking of whiskers has been shown to reduce the firing responses of cortical neurons in barrel cortex to deflection of the re-grown deprived whiskers (Simons and Land, 1987; Fox, 1992; Diamond et al., 1993; Glazewski and Fox, 1996) and later to increase the responses to the remaining surround

whiskers (Glazewski and Fox, 1996). Similarly, monocular deprivation in primary visual cortex causes an early component of depression of cortical response to the deprived-eye input, followed by potentiation to the spared open-eye input (Frenkel and Bear, 2004). However, most studies to investigate the mechanisms for experience-dependent plasticity in primary sensory cortex have focused on the effects of deprivation.

Layer 4-2/3 synapses, the major excitatory pathway onto layer 2/3 pyramidal cells are widely thought to be the sites for experience-dependent change in neuronal firing and map plasticity in primary cortices. Synaptic depression of layer 4-2/3 synapses has been observed after whisker deprivation in vivo and this deprivation-induced synaptic weakening occludes LTD induced in vitro (Allen et al., 2003). Our laboratory has initially characterized a new form of experience-dependent plasticity in the supragranular layer of barrel cortex, where 24 hr SWE results in an increase in the firing rate of layer 2/3 neurons within the spared barrel representation, to deflection of the spared whisker (Glazewski et al., 2007). This potentiation is correlated with robust synaptic strengthening, an increase in the quantal amplitude of evoked mEPSCs at layer 4-2/3 synapses (Clem and Barth, 2006). Because layer 4-2/3 synapses constitutes the major excitatory inputs to layer 2/3 pyramidal neurons (~15% for layer 3 and ~12% for layer 2 neurons) in mouse barrel cortex (Lefort et al., 2009), a net increase in the excitatory drive caused by an increase in the quantal amplitude at layer 4-2/3 excitatory synapses may provide a synaptic basis for the increase of layer 2/3 firing at the developmental ages we looked at (P12-14). We have found that 24 hr SWE is sufficient to drive an increase in the quantal amplitude of evoked mEPSCs at layer 4-2/3 excitatory synapses of the spared barrel column (Clem and Barth, 2006). Because changes in AMPAR trafficking is the prevalent mechanism for synaptic strengthening in vivo and in vitro, we tested if strengthening

of layer 4-2/3 synapses involves trafficking of AMPARs. The initial analysis for this synaptic strengthening suggests specific trafficking of one type of AMPARs, GluR2-lacking CP-AMPARs to layer 4-2/3 excitatory synapses within the spared barrel column, but not to cross-columnar layer 2/3-2/3 synapses (Clem and Barth, 2006). Although trafficking of CP-AMPARs is observed under some circumstances, its necessity for synaptic strengthening remains unknown. There is some evidence showing that CP-AMPARs may be required for a brief initial period (25 min) after LTP induction (Plant et al., 2006) and are subsequently replaced by Glu2-containing receptors, most other studies fail to see an essential role being played by CP-AMPARs during LTP. Our study shows that after 24 hr SWE, CP-AMPARs are detected at P13 but not at P14 when synapses could still be potentiated, suggesting that insertion of CP-AMPARs is not required for synaptic strengthening at layer 4-2/3 synapses.

Interestingly, SWE-induced synaptic potentiation at layer 4-2/3 synapses is developmentally regulated and exhibits a critical period that ends at P15 (Wen and Barth, 2011). Since CP-AMPARs are not required for synaptic strengthening, they are not the limiting factor that regulates the closure of this critical period. Our preliminary data show that CPP injection at P15 (6 hrs prior to recording) could further potentiate synaptic strength thus reopening the critical period; however, injection at P16 fails to do that (Appendix C). These data suggest that NMDAR-dependent synaptic depression might be enhanced at P15 to close the critical period for synaptic strengthening and that NMDARs are not required after the closure of this critical period. Therefore, experience-induced, NMDAR-dependent synaptic depression could be one mechanism that regulates critical period offset in the neocortex, amongst other identified mechanisms (for example, increase in cortical inhibition) to regulate various critical periods in the brain. Further experiments will be required to identify the

detailed molecular mechanism for changes in NMDAR properties around the closure of this critical period.

In order to investigate if the same sensory activity in vivo modifies other excitatory synapses made onto layer 2/3 pyramidal cells besides layer 4-2/3 synapses, we looked at within-columnar layer 2/3-2/3 synapses. Anatomical studies show that layer 2/3-2/3 synapses occupy a highly overlapping dendritic domain compared with layer 4-2/3 synapses on the basal dendrites of layer 2/3 pyramidal cells (Lubke et al., 2003; Feldmeyer et al., 2006). We find that similar to layer 4-2/3 synapses, layer 2/3-2/3 synapses can be potentiated by SWE with similar magnitude and exhibit a critical period as well, but the onset and duration of the critical period is different in that it starts later and lasts longer (Wen and Barth, 2011). Further analysis of synaptic properties of these two pathways indicates that CP-AMPARs are not involved in strengthening of layer 2/3-2/3 synapses at any age and that these two synapses mature differently during development. Together, these data reveal remarkable input-specificity of experience-dependent synaptic plasticity at excitatory synapses made onto the same postsynaptic neuron with overlapping dendritic domains. Evidence of synapse-specific plasticity in a single postsynaptic cell has been described elsewhere. For example, two excitatory inputs converge onto a single pyramidal cell in the anterior piriform cortex, one being the lateral olfactory tract (LOT) from mitral cells in the olfactory bulb and the other being associational fibers (ASSN) from other cortical regions. LOT targeting the distal apical dendrites of pyramidal cells shows a decline in LTP that ends at P30, whereas ASSN targeting the proximal apical and basal dendrites on the same pyramidal cell shows comparable level of LTP in adults as in neonates (Poo and Isaacson, 2007). The differences in synaptic properties at layer 4-2/3 and 2/3-2/3 synapses may suggest clustering of different receptors and molecules at

these synapses (pre or postsynaptically), as evidenced in other systems (Chiu and Castillo, 2008; Jung et al., 2010; Zhao et al., 2011). Consistently, properties of STDP and STDD induced at layer 4-2/3 and cross-columnar layer 2/3-2/3 synapses exhibit different requirements in vitro (Banerjee et al., 2009). In addition, presynaptic activity arising from layer 4 vs. layer 2/3 inputs at P12 (the onset timing of layer 4-2/3 plasticitiy) is different in that layer 4 neurons mature earlier and show more reliable firing to sensory inputs compared with layer 2/3 cells (Stern et al., 2001), which may result in an earlier onset of experience-dependent synaptic strengthening specifically at layer 4-2/3 synapses.

5.2 Other factors that might contribute to the increased firing of layer 2/3 neurons

Although an experience-dependent increase in the quantal amplitude of layer 4-2/3 excitatory synapses might contribute to the increased firing of layer 2/3 neurons, the answers to several questions remain unclear as to whether experience-dependent changes in the quantal amplitude of layer 4-2/3 synapses serve as a general mechanism for experience-dependent plasticity in barrel cortex.

First, 24 hr SWE-induced potentiation of layer 2/3 firing is still present at P15-16 (Benedetti et al., 2009), however, layer 4-2/3 synapses no longer show potentiation. This result suggests that at least at P15-16, the increase in the quantal amplitude of layer 4-2/3 synapses is not sufficient to account for an increase in layer 2/3 firing. One hypothesis that could explain this discrepancy between plasticity of neuronal firing and of the strength of layer 4-2/3 synapses is that synaptic plasticity at the horizontal inputs from neighboring layer 2/3 pyramidal cells could take over the role to potentiate firing rate of layer 2/3 neurons, at and after P15. Indeed, 24 hr SWE-induced synaptic potentiation of similar magnitude at

within-columnar layer 2/3-2/3 synapses has been observed in the spared barrel representation that lasts from P13 to P17 (see chapter 2). Supporting this hypothesis, the connectivity between neighboring layer 2/3 pyramidal neurons (~19%) within barrel cortex is similar to, if not higher than that between layer 4 and 2/3 cells (Lefort et al., 2009). Additionally, it remains unknown in adult cortex if synaptic strengthening at layer 4-2/3 excitatory synapses contribute to increased layer 2/3 firing induced by longer deprivation (7 days) in adult animals (Benedetti et al., 2009), although a capacity for LTP induction at layer 4-2/3 synapses in vitro has been reported (Banerjee et al., 2009). Interestingly, three weeks or more of whisker deprivation in adult rats results in an increase in the strength of unitary connections between neighboring layer 2/3 neurons without changing the overall connectivity in the spared barrel representation (Cheetham et al., 2007). In addition, sensory deprivation-induced response depression to the deprived whisker input does not seem to involve changes in the quantal amplitude of layer 4-2/3 synapses (Bender et al., 2006a). Therefore, experience-dependent change in the quantal amplitude of excitatory synapses could modulate neuronal output, especially increase neuronal responses, but may not be a required for response depression in the neocortex.

Alternative to a change in synaptic strength, structural plasticity including changes in neuronal connectivity or rearrangement of axonal or dendritic arbors could also contribute to changes in the integration of inputs and generation of neuronal output. Experience-dependent rewiring of local cortical circuitry has been reported after sensory deprivation in pimary barrel cortex of young and mature animals (Cheetham et al., 2007; Broser et al., 2008; Cheetham et al., 2008; Barnes and Finnerty, 2010). In addition, experience-dependent changes in intrinsic properties of layer 2/3 pyramidal neurons could contribute to alterations in firing output (Nader et al., 2000; Nader and Einarsson, 2010), although our preliminary data do not suggest a

change in intrinsic properties of layer 2/3 pyramidal neurons (Appendix D, Fig. D1). Experience-dependent changes in the input-output function (EPSP-spike coupling) that has been discovered in the hippocampus after LTP induction (Daoudal et al., 2002) and experience-dependent plasticity in inhibitory circuitry (Jiao et al., 2006; Helmstaedter et al., 2008; Yazaki-Sugiyama et al., 2009) are other potential mechanisms to regulate neuronal firing. Whether these mechanisms exist in barrel cortex will require further investigation.

5.3 Experience-dependent synaptic depression or depotentiation

Is experience-dependent synaptic potentiation stable? Experiments where synaptic plasticity (LTP or LTD) is induced in vitro show that activity-dependent changes in synaptic strength is unstable and subject to reversal upon subsequent neural activity including spontaneous activity and sensory experience (Barrionuevo et al., 1980; Zhou et al., 2003; Whitlock et al., 2006). Consistently, the ability of synapses to undergo LTP or LTD in response to a later bout of activity can be altered depending on prior plasticity, a phenomena termed “metaplasticity” (Abraham, 2008). Metaplasticity sets a brake on excessive LTP or LTD to prevent runaway potentiation or depression that would obliterate memory traces within the neural system.

NMDARs have been widely associated with LTP and LTD (Malenka and Bear, 2004), as well as metaplasticity (Abraham, 2008). Our laboratory has discovered a form of NMDAR-dependent metaplasticity during ongoing sensory experience where NMDARs are required for the initiation of synaptic strengthening right after the onset of SWE whereas subsequent experience triggers a switch in NMDAR function to mediate synaptic depression (Clem et al., 2008). This experience-dependent metaplasticity at layer 4-2/3 excitatory

synapses has been supported by two important observations. First, in vitro LTP experiments show that the same pairing protocol (0 mV postsynaptic depolarization and 2 Hz synaptic stimulation) results in LTP at 0 hr, but LTD at 24 hr of SWE and that APV blocks LTP at 0 hr but unmasks synaptic potentiation at 24 hr (Clem et al., 2008). Second, blocking NMDARs in vivo by CPP injection at 0 hr blocks, but at 18 hr enhances synaptic strengthening (Clem et al., 2008). Both the in vitro and in vivo data are consistent with a switch in NMDAR function from mediating potentiation to depression (Clem et al., 2008). However, although these initial findings indicate NMDARs depress synaptic strength between 18-24 hr after the onset of SWE, it is not clear when this potentiation-to-depression transition is initiated and how long it lasts. Therefore, a direct measurement of synaptic strength over the course of sensory experience is required to address these questions. Since fosGFP expression in the transgenic animals is not strong enough for unambiguous identification of the spared barrel column before 18 hr of SWE, we took advantage of another sensory paradigm, i.e., single row experience (SRE), which in combination with a special slicing protocol allows us to identify the spared barrel columns and to study experience-dependent plasticity irrespective of fosGFP expression. Our results corroborate that both sensory paradigms are similar in that they trigger metaplasticity of identical time course and they employ identical molecular mechanisms.

We find that ongoing SRE triggers three distinct phases of synaptic plasticity consisting of the early phase of synaptic strengthening (0-12 hr), followed by a labile phase of synaptic weakening (12-24 hr) and a later stabilization phase (24-72 hr) at layer 4-2/3 excitatory synapses. The identification of these discrete phases constrains the time window to studying synaptic mechanisms that regulate plasticity at individual phases. As predicted from our initial finding (Clem et al., 2008), we show that NMDAR function is tightly correlated with the three

phases during SRE, where NMDARs first mediate synaptic potentiation then depression and later do not contribute to plasticity. These findings not only verify NMDAR-dependent metaplasticity that we have observed during SWE, but advance our understanding of the time scale of when these changes occur in vivo. Taken together, these findings strongly indicate that in vivo experience does not progressively increase synaptic strength, but rather increases and then decreases synaptic strength before it reaches a plateau.

What is the advantage of synaptic weakening following an initial synaptic strengthening? Studies suggest that synaptic weakening might play an important role during memory consolidation. Specific blockade of LTD by blocking specific NMDARs or AMPAR endocytosis in the hippocampus of freely moving rats blocks spatial memory formation, however, blockade of LTP mediated by blocking NR2A that was shown to be specifically associated with LTP (Liu et al., 2004) does not affect spatial memory (Ge et al., 2010). Global downscaling of synaptic strength that occurs during sleep has been shown to contribute to overnight behavioral improvement (Tononi and Cirelli, 2003). These findings suggest that synaptic weakening might be a critical step before memories could be consolidated in the brain.

In a novel in vitro assay where Sr^{++} is present, we find that recently potentiated layer 4-2/3 synapses after 24 hr SWE can undergo synaptic depression (Sr-depression) as well, akin to the decrease of Sr-EPSCs that happen in vivo (Wen and Barth, 2012). Postsynaptic depolarization (to -20 mV) for 5 min paired with layer 4 stimulation at the same frequency as the baseline (0.1 Hz) is sufficient to cause a 20% decrease in the amplitude of Sr-EPSCs. This synaptic depression occurs very fast, being apparent right after the conclusion of the induction period and lasts for at least 5 min post the induction. We show that Sr-depression is only

present in SWE-treated animals, but not in the control animals, suggesting Sr-depression specifically occurs in recently potentiated synapses. Because Sr-depression is completely blocked by D-APV and partially blocked by modest postsynaptic depolarization (-40 mV), NMDARs are required for this form of synaptic depression. In addition, trafficking of CP-AMPARs has been implicated in some forms of synaptic depression (Bellone and Luscher, 2006; Rakhade et al., 2008; Clem and Huganir, 2010) and our laboratory has shown that CP-AMPARs can be trafficked to layer 4-2/3 synapses in the spared barrel column after 24 hr SWE at P13 (Wen and Barth, 2011). These results suggest that CP-AMPARs might confer lability to recently potentiated synapses. Our data show that Sr-depression never occurs in control cells despite the fact that some control cells also express CP-AMPARs, suggesting the presence of CP-AMPARs is not sufficient for Sr-depression. Further analyses from recordings of rectification index (RI) and Sr-depression within the same cell in SWE-treated animals demonstrate that cells that do or do not express CP-AMPARs both exhibit Sr-depression, with a similar magnitude. This result indicates that CP-AMPARs are not necessary for Sr-depression either. Although it seems that CP-AMPARs are not sufficient or necessary for Sr-depression, CP-AMPARs can be removed in some circumstances since the cells that express rectifying CP-AMPARs become non-rectifying after Sr-depression. Taken together, these results obtained during Sr-depression support that Sr-depression in vitro can provide a nice in vitro system to study molecular mechanisms of activity-dependent synaptic weakening that also occur in vivo. Our findings suggest a postsynaptic mechanism of synaptic weakening because of the postsynaptic nature of our measurement of Sr-EPSCs, however, we could not rule out possible changes in presynaptic cells since presynaptic mechanisms for LTD have been reported at layer 4-2/3 synapses (Bender et al., 2006a; Bender et al., 2006b). In addition,

since our recordings only last for 5 min post-induction, it remains unknown how long the Sr-depression lasts. Further experiments of longer post-induction recordings will address this question.

Finally, we observe that synaptic strength of layer 4-2/3 synapses seems to be stabilized starting at 24 hrs and lasts up to at least 72 hrs after the initiation of sensory experience in vivo. However, it remains unclear how long-lasting the stabilization phase is. Our preliminary results of synaptic strength at 5 day of SRE showed that synaptic strength returned to the control level (data not shown). These interesting data suggest that sensory information may be stored in primary somatosensory cortex in the form of synaptic strengthening, but might be transferred to other brain areas, for example, secondary somatosensory cortex (SII) (Sacco and Sacchetti, 2010). Further experiments need to be performed to investigate how long-lasting synaptic strengthening is in primary somatosensory cortex and the corresponding molecular mechanisms for regulating the duration of the stabilization phase.

5.4. NMDAR function and metaplasticity

NMDAR activation and postsynaptic Ca^{++} entry are both required for induction of LTP and LTD, synaptic plasticity of opposite signs. Hypotheses regarding differential activation of NMDAR have been proposed to explain the different outcome of NMDAR-dependent synaptic plasticity. Quantitative analysis of Ca^{++} entry during the induction of plasticity in vitro has revealed that brief and larger Ca^{++} increase is associated with LTP whereas smaller and prolonged Ca^{++} increase is associated with LTD (Cormier et al., 2001; Franks and Sejnowski, 2002). If the amount of NMDAR currents is altered by sensory experience, a measurement of NMDA-EPSC at a single release site might reflect the change. Therefore, we examined evoked

NMDAR-mediated Sr-EPSCs at layer 4-2/3 synapses, but there was no significant difference between the amplitude of these currents between the control and SWE-treated animals (data not shown), suggesting a lack of change in total NMDA currents at a single synaptic contact.

Although sensory experience has been found to alter NR2 subunit composition (NR2A and NR2B) that can modulate the thresholds for inducing LTP vs. LTD (Quinlan et al., 1999a; Philpot et al., 2003; Philpot et al., 2007), previous data from our laboratory did not show such a change at 24 hr of SWE (Clem et al., 2008). Based on our findings of the distinct phases during ongoing sensory experience, we reason that the lack of detected alteration in NMDAR subunit composition could be due to the time point at which the experiments were performed, since 24 hr is the transition point from the second phase of synaptic weakening to the third phase of stabilization. Thus comparisons need to be made between two time points when synaptic potentiation and depression are the most profound. We employed NR2B specific antagonist, ifenprodil to investigate the amount of NR2B-containing NMDARs at different phases of synaptic plasticity after SRE initiation. Our analysis reveals ifenprodil sensitivity has both postsynaptic and non-postsynaptic components. We find that postsynaptic ifenprodil sensitivity decreases from the early phase (6 hr) to the labile phase (18 hr) when there is virtually no ifenprodil sensitivity. These results are consistent with a loss of postsynaptic NR2B-containing NMDARs and thus a drastic decrease in the NR2B/NR2A ratio during the transition from synaptic potentiation to depression. This switch in NR2 subunit composition (NR2B to NR2A) supports a right shift of the BCM curve to favor LTD, similar to the metaplasticity in visual cortex following alteration in sensory experience. Therefore, a switch in postsynaptic NR2 subunit composition might be a regulatory mechanism for experience-dependent metaplasticity at layer 4-2/3 synapses of barrel cortex. However, several questions remain unanswered

regarding the alteration in NMDAR channel properties during the phase transition. First, the decay kinetics of NMDA-EPSCs do not change at 18 hr compared with 6 hr, which would be expected to shorten considering the switch from NR2B to NR2A. This discrepancy between the decay kinetics and ifenprodil sensitivity might be explained by the presence of triheteromeric NR1/NR2B/NR2A receptors at 18 hr which show much less ifenprodil sensitivity compared to NR1/NR2B (Hatton and Paoletti, 2005) but may show similar decay kinetics. Alternatively, there could be an increase in the NR2C or NR2D expression at 18 hr whose prolonged decay kinetics will balance out the fast decay kinetics of NR2A-containing NMDARs, thus making the decay kinetics at 6 hr vs. 18 hr indistinguishable. Second, the change in NR2A subunit prevalence has not been directly tested, which will affect the NR2B/NR2A ratio. Effects of pharmacological blockade of NR2A on NMDA-EPSCs will need to be investigated. Third, despite the fact that postsynaptic NR2B seems to return to the 0 hr level when assayed at 24 hr, LTD could still be induced in vitro by the pairing protocol (Clem et al., 2008), inconsistent with an association of LTP with more NR2B (Philpot et al., 2007). Furthermore, transgenic mice which overexpress NR2B or NR2A knock-out mice will be required to test if the switch from NR2B to NR2A is required for the metaplasticity. The expectation from these animals would be a complete loss of the labile phase and an extended synaptic potentiation phase.

Interestingly, our analysis of NMDAR properties reveals an up-regulation of non-postsynaptic NMDARs that promote presynaptic release probability during the early phase (6 hr) and labile phase (18 hr) at layer 4-2/3 synapses. Non-postsynaptic NMDARs that promote spontaneous and evoked release probability have been detected previously in wildtype rodents at layer 4-2/3 synapses (Bender et al., 2006a; Brasier and Feldman, 2008). Compared with the previous studies in which detection of non-postsynaptic NMDARs was facilitated by

recording at physiological temperature and adding TBOA to increase ambient glutamate concentration, our study can detect these receptors at room temperature during basal synaptic stimulation (0.1 Hz), without adding TBOA. However, the exact role of this up-regulation is unclear. Since most studies about presynaptic NMDAR function in visual and barrel cortex have pointed to a role in spike-timing dependent depression (STDD), it is surprising to see an increase in the expression of these receptors during the initial phase of synaptic potentiation (6 hr). A possible scenario could be that these non-postsynaptic NMDARs which promote presynaptic glutamate release act to increase the firing of postsynaptic cells and thus faciliate synaptic strengthening in a concerted fashion along with the postsynaptic mechanism of LTP, during the initial bout of sensory experience. Continued expression of non-postsynaptic NMDARs might function in concert with postsynaptic NMDAR-dependent mechanisms to generate LTD during the labile phase of synaptic weakening. However, these speculations of dissociation between non-postsynaptic and postsynaptic mechanisms need to be tested, for example, by in vitro LTP experiments where postsynaptic mechanisms are selectively blocked at different phases.

Appendix A. Effects of the sleep-wake cycle on SWE-induced synaptic strengthening

It has been found that different behavioral states can modulate synaptic plasticity, in particular, wakefulness has been associated with synaptic potentiation and sleep with homeostatic downscaling of synaptic strength (Tononi and Cirelli, 2003; Vyazovskiy et al., 2008). We set out to test if SWE-induced synaptic strengthening at 24 hr is regulated by the sleep-wake cycle and thus influenced by animals' behavioral state. We put the animals on the same 12hr light/12 hr dark schedule and recorded synaptic strength 24 hr after the onset of SWE at 9 am or 7 pm when animals were in the sleep or wakeful state, respectively. Our results showed that 24 hr SWE can equally increase the synaptic strength of layer 4-2/3 synapses no matter whether the animals were in the sleep state (9 am) or wakeful state (7 pm) (Fig. A-B). Shortened decay kinetics can be induced by 24 hr SWE under both conditions, suggesting an increase in synaptic CP-AMPARs (Fig A-C).

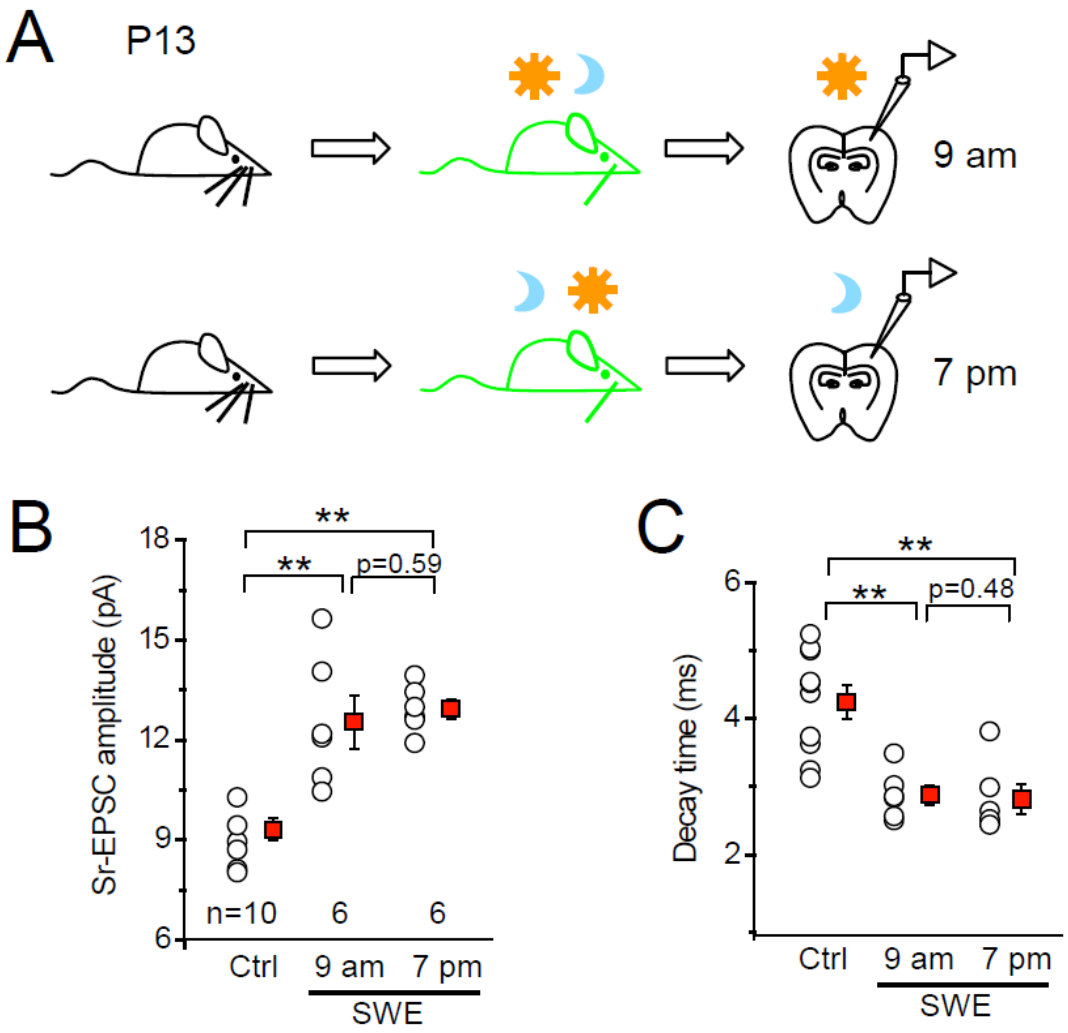

Figure A. SWE-induced synaptic strengthening is not affected by the sleep or wakeful state. A) Schematics showing experimental design. Whisker deprivation was performed at 9 am or 7 pm on the previous day and recordings of Sr-EPSCs were performed 24 hr after the SWE onset on next day at the same time. Animals (P13) were maintained at the same 12 hr light/12 hr dark cycle. **B)** Summary of Sr-EPSC amplitude recorded at 9 am and 7 pm. **C)** Summary of the decay time of average Sr-EPSCs recorded at 9 am and 7 pm.

Appendix B. Effect of whisker activity modulation during SWE-induced synaptic plasticity

Unbalanced whisker activity such as SWE or SRE induces robust synaptic strengthening, suggesting the importance of sensory activation in triggering synaptic plasticity in cortical circuits. We wanted to test if modulation of whisker activity by depriving or artificially stimulating the remaining single whisker after the initiation of synaptic plasticity would change synaptic strengthening (at 24 hr). First, we tested if continued whisker activity is required to maintain synaptic strengthening during the first 24 hr of experience. We clipped the remaining D1 whisker (to about the skin level) after 18 hr of SWE and put the animals back to their home cages, recording Sr-EPSCs at layer 4-2/3 excitatory synapses at 24 hr (Fig. B1-A). We found that clipping the whisker 6 hr prior to experiements was not sufficient to wipe out synaptic strengthening (Fig. B1-B) and the synaptic gain was maintained, suggesting continued sensory experience (for the last 6 hr) is not required to maintain the synaptic gain. In addition, there was no change in synaptic CP-AMPARs, which were maintained at the synapses irrespective of the loss of sensory activity (Fig. B1-C and B1-D). Second, we tested if artificially stimulating the remaining D1 whisker at 1 Hz, a frequency that causes synaptic depression at cortical synapses in vitro would change synaptic strength (Fig. B2-A). We found stimulating the whisker at 1 Hz for 25 min immediately before sacrifice can not change synaptic strength (Fig. B2-B).

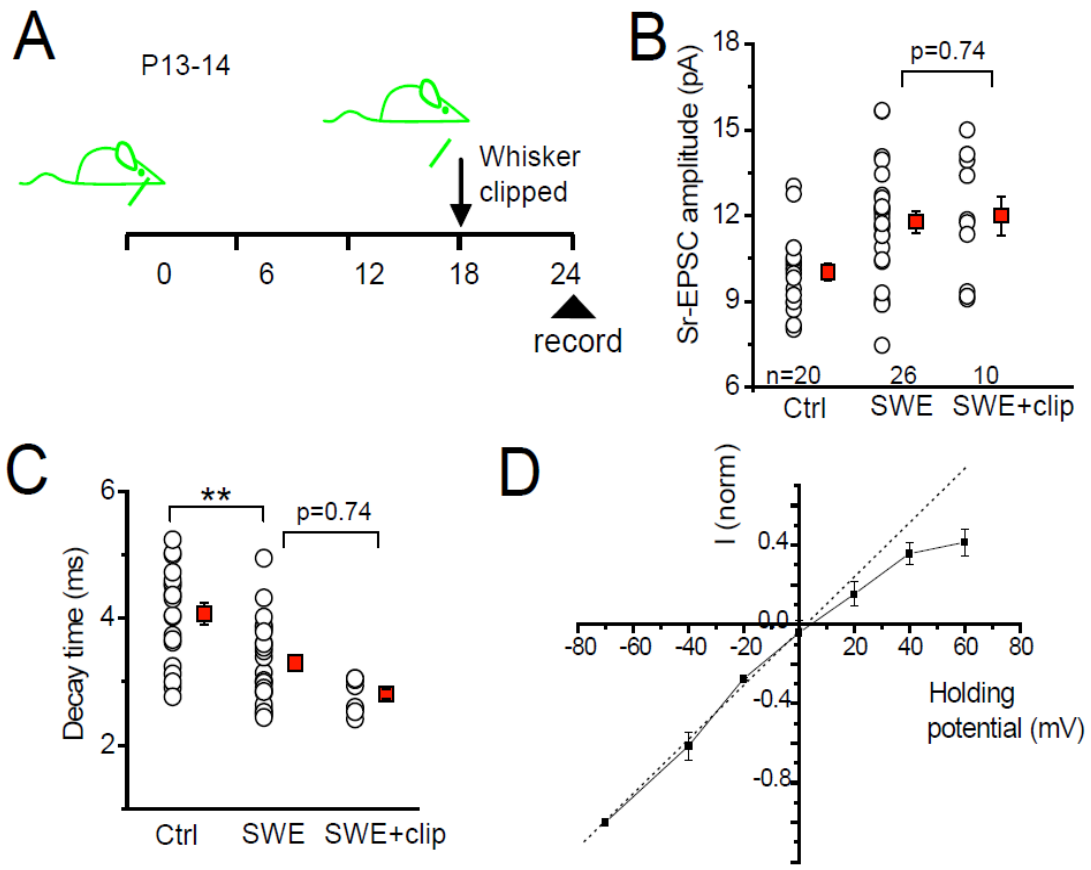

Figure B1. Deprivation of remaining D1 whisker does not affect SWE-induced synaptic strengthening at 24 hr. A) Schematic of experimental design. **B)** Summary of the Sr-EPSC amplitude across conditions. **C)** Decay time across conditions. **D)** Current-voltage (I-V) relationship of AMPA-EPSC from SWE-treated animals whose D1 whisker was clipped 6 hr prior to recordings. N=3 cells. Current amplitude (I-norm) was normalized to that recorded at -70 mV. The I-V plot shows a clear rectification, suggesting the presence of calcium-permeable AMPARs (CP-AMPARs), similar to unplucked SWE-treated animals at this age. These results suggest that continued whisker activity is not required for synaptic strengthening or the maintenance of synaptic CP-AMPARs.

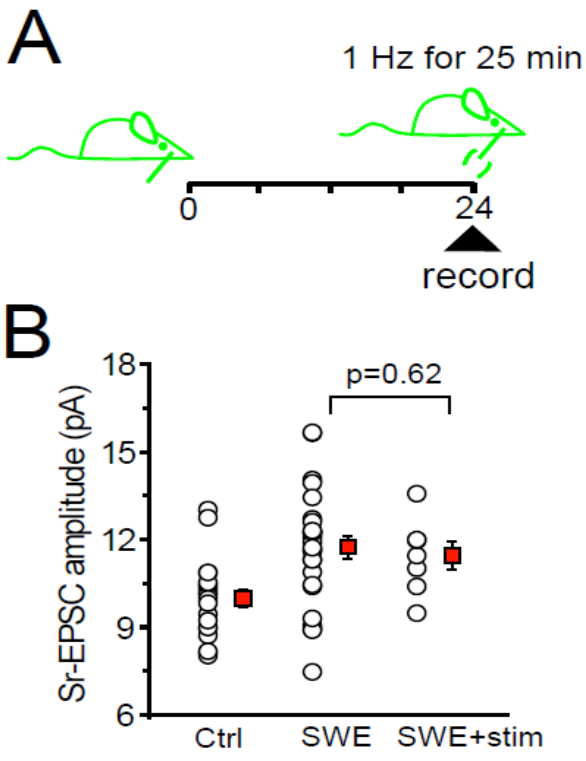

Figure B2 Simulating the D1 whisker at 1 Hz (25 min) does not change synaptic strength. A) Schematic of the experiment. The remaining D1 whisker was manually stroked at 1Hz for 25 min immediately before sacrifice. **B)** Summary of Sr-EPSC amplitude.

Appendix C. Rescue of the critical period for SWE-induced synaptic strengthening at layer 4-2/3 excitatory synapses

Previously we have found that there is a critical period for experience-dependent synaptic strengthening that ends at P15 at layer 4-2/3 excitatory synapses (Wen and Barth, 2011). We hypothesize that the closure of this critical period could be due to an increased NMDAR-dependent synaptic depression at P15. Control experiments where CPP was injected into whisker-intact naïve animals showed that CPP injection did not change synaptic strength in the control animals (Fig. C-A,B). Then we injected CPP in SWE-treated animals 6 hr prior to synaptic strength recordings at P15 after 24 hr of SWE and found that this manipulation

resulted in further potentiation of synaptic strength, thus reopening the critical window (Fig. C-C,D). However, the same manipulation failed to increase synaptic strength at P16 (Fig. C-E,F). Theses results suggest that SWE-induced, NMDAR-dependent synaptic depression is developmentally regulated, which contributes to the closure of critical period for experience-dependent synaptic strengthening at layer 4-2/3 excitatory synapses. However, this developmental regulation is transient and not responsible for the sustained closure of the critical period.

Furthermore, since single layer 2/3 pyramidal neurons show input-specific critical periods for SWE-induced synaptic strengthening at excitatory inputs from layer 4 vs. layer 2/3 (Wen and Barth, 2011), we asked if NMDAR-dependent depression at layer 4-2/3 synapses is input-specific. CPP injection was performed in SWE-treated animals at P16, when synaptic strengthening is present at layer 2/3-2/3 but not at layer 4-2/3 synapses (Fig. C-G). Our results showed that CPP injection at 18 hr after the onset of SWE did not change synaptic strength of layer 2/3-2/3 synapses at 24 hr (Fig. C-G). Although our data did not completely rule out the presence of NMDAR-dependent depression at layer 2/3-2/3 synapses because we only looked at one time point (18 hr), these results do suggest that NMDAR-dependent depression might be specific to layer 4-2/3 synapses and that molecular mechanisms regulating synaptic plasticity in layer 2/3 pyramidal neurons are synapse-specific.

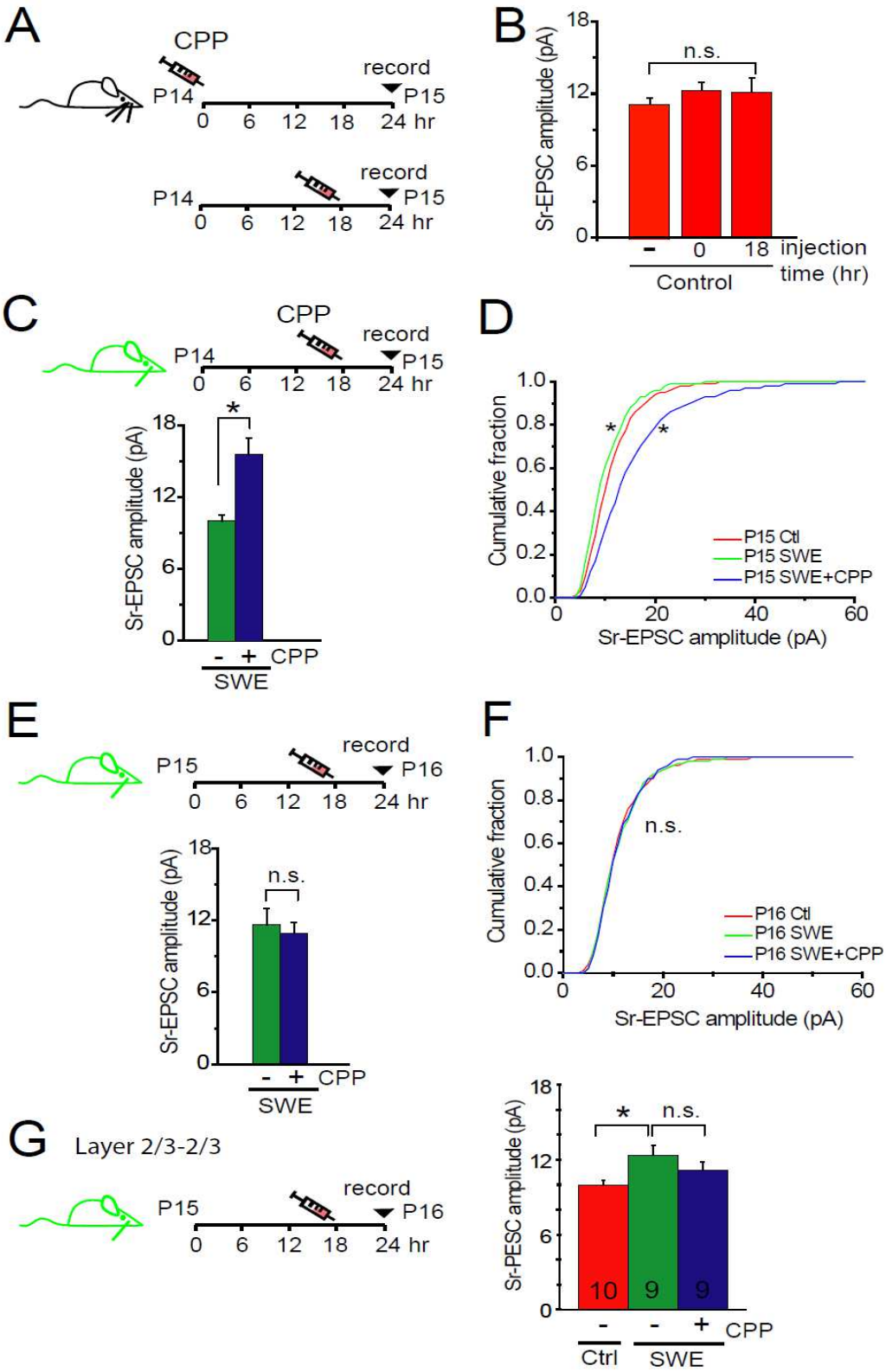

Figure C. CPP injection transiently rescues the closure of the critical period for SWE-induced synaptic strengthening. A) Schematics of CPP injection in control mice at 0 and 18 hr after the onset of a control mouse at the age of P14. This is a control experiment to show that CPP injection does not

affect synaptic strength in undeprived naïve animals of the same age. **B)** Summary of Sr-EPSC amplitude for control mice with or without CPP injection. **C)** Schematic of CPP injection in P15 SWE-treated mice at 18 hr. Bottom: summary of the Sr-EPSC amplitude showing that CPP injection increases synaptic strength. **D)** Cumulative fraction of Sr-EPSC amplitude. * indicated $p<0.05$. **E)** Schematic of CPP injection in P16 SWE-treated mice at 18 hr. Bottom: summary of Sr-EPSC amplitude showing that CPP injection does not change synaptic strength. **F)** Cumulative fraction of the Sr-EPSC amplitude. There was no significant different between the conditions. **G)** Schematic of CPP injection in P16 SWE-treated mice at layer 2/3-2/3 synapses. Right: summary of the Sr-EPSC amplitude.

Appendix D. Firing properties of layer 2/3 pyramidal cells by layer 4 stimulation in slices after 24 hr SWE

Our lab has previously demonstrated by in vivo single unit recording that sensory activity (24 hr of SWE) induces an increase in the firing rate, a decrease in spike time (relative to the stimulus in vivo, i.e., whisker deflection) and improved trial-to-trial reliability and spike timing precision of layer 2/3 cells in the spared barrel representation (Benedetti et al., 2009). These changes (rate, reliability and precision) of layer 2/3 neurons are correlated with a concurrent increase in synaptic strength of layer 4-2/3 excitatory synapses (Clem and Barth, 2006). Thus one hypothesis to explain the increase in spike output of layer 2/3 neurons is the increase in the excitatory drive received by individual layer 2/3 neurons which receive excitatory inputs from multiple presynaptic sources, including layer 4, layer 2/3 in barrel cortex and other areas. In addition to layer 4-2/3 synapses, experience-induced synaptic strengthening was also observed at putative layer 2/3-2/3 excitatory synapses from within the spared barrel column (Wen and Barth, 2011). Therefore, an important question is whether experience-dependent changes in the evoked firing of layer 2/3 neurons require synaptic potentiation at multiple converging inputs or whether synaptic potentiation in one input is predominantly involved. We wanted to test if synaptic potentiation in layer 4-2/3 synapses is sufficient to cause most of the changes in firing output that are observed in vivo. However, a direct test in vivo would involve inactivation of all other presynaptic populations that synapse

onto layer 2/3 pyramidal cells while specifically stimulating one input, which is technically challenging. Therefore, we tested the hypothesis in slices by electrically stimulating layer 4 as a proxy for sensory stimulation (whisker deflection) and recording the firing responses to 0.1 Hz synaptic stimulation at different stimulus intensities. We compared the input-output function, spike time and spike timing precision in layer 2/3 pyramidal cells (both fosGFP+ and fosGFP- cells) by stimulating layer 4, between control and 24 hr SWE-treated animals. Our data show that except for a near-significant increase in the gain (Fig. D2-B,C,D,F), which is quantified as the slope of the linear portion of the input-output function in the SWE-treated animals, there is no obvious change in the spike time (relative to the stimulus artifact, i.e., layer 4 stimulation) (Fig. D2-G) or spike timing precision (Fig. D2-H). In addition, our measurement of intrinsic properties between control and SWE-treated animals do not yield any change after SWE (Fig. D1). Taken together, these data suggest that experience-dependent synaptic strengthening at layer 4-2/3 synapses alone is not sufficient to explain the observed experience-induced changes in evoked firing of layer 2/3 neurons. Although it is possible that our synaptic stimulation frequency in slices might not correctly recapitulate the actual layer 4 activity during sensory activation in vivo, it remains to be tested if experience-dependent synaptic plasticity at other excitatory inputs, for example, layer 2/3-2/3 synapses also contributes to the changes in firing output of layer 2/3 neurons.

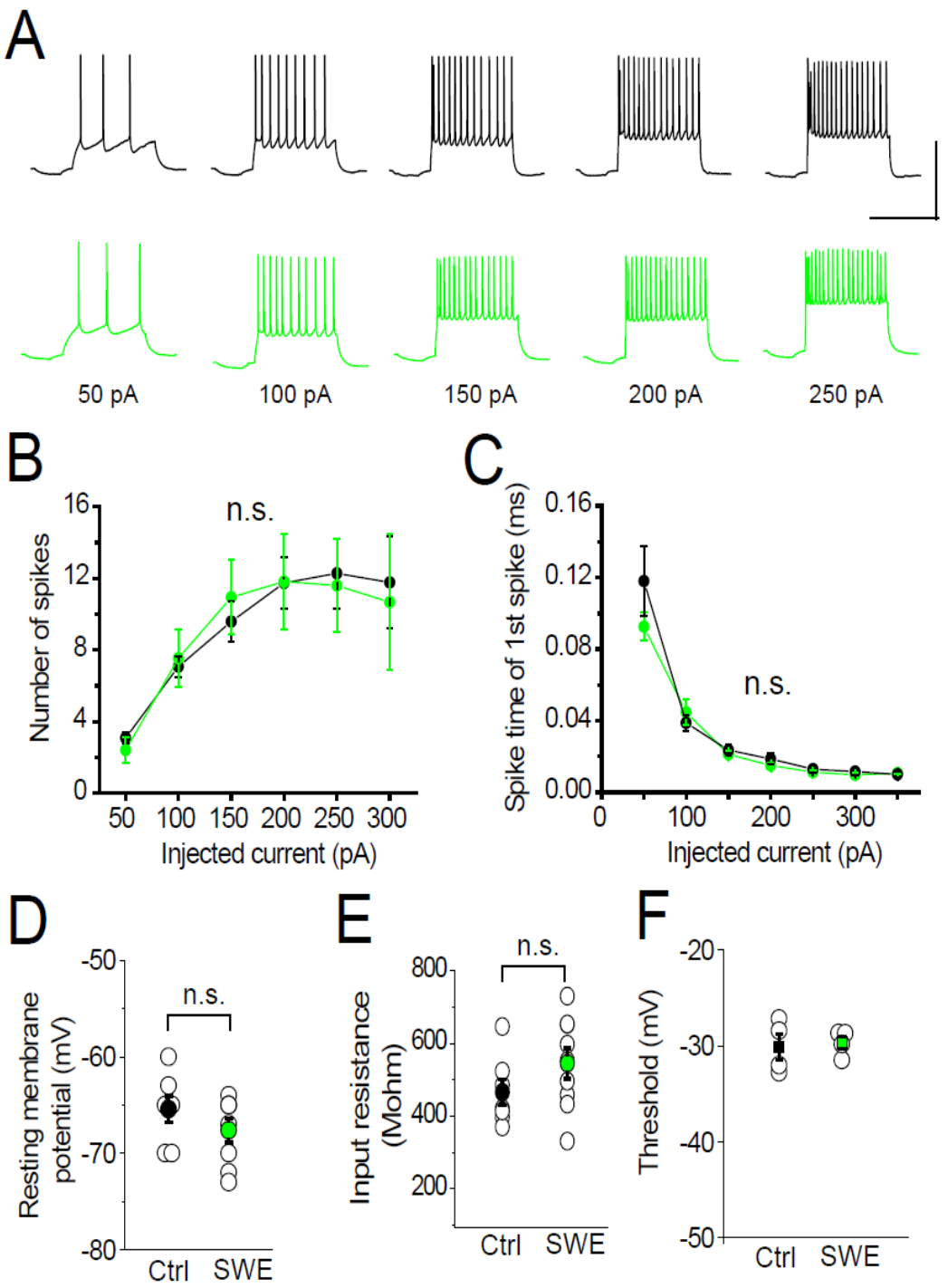

Figure D1. Intrinsic properties of layer 2/3 pyramidal neurons (P12-14) do not change after 24 hr SWE. A) Example cells from control (black) and SWE-treated (green) mice showing evoked responses to different levels of square pulse injection. Scale bars: 20 mV, 200 ms. **B)** Number of spikes during current injection for control and SWE-treated mice. **C)** The time (relative to the onset of square pulse) of the 1st spike triggered by current injection. **D)** Resting membrane potentials. N=7 (control) and 8 (SWE) cells. **E)** Input resistance. N=7 (control) and 9 (SWE) cells. **F)** Spike threshold (calculated at Rheobase current). N=4 (control) and 4 (SWE) cells.

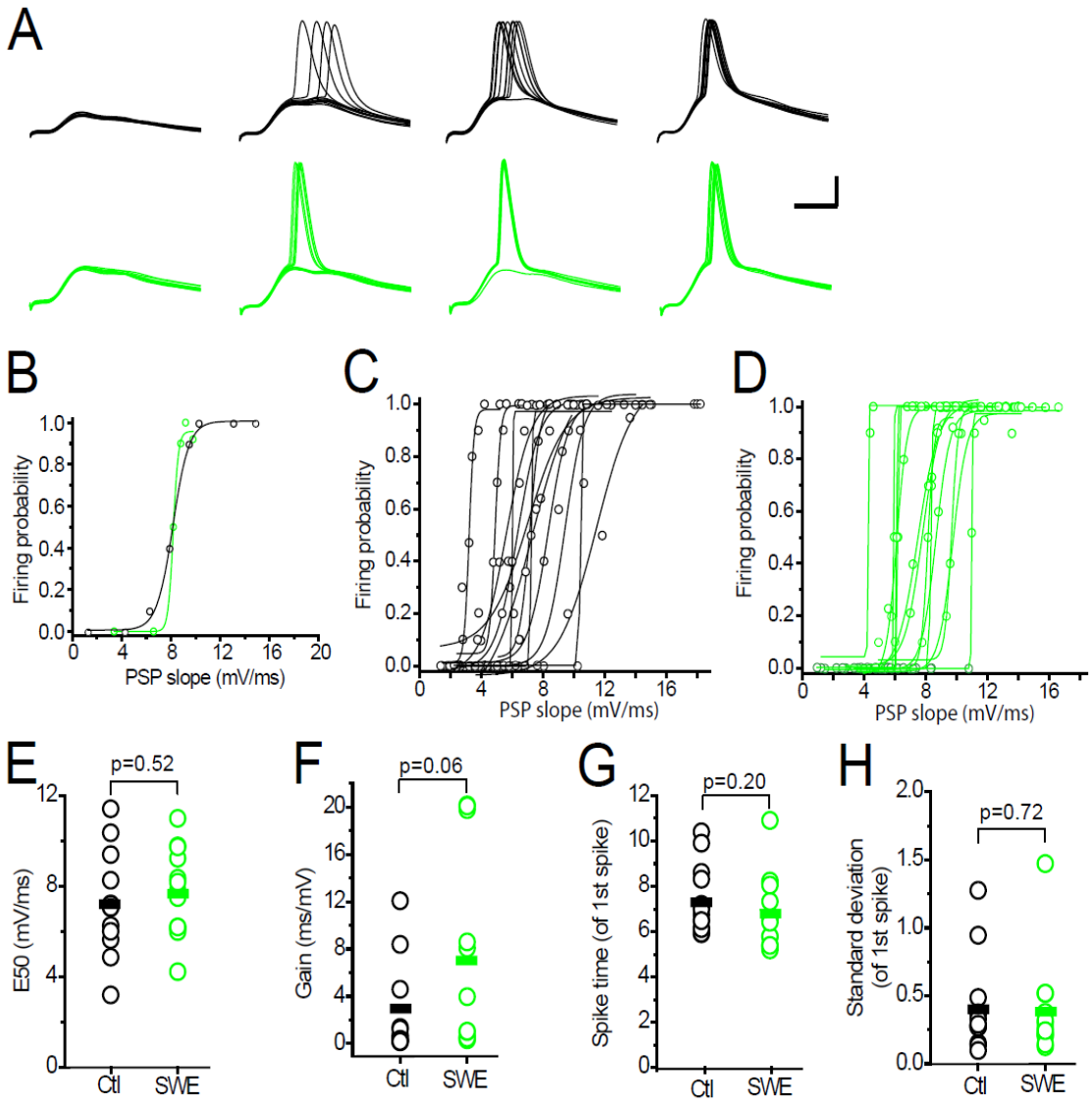

Figure D2. Evoked firing of layer 2/3 pyramidal neurons by layer 4 stimulation in slices. A) Example traces of layer 4-evoked firing in two cells from control (black) and SWE-treated (green) animals at different stimulus intensities. Scale bars: 20 mV, 5 ms. **B)** Firing probability as a function of PSP slope with fitted sigmoidal curves for the two cells shown in **A)**. Firing probability was calculated as the number of sweeps that show at least one spike out of 10 sweeps at a given stimulus intensity. PSP slope was calculated from the first 2 ms time window of voltage deflection of the average trace at a given stimulus intensity. For each cell, a sigmoidal function was fitted to the data. **C)** Firing probability as a function of PSP slope for all the cells in the control animals. **D)** Firing probability as a function of PSP slope for all the cells in the SWE-treated animals. **E)** E50 (mV/ms) for all the control and SWE cells. E50 is the value of PSP slope required to elicit 0.5 firing probability extrapolated from the fitted sigmoidal curve for a given cell. **F)** Gain for all the control and SWE cells. Gain is the value of the slope for the portion between 0.2 and 0.8 firing probability in the fitted sigmoidal curve. **G)** The time of the 1st spike relative to the stimulus (layer 4 stimulation) artifact. **H)** Standard deviation (SD) of the time of the 1st spike at the first stimulus intensity at which the firing probability reaches 1. Standard deviation was calculated from the time of the 1st spike.

References

Abdul-Ghani MA, Valiante TA, Pennefather PS (1996) Sr2+ and quantal events at excitatory synapses between mouse hippocampal neurons in culture. J Physiol 495 (Pt 1):113-125.

Abraham WC (2008) Metaplasticity: tuning synapses and networks for plasticity. Nat Rev Neurosci 9:387.

Adesnik H, Nicoll RA (2007) Conservation of glutamate receptor 2-containing AMPA receptors during long-term potentiation. J Neurosci 27:4598-4602.

Al-Hallaq RA, Conrads TP, Veenstra TD, Wenthold RJ (2007) NMDA di-heteromeric receptor populations and associated proteins in rat hippocampus. J Neurosci 27:8334-8343.

Allen CB, Celikel T, Feldman DE (2003) Long-term depression induced by sensory deprivation during cortical map plasticity in vivo. Nat Neurosci 6:291-299.

Aoki C, Venkatesan C, Go CG, Mong JA, Dawson TM (1994) Cellular and subcellular localization of NMDA-R1 subunit immunoreactivity in the visual cortex of adult and neonatal rats. J Neurosci 14:5202-5222.

Armstrong-James M, Fox K (1987) Spatiotemporal convergence and divergence in the rat S1 "barrel" cortex. J Comp Neurol 263:265-281.

Armstrong-James M, Fox K, Das-Gupta A (1992) Flow of excitation within rat barrel cortex on striking a single vibrissa. J Neurophysiol 68:1345-1358.

Asrar S, Zhou Z, Ren W, Jia Z (2009) Ca(2+) permeable AMPA receptor induced long-term potentiation requires PI3/MAP kinases but not Ca/CaM-dependent kinase II. PLoS One 4:e4339.

Awad H, Hubert GW, Smith Y, Levey AI, Conn PJ (2000) Activation of metabotropic glutamate receptor 5 has direct excitatory effects and potentiates NMDA receptor currents in neurons of the subthalamic nucleus. J Neurosci 20:7871-7879.

Banerjee A, Meredith RM, Rodriguez-Moreno A, Mierau SB, Auberson YP, Paulsen O (2009) Double dissociation of spike timing-dependent potentiation and depression by subunit-preferring NMDA receptor antagonists in mouse barrel cortex. Cereb Cortex 19:2959-2969.

Barkat TR, Polley DB, Hensch TK (2011) A critical period for auditory thalamocortical connectivity. Nat Neurosci 14:1189-1194.

Barnes SJ, Finnerty GT (2010) Sensory experience and cortical rewiring. Neuroscientist 16:186-198.

Barria A, Malinow R (2002) Subunit-specific NMDA receptor trafficking to synapses. Neuron 35:345-353.

Barria A, Malinow R (2005) NMDA receptor subunit composition controls synaptic plasticity by regulating binding to CaMKII. Neuron 48:289-301.

Barrionuevo G, Schottler F, Lynch G (1980) The effects of repetitive low frequency stimulation on control and "potentiated" synaptic responses in the hippocampus. Life Sci 27:2385-2391.

Barth AL, Malenka RC (2001) NMDAR EPSC kinetics do not regulate the critical period for LTP at thalamocortical synapses. Nat Neurosci 4:235-236.

Barth AL, Gerkin RC, Dean KL (2004) Alteration of neuronal firing properties after in vivo experience in a FosGFP transgenic mouse. J Neurosci 24:6466-6475.

Barth AL, McKenna M, Glazewski S, Hill P, Impey S, Storm D, Fox K (2000) Upregulation of cAMP response element-mediated gene expression during experience-dependent plasticity in adult neocortex. J Neurosci 20:4206-4216.
Bartlett TE, Bannister NJ, Collett VJ, Dargan SL, Massey PV, Bortolotto ZA, Fitzjohn SM, Bashir ZI, Collingridge GL, Lodge D (2007) Differential roles of NR2A and NR2B-containing NMDA receptors in LTP and LTD in the CA1 region of two-week old rat hippocampus. Neuropharmacology 52:60-70.
Bashir ZI, Collingridge GL (1994) An investigation of depotentiation of long-term potentiation in the CA1 region of the hippocampus. Exp Brain Res 100:437-443.
Bates CA, Killackey HP (1985) The organization of the neonatal rat's brainstem trigeminal complex and its role in the formation of central trigeminal patterns. J Comp Neurol 240:265-287.
Bellone C, Luscher C (2006) Cocaine triggered AMPA receptor redistribution is reversed in vivo by mGluR-dependent long-term depression. Nat Neurosci 9:636-641.
Bellone C, Nicoll RA (2007) Rapid bidirectional switching of synaptic NMDA receptors. Neuron 55:779-785.
Bender KJ, Allen CB, Bender VA, Feldman DE (2006a) Synaptic basis for whisker deprivation-induced synaptic depression in rat somatosensory cortex. J Neurosci 26:4155-4165.
Bender VA, Bender KJ, Brasier DJ, Feldman DE (2006b) Two coincidence detectors for spike timing-dependent plasticity in somatosensory cortex. J Neurosci 26:4166-4177.
Benedetti BL, Glazewski S, Barth AL (2009) Reliable and precise neuronal firing during sensory plasticity in superficial layers of primary somatosensory cortex. J Neurosci 29:11817-11827.
Berretta N, Jones RS (1996) Tonic facilitation of glutamate release by presynaptic N-methyl-D-aspartate autoreceptors in the entorhinal cortex. Neuroscience 75:339-344.
Bi GQ, Poo MM (1998) Synaptic modifications in cultured hippocampal neurons: dependence on spike timing, synaptic strength, and postsynaptic cell type. J Neurosci 18:10464-10472.
Bidoret C, Ayon A, Barbour B, Casado M (2009) Presynaptic NR2A-containing NMDA receptors implement a high-pass filter synaptic plasticity rule. Proc Natl Acad Sci U S A 106:14126-14131.
Biou V, Bhattacharyya S, Malenka RC (2008) Endocytosis and recycling of AMPA receptors lacking GluR2/3. Proc Natl Acad Sci U S A 105:1038-1043.
Bolshakov VY, Siegelbaum SA (1994) Postsynaptic induction and presynaptic expression of hippocampal long-term depression. Science 264:1148-1152.
Boulter J, Hollmann M, O'Shea-Greenfield A, Hartley M, Deneris E, Maron C, Heinemann S (1990) Molecular cloning and functional expression of glutamate receptor subunit genes. Science 249:1033-1037.
Bowie D, Mayer ML (1995) Inward rectification of both AMPA and kainate subtype glutamate receptors generated by polyamine-mediated ion channel block. Neuron 15:453-462.
Brasier DJ, Feldman DE (2008) Synapse-specific expression of functional presynaptic NMDA receptors in rat somatosensory cortex. J Neurosci 28:2199-2211.

Brecht M, Sakmann B (2002a) Whisker maps of neuronal subclasses of the rat ventral posterior medial thalamus, identified by whole-cell voltage recording and morphological reconstruction. J Physiol 538:495-515.
Brecht M, Sakmann B (2002b) Dynamic representation of whisker deflection by synaptic potentials in spiny stellate and pyramidal cells in the barrels and septa of layer 4 rat somatosensory cortex. J Physiol 543:49-70.
Brill J, Huguenard JR (2008) Sequential changes in AMPA receptor targeting in the developing neocortical excitatory circuit. J Neurosci 28:13918-13928.
Broser P, Grinevich V, Osten P, Sakmann B, Wallace DJ (2008) Critical period plasticity of axonal arbors of layer 2/3 pyramidal neurons in rat somatosensory cortex: layer-specific reduction of projections into deprived cortical columns. Cereb Cortex 18:1588-1603.
Bruno RM, Sakmann B (2006) Cortex is driven by weak but synchronously active thalamocortical synapses. Science 312:1622-1627.
Bureau I, von Saint Paul F, Svoboda K (2006) Interdigitated paralemniscal and lemniscal pathways in the mouse barrel cortex. PLoS Biol 4:e382.
Burnashev N, Villarroel A, Sakmann B (1996) Dimensions and ion selectivity of recombinant AMPA and kainate receptor channels and their dependence on Q/R site residues. J Physiol 496 (Pt 1):165-173.
Burnashev N, Zhou Z, Neher E, Sakmann B (1995) Fractional calcium currents through recombinant GluR channels of the NMDA, AMPA and kainate receptor subtypes. J Physiol 485 (Pt 2):403-418.
Campbell UC, Lalwani K, Hernandez L, Kinney GG, Conn PJ, Bristow LJ (2004) The mGluR5 antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP) potentiates PCP-induced cognitive deficits in rats. Psychopharmacology (Berl) 175:310-318.
Carmignoto G, Vicini S (1992) Activity-dependent decrease in NMDA receptor responses during development of the visual cortex. Science 258:1007-1011.
Carter AG, Regehr WG (2002) Quantal events shape cerebellar interneuron firing. Nat Neurosci 5:1309-1318.
Charton JP, Herkert M, Becker CM, Schroder H (1999) Cellular and subcellular localization of the 2B-subunit of the NMDA receptor in the adult rat telencephalon. Brain Res 816:609-617.
Cheetham CE, Hammond MS, Edwards CE, Finnerty GT (2007) Sensory experience alters cortical connectivity and synaptic function site specifically. J Neurosci 27:3456-3465.
Cheetham CE, Hammond MS, McFarlane R, Finnerty GT (2008) Altered sensory experience induces targeted rewiring of local excitatory connections in mature neocortex. J Neurosci 28:9249-9260.
Chen WS, Bear MF (2007) Activity-dependent regulation of NR2B translation contributes to metaplasticity in mouse visual cortex. Neuropharmacology 52:200-214.
Chiu CQ, Castillo PE (2008) Input-specific plasticity at excitatory synapses mediated by endocannabinoids in the dentate gyrus. Neuropharmacology 54:68-78.
Chiu SL, Chen CM, Cline HT (2008) Insulin receptor signaling regulates synapse number, dendritic plasticity, and circuit function in vivo. Neuron 58:708-719.

Christie JM, Jahr CE (2008) Dendritic NMDA receptors activate axonal calcium channels. Neuron 60:298-307.
Christie JM, Jahr CE (2009) Selective expression of ligand-gated ion channels in L5 pyramidal cell axons. J Neurosci 29:11441-11450.
Clarke JR, Cammarota M, Gruart A, Izquierdo I, Delgado-Garcia JM (2010) Plastic modifications induced by object recognition memory processing. Proc Natl Acad Sci U S A 107:2652-2657.
Clem RL, Barth A (2006) Pathway-specific trafficking of native AMPARs by in vivo experience. Neuron 49:663-670.
Clem RL, Huganir RL (2010) Calcium-permeable AMPA receptor dynamics mediate fear memory erasure. Science 330:1108-1112.
Clem RL, Celikel T, Barth AL (2008) Ongoing in vivo experience triggers synaptic metaplasticity in the neocortex. Science 319:101-104.
Clem RL, Anggono V, Huganir RL (2010) PICK1 regulates incorporation of calcium-permeable AMPA receptors during cortical synaptic strengthening. J Neurosci 30:6360-6366.
Coleman JE, Nahmani M, Gavornik JP, Haslinger R, Heynen AJ, Erisir A, Bear MF (2010) Rapid structural remodeling of thalamocortical synapses parallels experience-dependent functional plasticity in mouse primary visual cortex. J Neurosci 30:9670-9682.
Cooke SF, Bear MF (2010) Visual experience induces long-term potentiation in the primary visual cortex. J Neurosci 30:16304-16313.
Corlew R, Brasier DJ, Feldman DE, Philpot BD (2008) Presynaptic NMDA receptors: newly appreciated roles in cortical synaptic function and plasticity. Neuroscientist 14:609-625.
Corlew R, Wang Y, Ghermazien H, Erisir A, Philpot BD (2007) Developmental switch in the contribution of presynaptic and postsynaptic NMDA receptors to long-term depression. J Neurosci 27:9835-9845.
Cormier RJ, Greenwood AC, Connor JA (2001) Bidirectional synaptic plasticity correlated with the magnitude of dendritic calcium transients above a threshold. J Neurophysiol 85:399-406.
Crair MC, Malenka RC (1995) A critical period for long-term potentiation at thalamocortical synapses. Nature 375:325-328.
Crepel F, Mariani J, Delhaye-Bouchaud N (1976) Evidence for a multiple innervation of Purkinje cells by climbing fibers in the immature rat cerebellum. J Neurobiol 7:567-578.
Cui Y, Jin J, Zhang X, Xu H, Yang L, Du D, Zeng Q, Tsien JZ, Yu H, Cao X (2011) Forebrain NR2B overexpression facilitating the prefrontal cortex long-term potentiation and enhancing working memory function in mice. PLoS One 6:e20312.
Dachtler J, Hardingham NR, Glazewski S, Wright NF, Blain EJ, Fox K (2011) Experience-dependent plasticity acts via GluR1 and a novel neuronal nitric oxide synthase-dependent synaptic mechanism in adult cortex. J Neurosci 31:11220-11230.

Daoudal G, Hanada Y, Debanne D (2002) Bidirectional plasticity of excitatory postsynaptic potential (EPSP)-spike coupling in CA1 hippocampal pyramidal neurons. Proc Natl Acad Sci U S A 99:14512-14517.
Daw N, Rao Y, Wang XF, Fischer Q, Yang Y (2004) LTP and LTD vary with layer in rodent visual cortex. Vision Res 44:3377-3380.
Debanne D, Gahwiler BH, Thompson SM (1998) Long-term synaptic plasticity between pairs of individual CA3 pyramidal cells in rat hippocampal slice cultures. J Physiol 507 (Pt 1):237-247.
DeBiasi S, Minelli A, Melone M, Conti F (1996) Presynaptic NMDA receptors in the neocortex are both auto- and heteroreceptors. Neuroreport 7:2773-2776.
Delaney AJ, Power JM, Sah P (2011) Ifenprodil reduces excitatory synaptic transmission by blocking presynaptic P/Q type calcium channels. J Neurophysiol.
Deng W, Rosenberg PA, Volpe JJ, Jensen FE (2003) Calcium-permeable AMPA/kainate receptors mediate toxicity and preconditioning by oxygen-glucose deprivation in oligodendrocyte precursors. Proc Natl Acad Sci U S A 100:6801-6806.
Desai NS, Cudmore RH, Nelson SB, Turrigiano GG (2002) Critical periods for experience-dependent synaptic scaling in visual cortex. Nat Neurosci 5:783-789.
Diamond ME, Armstrong-James M, Ebner FF (1993) Experience-dependent plasticity in adult rat barrel cortex. Proc Natl Acad Sci U S A 90:2082-2086.
Diamond ME, Huang W, Ebner FF (1994) Laminar comparison of somatosensory cortical plasticity. Science 265:1885-1888.
Diamond ME, Armstrong-James M, Budway MJ, Ebner FF (1992) Somatic sensory responses in the rostral sector of the posterior group (POm) and in the ventral posterior medial nucleus (VPM) of the rat thalamus: dependence on the barrel field cortex. J Comp Neurol 319:66-84.
Dingledine R, Borges K, Bowie D, Traynelis SF (1999) The glutamate receptor ion channels. Pharmacol Rev 51:7-61.
Doyere V, Srebro B, Laroche S (1997) Heterosynaptic LTD and depotentiation in the medial perforant path of the dentate gyrus in the freely moving rat. J Neurophysiol 77:571-578.
Dudek SM, Friedlander MJ (1996) Developmental down-regulation of LTD in cortical layer IV and its independence of modulation by inhibition. Neuron 16:1097-1106.
Engelman HS, MacDermott AB (2004) Presynaptic ionotropic receptors and control of transmitter release. Nat Rev Neurosci 5:135-145.
Fagiolini M, Hensch TK (2000) Inhibitory threshold for critical-period activation in primary visual cortex. Nature 404:183-186.
Fagiolini M, Pizzorusso T, Berardi N, Domenici L, Maffei L (1994) Functional postnatal development of the rat primary visual cortex and the role of visual experience: dark rearing and monocular deprivation. Vision Res 34:709-720.
Fagiolini M, Fritschy JM, Low K, Mohler H, Rudolph U, Hensch TK (2004) Specific GABAA circuits for visual cortical plasticity. Science 303:1681-1683.
Farb CR, Aoki C, Ledoux JE (1995) Differential localization of NMDA and AMPA receptor subunits in the lateral and basal nuclei of the amygdala: a light and electron microscopic study. J Comp Neurol 362:86-108.
Feldman DE (2000) Timing-based LTP and LTD at vertical inputs to layer II/III pyramidal cells in rat barrel cortex. Neuron 27:45-56.

Feldman DE (2009) Synaptic mechanisms for plasticity in neocortex. Annu Rev Neurosci 32:33-55.
Feldman DE, Nicoll RA, Malenka RC, Isaac JT (1998) Long-term depression at thalamocortical synapses in developing rat somatosensory cortex. Neuron 21:347-357.
Feldmeyer D, Lubke J, Sakmann B (2006) Efficacy and connectivity of intracolumnar pairs of layer 2/3 pyramidal cells in the barrel cortex of juvenile rats. J Physiol 575:583-602.
Feldmeyer D, Egger V, Lubke J, Sakmann B (1999) Reliable synaptic connections between pairs of excitatory layer 4 neurones within a single 'barrel' of developing rat somatosensory cortex. J Physiol 521 Pt 1:169-190.
Feldmeyer D, Lubke J, Silver RA, Sakmann B (2002) Synaptic connections between layer 4 spiny neurone-layer 2/3 pyramidal cell pairs in juvenile rat barrel cortex: physiology and anatomy of interlaminar signalling within a cortical column. J Physiol 538:803-822.
Ferezou I, Bolea S, Petersen CC (2006) Visualizing the cortical representation of whisker touch: voltage-sensitive dye imaging in freely moving mice. Neuron 50:617-629.
Fetterolf F, Foster KA (2011) Regulation of long-term plasticity induction by the channel and C-terminal domains of GluN2 subunits. Mol Neurobiol 44:71-82.
Finnerty GT, Roberts LS, Connors BW (1999) Sensory experience modifies the short-term dynamics of neocortical synapses. Nature 400:367-371.
Fischer QS, Beaver CJ, Yang Y, Rao Y, Jakobsdottir KB, Storm DR, McKnight GS, Daw NW (2004) Requirement for the RIIbeta isoform of PKA, but not calcium-stimulated adenylyl cyclase, in visual cortical plasticity. J Neurosci 24:9049-9058.
Fitzjohn SM, Bortolotto ZA, Palmer MJ, Doherty AJ, Ornstein PL, Schoepp DD, Kingston AE, Lodge D, Collingridge GL (1998) The potent mGlu receptor antagonist LY341495 identifies roles for both cloned and novel mGlu receptors in hippocampal synaptic plasticity. Neuropharmacology 37:1445-1458.
Flint AC, Maisch US, Weishaupt JH, Kriegstein AR, Monyer H (1997) NR2A subunit expression shortens NMDA receptor synaptic currents in developing neocortex. J Neurosci 17:2469-2476.
Foster KA, McLaughlin N, Edbauer D, Phillips M, Bolton A, Constantine-Paton M, Sheng M (2010) Distinct roles of NR2A and NR2B cytoplasmic tails in long-term potentiation. J Neurosci 30:2676-2685.
Fox K (1992) A critical period for experience-dependent synaptic plasticity in rat barrel cortex. J Neurosci 12:1826-1838.
Fox K (2009) Experience-dependent plasticity mechanisms for neural rehabilitation in somatosensory cortex. Philos Trans R Soc Lond B Biol Sci 364:369-381.
Franks KM, Sejnowski TJ (2002) Complexity of calcium signaling in synaptic spines. Bioessays 24:1130-1144.
Frenkel MY, Bear MF (2004) How monocular deprivation shifts ocular dominance in visual cortex of young mice. Neuron 44:917-923.
Fujii S, Saito K, Miyakawa H, Ito K, Kato H (1991) Reversal of long-term potentiation (depotentiation) induced by tetanus stimulation of the input to CA1 neurons of guinea pig hippocampal slices. Brain Res 555:112-122.

Fujisawa S, Aoki C (2003) In vivo blockade of N-methyl-D-aspartate receptors induces rapid trafficking of NR2B subunits away from synapses and out of spines and terminals in adult cortex. Neuroscience 121:51-63.
Gagolewicz PJ, Dringenberg HC (2011) NR2B-subunit dependent facilitation of long-term potentiation in primary visual cortex following visual discrimination training of adult rats. Eur J Neurosci 34:1222-1229.
Gaiarsa JL, Caillard O, Ben-Ari Y (2002) Long-term plasticity at GABAergic and glycinergic synapses: mechanisms and functional significance. Trends Neurosci 25:564-570.
Gandhi SP, Yanagawa Y, Stryker MP (2008) Delayed plasticity of inhibitory neurons in developing visual cortex. Proc Natl Acad Sci U S A 105:16797-16802.
Ge Y, Dong Z, Bagot RC, Howland JG, Phillips AG, Wong TP, Wang YT (2010) Hippocampal long-term depression is required for the consolidation of spatial memory. Proc Natl Acad Sci U S A 107:16697-16702.
Geiger JR, Melcher T, Koh DS, Sakmann B, Seeburg PH, Jonas P, Monyer H (1995) Relative abundance of subunit mRNAs determines gating and Ca2+ permeability of AMPA receptors in principal neurons and interneurons in rat CNS. Neuron 15:193-204.
Gianfranceschi L, Siciliano R, Walls J, Morales B, Kirkwood A, Huang ZJ, Tonegawa S, Maffei L (2003) Visual cortex is rescued from the effects of dark rearing by overexpression of BDNF. Proc Natl Acad Sci U S A 100:12486-12491.
Glazewski S, Fox K (1996) Time course of experience-dependent synaptic potentiation and depression in barrel cortex of adolescent rats. J Neurophysiol 75:1714-1729.
Glazewski S, Benedetti BL, Barth AL (2007) Ipsilateral whiskers suppress experience-dependent plasticity in the barrel cortex. J Neurosci 27:3910-3920.
Glazewski S, McKenna M, Jacquin M, Fox K (1998) Experience-dependent depression of vibrissae responses in adolescent rat barrel cortex. Eur J Neurosci 10:2107-2116.
Glazewski S, Giese KP, Silva A, Fox K (2000) The role of alpha-CaMKII autophosphorylation in neocortical experience-dependent plasticity. Nat Neurosci 3:911-918.
Glitsch MD (2008) Calcium influx through N-methyl-D-aspartate receptors triggers GABA release at interneuron-Purkinje cell synapse in rat cerebellum. Neuroscience 151:403-409.
Goda Y, Stevens CF (1994) Two components of transmitter release at a central synapse. Proc Natl Acad Sci U S A 91:12942-12946.
Gordon JA, Stryker MP (1996) Experience-dependent plasticity of binocular responses in the primary visual cortex of the mouse. J Neurosci 16:3274-3286.
Gray EE, Fink AE, Sarinana J, Vissel B, O'Dell TJ (2007) Long-term potentiation in the hippocampal CA1 region does not require insertion and activation of GluR2-lacking AMPA receptors. J Neurophysiol 98:2488-2492.
Gray JA, Shi Y, Usui H, During MJ, Sakimura K, Nicoll RA (2011) Distinct modes of AMPA receptor suppression at developing synapses by GluN2A and GluN2B: single-cell NMDA receptor subunit deletion in vivo. Neuron 71:1085-1101.

Groc L, Heine M, Cousins SL, Stephenson FA, Lounis B, Cognet L, Choquet D (2006) NMDA receptor surface mobility depends on NR2A-2B subunits. Proc Natl Acad Sci U S A 103:18769-18774.
Hall BJ, Ripley B, Ghosh A (2007) NR2B signaling regulates the development of synaptic AMPA receptor current. J Neurosci 27:13446-13456.
Hanover JL, Huang ZJ, Tonegawa S, Stryker MP (1999) Brain-derived neurotrophic factor overexpression induces precocious critical period in mouse visual cortex. J Neurosci 19:RC40.
Hardingham N, Fox K (2006) The role of nitric oxide and GluR1 in presynaptic and postsynaptic components of neocortical potentiation. J Neurosci 26:7395-7404.
Hardingham N, Glazewski S, Pakhotin P, Mizuno K, Chapman PF, Giese KP, Fox K (2003) Neocortical long-term potentiation and experience-dependent synaptic plasticity require alpha-calcium/calmodulin-dependent protein kinase II autophosphorylation. J Neurosci 23:4428-4436.
Harlow EG, Till SM, Russell TA, Wijetunge LS, Kind P, Contractor A (2010) Critical period plasticity is disrupted in the barrel cortex of FMR1 knockout mice. Neuron 65:385-398.
Harris AZ, Pettit DL (2007) Extrasynaptic and synaptic NMDA receptors form stable and uniform pools in rat hippocampal slices. J Physiol 584:509-519.
Hatton CJ, Paoletti P (2005) Modulation of triheteromeric NMDA receptors by N-terminal domain ligands. Neuron 46:261-274.
Hattox AM, Nelson SB (2007) Layer V neurons in mouse cortex projecting to different targets have distinct physiological properties. J Neurophysiol 98:3330-3340.
Hayashi Y, Shi SH, Esteban JA, Piccini A, Poncer JC, Malinow R (2000) Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction. Science 287:2262-2267.
Helmstaedter M, Staiger JF, Sakmann B, Feldmeyer D (2008) Efficient recruitment of layer 2/3 interneurons by layer 4 input in single columns of rat somatosensory cortex. J Neurosci 28:8273-8284.
Henry SA, Lehmann-Masten V, Gasparini F, Geyer MA, Markou A (2002) The mGluR5 antagonist MPEP, but not the mGluR2/3 agonist LY314582, augments PCP effects on prepulse inhibition and locomotor activity. Neuropharmacology 43:1199-1209.
Hensch TK (2004) Critical period regulation. Annu Rev Neurosci 27:549-579.
Hensch TK (2005) Critical period plasticity in local cortical circuits. Nat Rev Neurosci 6:877-888.
Hensch TK, Fagiolini M, Mataga N, Stryker MP, Baekkeskov S, Kash SF (1998) Local GABA circuit control of experience-dependent plasticity in developing visual cortex. Science 282:1504-1508.
Hesse GW, Teyler TJ (1976) Reversible loss of hippocampal long term potentiation following electronconvulsive seizures. Nature 264:562-564.
Hestrin S (1992) Developmental regulation of NMDA receptor-mediated synaptic currents at a central synapse. Nature 357:686-689.
Hestrin S (1993) Different glutamate receptor channels mediate fast excitatory synaptic currents in inhibitory and excitatory cortical neurons. Neuron 11:1083-1091.

Heynen AJ, Yoon BJ, Liu CH, Chung HJ, Huganir RL, Bear MF (2003) Molecular mechanism for loss of visual cortical responsiveness following brief monocular deprivation. Nat Neurosci 6:854-862.
Higuchi M, Single FN, Kohler M, Sommer B, Sprengel R, Seeburg PH (1993) RNA editing of AMPA receptor subunit GluR-B: a base-paired intron-exon structure determines position and efficiency. Cell 75:1361-1370.
Ho MT, Pelkey KA, Topolnik L, Petralia RS, Takamiya K, Xia J, Huganir RL, Lacaille JC, McBain CJ (2007) Developmental expression of Ca2+-permeable AMPA receptors underlies depolarization-induced long-term depression at mossy fiber CA3 pyramid synapses. J Neurosci 27:11651-11662.
Hollmann M, Heinemann S (1994) Cloned glutamate receptors. Annu Rev Neurosci 17:31-108.
Hou Q, Zhang D, Jarzylo L, Huganir RL, Man HY (2008) Homeostatic regulation of AMPA receptor expression at single hippocampal synapses. Proc Natl Acad Sci U S A 105:775-780.
Huang CC, Liang YC, Hsu KS (2001) Characterization of the mechanism underlying the reversal of long term potentiation by low frequency stimulation at hippocampal CA1 synapses. J Biol Chem 276:48108-48117.
Huang ZJ, Kirkwood A, Pizzorusso T, Porciatti V, Morales B, Bear MF, Maffei L, Tonegawa S (1999) BDNF regulates the maturation of inhibition and the critical period of plasticity in mouse visual cortex. Cell 98:739-755.
Hubel DH, Wiesel TN, LeVay S (1977) Plasticity of ocular dominance columns in monkey striate cortex. Philos Trans R Soc Lond B Biol Sci 278:377-409.
Huber D, Petreanu L, Ghitani N, Ranade S, Hromadka T, Mainen Z, Svoboda K (2008) Sparse optical microstimulation in barrel cortex drives learned behaviour in freely moving mice. Nature 451:61-64.
Huber KM, Kayser MS, Bear MF (2000) Role for rapid dendritic protein synthesis in hippocampal mGluR-dependent long-term depression. Science 288:1254-1257.
Hull C, Isaacson JS, Scanziani M (2009) Postsynaptic mechanisms govern the differential excitation of cortical neurons by thalamic inputs. J Neurosci 29:9127-9136.
Humeau Y, Reisel D, Johnson AW, Borchardt T, Jensen V, Gebhardt C, Bosch V, Gass P, Bannerman DM, Good MA, Hvalby O, Sprengel R, Luthi A (2007) A pathway-specific function for different AMPA receptor subunits in amygdala long-term potentiation and fear conditioning. J Neurosci 27:10947-10956.
Hupbach A, Gomez R, Hardt O, Nadel L (2007) Reconsolidation of episodic memories: a subtle reminder triggers integration of new information. Learn Mem 14:47-53.
Isaac JT, Crair MC, Nicoll RA, Malenka RC (1997) Silent synapses during development of thalamocortical inputs. Neuron 18:269-280.
Iwai Y, Fagiolini M, Obata K, Hensch TK (2003) Rapid critical period induction by tonic inhibition in visual cortex. J Neurosci 23:6695-6702.
Jiang B, Huang ZJ, Morales B, Kirkwood A (2005) Maturation of GABAergic transmission and the timing of plasticity in visual cortex. Brain Res Brain Res Rev 50:126-133.
Jiao Y, Zhang C, Yanagawa Y, Sun QQ (2006) Major effects of sensory experiences on the neocortical inhibitory circuits. J Neurosci 26:8691-8701.

Johenning FW, Beed PS, Trimbuch T, Bendels MH, Winterer J, Schmitz D (2009) Dendritic compartment and neuronal output mode determine pathway-specific long-term potentiation in the piriform cortex. J Neurosci 29:13649-13661.
Jonas P, Burnashev N (1995) Molecular mechanisms controlling calcium entry through AMPA-type glutamate receptor channels. Neuron 15:987-990.
Jourdain P, Bergersen LH, Bhaukaurally K, Bezzi P, Santello M, Domercq M, Matute C, Tonello F, Gundersen V, Volterra A (2007) Glutamate exocytosis from astrocytes controls synaptic strength. Nat Neurosci 10:331-339.
Jung SY, Kim J, Kwon OB, Jung JH, An K, Jeong AY, Lee CJ, Choi YB, Bailey CH, Kandel ER, Kim JH (2010) Input-specific synaptic plasticity in the amygdala is regulated by neuroligin-1 via postsynaptic NMDA receptors. Proc Natl Acad Sci U S A 107:4710-4715.
Kennedy MB, Beale HC, Carlisle HJ, Washburn LR (2005) Integration of biochemical signalling in spines. Nat Rev Neurosci 6:423-434.
Kessels HW, Malinow R (2009) Synaptic AMPA receptor plasticity and behavior. Neuron 61:340-350.
Kidd FL, Coumis U, Collingridge GL, Crabtree JW, Isaac JT (2002) A presynaptic kainate receptor is involved in regulating the dynamic properties of thalamocortical synapses during development. Neuron 34:635-646.
Kielland A, Bochorishvili G, Corson J, Zhang L, Rosin DL, Heggelund P, Zhu JJ (2009) Activity patterns govern synapse-specific AMPA receptor trafficking between deliverable and synaptic pools. Neuron 62:84-101.
Kim J, Lee S, Park K, Hong I, Song B, Son G, Park H, Kim WR, Park E, Choe HK, Kim H, Lee C, Sun W, Kim K, Shin KS, Choi S (2007) Amygdala depotentiation and fear extinction. Proc Natl Acad Sci U S A 104:20955-20960.
Kim JH, Liao D, Lau LF, Huganir RL (1998) SynGAP: a synaptic RasGAP that associates with the PSD-95/SAP90 protein family. Neuron 20:683-691.
Kirkwood A, Lee HK, Bear MF (1995) Co-regulation of long-term potentiation and experience-dependent synaptic plasticity in visual cortex by age and experience. Nature 375:328-331.
Kirkwood A, Rioult MC, Bear MF (1996) Experience-dependent modification of synaptic plasticity in visual cortex. Nature 381:526-528.
Kiser PJ, Cooper NG, Mower GD (1998) Expression of two forms of glutamic acid decarboxylase (GAD67 and GAD65) during postnatal development of rat somatosensory barrel cortex. J Comp Neurol 402:62-74.
Knott GW, Quairiaux C, Genoud C, Welker E (2002) Formation of dendritic spines with GABAergic synapses induced by whisker stimulation in adult mice. Neuron 34:265-273.
Koike M, Iino M, Ozawa S (1997) Blocking effect of 1-naphthyl acetyl spermine on Ca(2+)-permeable AMPA receptors in cultured rat hippocampal neurons. Neurosci Res 29:27-36.
Kole MH, Hallermann S, Stuart GJ (2006) Single Ih channels in pyramidal neuron dendrites: properties, distribution, and impact on action potential output. J Neurosci 26:1677-1687.
Krapivinsky G, Krapivinsky L, Manasian Y, Ivanov A, Tyzio R, Pellegrino C, Ben-Ari Y, Clapham DE, Medina I (2003) The NMDA receptor is coupled to the ERK

pathway by a direct interaction between NR2B and RasGRF1. Neuron 40:775-784.
Kullmann DM, Lamsa KP (2007) Long-term synaptic plasticity in hippocampal interneurons. Nat Rev Neurosci 8:687-699.
Kumar SS, Huguenard JR (2003) Pathway-specific differences in subunit composition of synaptic NMDA receptors on pyramidal neurons in neocortex. J Neurosci 23:10074-10083.
Kumar SS, Bacci A, Kharazia V, Huguenard JR (2002) A developmental switch of AMPA receptor subunits in neocortical pyramidal neurons. J Neurosci 22:3005-3015.
Lante F, Toledo-Salas JC, Ondrejcak T, Rowan MJ, Ulrich D (2011) Removal of synaptic Ca(2)+-permeable AMPA receptors during sleep. J Neurosci 31:3953-3961.
Larsen RS, Corlew RJ, Henson MA, Roberts AC, Mishina M, Watanabe M, Lipton SA, Nakanishi N, Perez-Otano I, Weinberg RJ, Philpot BD (2011) NR3A-containing NMDARs promote neurotransmitter release and spike timing-dependent plasticity. Nat Neurosci 14:338-344.
Larson J, Xiao P, Lynch G (1993) Reversal of LTP by theta frequency stimulation. Brain Res 600:97-102.
Laurie DJ, Bartke I, Schoepfer R, Naujoks K, Seeburg PH (1997) Regional, developmental and interspecies expression of the four NMDAR2 subunits, examined using monoclonal antibodies. Brain Res Mol Brain Res 51:23-32.
Lee HK, Barbarosie M, Kameyama K, Bear MF, Huganir RL (2000) Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity. Nature 405:955-959.
Lee SH, Liu L, Wang YT, Sheng M (2002) Clathrin adaptor AP2 and NSF interact with overlapping sites of GluR2 and play distinct roles in AMPA receptor trafficking and hippocampal LTD. Neuron 36:661-674.
Lefort S, Tomm C, Floyd Sarria JC, Petersen CC (2009) The excitatory neuronal network of the C2 barrel column in mouse primary somatosensory cortex. Neuron 61:301-316.
Lendvai B, Stern EA, Chen B, Svoboda K (2000) Experience-dependent plasticity of dendritic spines in the developing rat barrel cortex in vivo. Nature 404:876-881.
Lisman J, Yasuda R, Raghavachari S (2012) Mechanisms of CaMKII action in long-term potentiation. Nat Rev Neurosci 13:169-182.
Liu CH, Heynen AJ, Shuler MG, Bear MF (2008) Cannabinoid receptor blockade reveals parallel plasticity mechanisms in different layers of mouse visual cortex. Neuron 58:340-345.
Liu H, Wang H, Sheng M, Jan LY, Jan YN, Basbaum AI (1994) Evidence for presynaptic N-methyl-D-aspartate autoreceptors in the spinal cord dorsal horn. Proc Natl Acad Sci U S A 91:8383-8387.
Liu L, Wong TP, Pozza MF, Lingenhoehl K, Wang Y, Sheng M, Auberson YP, Wang YT (2004) Role of NMDA receptor subtypes in governing the direction of hippocampal synaptic plasticity. Science 304:1021-1024.
Lorincz A, Notomi T, Tamas G, Shigemoto R, Nusser Z (2002) Polarized and compartment-dependent distribution of HCN1 in pyramidal cell dendrites. Nat Neurosci 5:1185-1193.

Lu HC, Gonzalez E, Crair MC (2001) Barrel cortex critical period plasticity is independent of changes in NMDA receptor subunit composition. Neuron 32:619-634.
Lu HC, She WC, Plas DT, Neumann PE, Janz R, Crair MC (2003) Adenylyl cyclase I regulates AMPA receptor trafficking during mouse cortical 'barrel' map development. Nat Neurosci 6:939-947.
Lubke J, Roth A, Feldmeyer D, Sakmann B (2003) Morphometric analysis of the columnar innervation domain of neurons connecting layer 4 and layer 2/3 of juvenile rat barrel cortex. Cereb Cortex 13:1051-1063.
Maffei A, Nataraj K, Nelson SB, Turrigiano GG (2006) Potentiation of cortical inhibition by visual deprivation. Nature 443:81-84.
Magee JC (1998) Dendritic hyperpolarization-activated currents modify the integrative properties of hippocampal CA1 pyramidal neurons. J Neurosci 18:7613-7624.
Mahanty NK, Sah P (1998) Calcium-permeable AMPA receptors mediate long-term potentiation in interneurons in the amygdala. Nature 394:683-687.
Malenka RC, Bear MF (2004) LTP and LTD: an embarrassment of riches. Neuron 44:5-21.
Mameli M, Carta M, Partridge LD, Valenzuela CF (2005) Neurosteroid-induced plasticity of immature synapses via retrograde modulation of presynaptic NMDA receptors. J Neurosci 25:2285-2294.
Man HY (2011) GluA2-lacking, calcium-permeable AMPA receptors--inducers of plasticity? Curr Opin Neurobiol 21:291-298.
Manahan-Vaughan D, Braunewell KH (1999) Novelty acquisition is associated with induction of hippocampal long-term depression. Proc Natl Acad Sci U S A 96:8739-8744.
Mao L, Wang JQ (2002) Interactions between ionotropic and metabotropic glutamate receptors regulate cAMP response element-binding protein phosphorylation in cultured striatal neurons. Neuroscience 115:395-402.
Maravall M, Stern EA, Svoboda K (2004) Development of intrinsic properties and excitability of layer 2/3 pyramidal neurons during a critical period for sensory maps in rat barrel cortex. J Neurophysiol 92:144-156.
Markram H, Lubke J, Frotscher M, Sakmann B (1997) Regulation of synaptic efficacy by coincidence of postsynaptic APs and EPSPs. Science 275:213-215.
Massey PV, Johnson BE, Moult PR, Auberson YP, Brown MW, Molnar E, Collingridge GL, Bashir ZI (2004) Differential roles of NR2A and NR2B-containing NMDA receptors in cortical long-term potentiation and long-term depression. J Neurosci 24:7821-7828.
Matsuo N, Reijmers L, Mayford M (2008) Spine-type-specific recruitment of newly synthesized AMPA receptors with learning. Science 319:1104-1107.
Matta JA, Ashby MC, Sanz-Clemente A, Roche KW, Isaac JT (2011) mGluR5 and NMDA receptors drive the experience- and activity-dependent NMDA receptor NR2B to NR2A subunit switch. Neuron 70:339-351.
Mayer ML, Westbrook GL (1987) Permeation and block of N-methyl-D-aspartic acid receptor channels by divalent cations in mouse cultured central neurones. J Physiol 394:501-527.

McGee AW, Yang Y, Fischer QS, Daw NW, Strittmatter SM (2005) Experience-driven plasticity of visual cortex limited by myelin and Nogo receptor. Science 309:2222-2226.
Miwa H, Fukaya M, Watabe AM, Watanabe M, Manabe T (2008) Functional contributions of synaptically localized NR2B subunits of the NMDA receptor to synaptic transmission and long-term potentiation in the adult mouse CNS. J Physiol 586:2539-2550.
Mohrmann R, Hatt H, Gottmann K (2000) Developmental regulation of subunit composition of extrasynaptic NMDA receptors in neocortical neurones. Neuroreport 11:1203-1208.
Mony L, Krzaczkowski L, Leonetti M, Le Goff A, Alarcon K, Neyton J, Bertrand HO, Acher F, Paoletti P (2009) Structural basis of NR2B-selective antagonist recognition by N-methyl-D-aspartate receptors. Mol Pharmacol 75:60-74.
Monyer H, Seeburg PH, Wisden W (1991) Glutamate-operated channels: developmentally early and mature forms arise by alternative splicing. Neuron 6:799-810.
Monyer H, Burnashev N, Laurie DJ, Sakmann B, Seeburg PH (1994) Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. Neuron 12:529-540.
Morales B, Choi SY, Kirkwood A (2002) Dark rearing alters the development of GABAergic transmission in visual cortex. J Neurosci 22:8084-8090.
Morishita W, Lu W, Smith GB, Nicoll RA, Bear MF, Malenka RC (2007) Activation of NR2B-containing NMDA receptors is not required for NMDA receptor-dependent long-term depression. Neuropharmacology 52:71-76.
Morris RG, Anderson E, Lynch GS, Baudry M (1986) Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. Nature 319:774-776.
Moser EI, Krobert KA, Moser MB, Morris RG (1998) Impaired spatial learning after saturation of long-term potentiation. Science 281:2038-2042.
Mower GD (1991) The effect of dark rearing on the time course of the critical period in cat visual cortex. Brain Res Dev Brain Res 58:151-158.
Nader K, Einarsson EO (2010) Memory reconsolidation: an update. Ann N Y Acad Sci 1191:27-41.
Nader K, Schafe GE, LeDoux JE (2000) The labile nature of consolidation theory. Nat Rev Neurosci 1:216-219.
Notomi T, Shigemoto R (2004) Immunohistochemical localization of Ih channel subunits, HCN1-4, in the rat brain. J Comp Neurol 471:241-276.
O'Dell TJ, Kandel ER (1994) Low-frequency stimulation erases LTP through an NMDA receptor-mediated activation of protein phosphatases. Learn Mem 1:129-139.
Oh MC, Derkach VA (2005) Dominant role of the GluR2 subunit in regulation of AMPA receptors by CaMKII. Nat Neurosci 8:853-854.
Oliet SH, Malenka RC, Nicoll RA (1997) Two distinct forms of long-term depression coexist in CA1 hippocampal pyramidal cells. Neuron 18:969-982.
Pastalkova E, Serrano P, Pinkhasova D, Wallace E, Fenton AA, Sacktor TC (2006) Storage of spatial information by the maintenance mechanism of LTP. Science 313:1141-1144.

Perez-Otano I, Schulteis CT, Contractor A, Lipton SA, Trimmer JS, Sucher NJ, Heinemann SF (2001) Assembly with the NR1 subunit is required for surface expression of NR3A-containing NMDA receptors. J Neurosci 21:1228-1237.
Petersen CC (2007) The functional organization of the barrel cortex. Neuron 56:339-355.
Petersen CC, Sakmann B (2000) The excitatory neuronal network of rat layer 4 barrel cortex. J Neurosci 20:7579-7586.
Petersen CC, Sakmann B (2001) Functionally independent columns of rat somatosensory barrel cortex revealed with voltage-sensitive dye imaging. J Neurosci 21:8435-8446.
Petersen CC, Grinvald A, Sakmann B (2003a) Spatiotemporal dynamics of sensory responses in layer 2/3 of rat barrel cortex measured in vivo by voltage-sensitive dye imaging combined with whole-cell voltage recordings and neuron reconstructions. J Neurosci 23:1298-1309.
Petersen CC, Hahn TT, Mehta M, Grinvald A, Sakmann B (2003b) Interaction of sensory responses with spontaneous depolarization in layer 2/3 barrel cortex. Proc Natl Acad Sci U S A 100:13638-13643.
Petralia RS, Yokotani N, Wenthold RJ (1994) Light and electron microscope distribution of the NMDA receptor subunit NMDAR1 in the rat nervous system using a selective anti-peptide antibody. J Neurosci 14:667-696.
Petreanu L, Huber D, Sobczyk A, Svoboda K (2007) Channelrhodopsin-2-assisted circuit mapping of long-range callosal projections. Nat Neurosci 10:663-668.
Petreanu L, Mao T, Sternson SM, Svoboda K (2009) The subcellular organization of neocortical excitatory connections. Nature 457:1142-1145.
Philpot BD, Espinosa JS, Bear MF (2003) Evidence for altered NMDA receptor function as a basis for metaplasticity in visual cortex. J Neurosci 23:5583-5588.
Philpot BD, Cho KK, Bear MF (2007) Obligatory role of NR2A for metaplasticity in visual cortex. Neuron 53:495-502.
Philpot BD, Sekhar AK, Shouval HZ, Bear MF (2001) Visual experience and deprivation bidirectionally modify the composition and function of NMDA receptors in visual cortex. Neuron 29:157-169.
Pickel VM, Colago EE, Mania I, Molosh AI, Rainnie DG (2006) Dopamine D1 receptors co-distribute with N-methyl-D-aspartic acid type-1 subunits and modulate synaptically-evoked N-methyl-D-aspartic acid currents in rat basolateral amygdala. Neuroscience 142:671-690.
Pisani A, Gubellini P, Bonsi P, Conquet F, Picconi B, Centonze D, Bernardi G, Calabresi P (2001) Metabotropic glutamate receptor 5 mediates the potentiation of N-methyl-D-aspartate responses in medium spiny striatal neurons. Neuroscience 106:579-587.
Pizzorusso T, Medini P, Berardi N, Chierzi S, Fawcett JW, Maffei L (2002) Reactivation of ocular dominance plasticity in the adult visual cortex. Science 298:1248-1251.
Plant K, Pelkey KA, Bortolotto ZA, Morita D, Terashima A, McBain CJ, Collingridge GL, Isaac JT (2006) Transient incorporation of native GluR2-lacking AMPA receptors during hippocampal long-term potentiation. Nat Neurosci 9:602-604.
Poo C, Isaacson JS (2007) An early critical period for long-term plasticity and structural modification of sensory synapses in olfactory cortex. J Neurosci 27:7553-7558.

Porter JT, Johnson CK, Agmon A (2001) Diverse types of interneurons generate thalamus-evoked feedforward inhibition in the mouse barrel cortex. J Neurosci 21:2699-2710.
Prybylowski K, Chang K, Sans N, Kan L, Vicini S, Wenthold RJ (2005) The synaptic localization of NR2B-containing NMDA receptors is controlled by interactions with PDZ proteins and AP-2. Neuron 47:845-857.
Putignano E, Lonetti G, Cancedda L, Ratto G, Costa M, Maffei L, Pizzorusso T (2007) Developmental downregulation of histone posttranslational modifications regulates visual cortical plasticity. Neuron 53:747-759.
Quinlan EM, Olstein DH, Bear MF (1999a) Bidirectional, experience-dependent regulation of N-methyl-D-aspartate receptor subunit composition in the rat visual cortex during postnatal development. Proc Natl Acad Sci U S A 96:12876-12880.
Quinlan EM, Philpot BD, Huganir RL, Bear MF (1999b) Rapid, experience-dependent expression of synaptic NMDA receptors in visual cortex in vivo. Nat Neurosci 2:352-357.
Rakhade SN, Zhou C, Aujla PK, Fishman R, Sucher NJ, Jensen FE (2008) Early alterations of AMPA receptors mediate synaptic potentiation induced by neonatal seizures. J Neurosci 28:7979-7990.
Reyes A, Sakmann B (1999) Developmental switch in the short-term modification of unitary EPSPs evoked in layer 2/3 and layer 5 pyramidal neurons of rat neocortex. J Neurosci 19:3827-3835.
Roberts AC, Diez-Garcia J, Rodriguiz RM, Lopez IP, Lujan R, Martinez-Turrillas R, Pico E, Henson MA, Bernardo DR, Jarrett TM, Clendeninn DJ, Lopez-Mascaraque L, Feng G, Lo DC, Wesseling JF, Wetsel WC, Philpot BD, Perez-Otano I (2009) Downregulation of NR3A-containing NMDARs is required for synapse maturation and memory consolidation. Neuron 63:342-356.
Roberts EB, Ramoa AS (1999) Enhanced NR2A subunit expression and decreased NMDA receptor decay time at the onset of ocular dominance plasticity in the ferret. J Neurophysiol 81:2587-2591.
Rodriguez-Moreno A, Paulsen O (2008) Spike timing-dependent long-term depression requires presynaptic NMDA receptors. Nat Neurosci 11:744-745.
Rozas C, Frank H, Heynen AJ, Morales B, Bear MF, Kirkwood A (2001) Developmental inhibitory gate controls the relay of activity to the superficial layers of the visual cortex. J Neurosci 21:6791-6801.
Rumpel S, LeDoux J, Zador A, Malinow R (2005) Postsynaptic receptor trafficking underlying a form of associative learning. Science 308:83-88.
Sacchetti B, Lorenzini CA, Baldi E, Bucherelli C, Roberto M, Tassoni G, Brunelli M (2001) Long-lasting hippocampal potentiation and contextual memory consolidation. Eur J Neurosci 13:2291-2298.
Sacco T, Sacchetti B (2010) Role of secondary sensory cortices in emotional memory storage and retrieval in rats. Science 329:649-656.
Sanes JR, Lichtman JW (1999) Development of the vertebrate neuromuscular junction. Annu Rev Neurosci 22:389-442.
Sans N, Vissel B, Petralia RS, Wang YX, Chang K, Royle GA, Wang CY, O'Gorman S, Heinemann SF, Wenthold RJ (2003) Aberrant formation of glutamate receptor

complexes in hippocampal neurons of mice lacking the GluR2 AMPA receptor subunit. J Neurosci 23:9367-9373.
Sasaki YF, Rothe T, Premkumar LS, Das S, Cui J, Talantova MV, Wong HK, Gong X, Chan SF, Zhang D, Nakanishi N, Sucher NJ, Lipton SA (2002) Characterization and comparison of the NR3A subunit of the NMDA receptor in recombinant systems and primary cortical neurons. J Neurophysiol 87:2052-2063.
Sawtell NB, Frenkel MY, Philpot BD, Nakazawa K, Tonegawa S, Bear MF (2003) NMDA receptor-dependent ocular dominance plasticity in adult visual cortex. Neuron 38:977-985.
Schubert D, Kotter R, Zilles K, Luhmann HJ, Staiger JF (2003) Cell type-specific circuits of cortical layer IV spiny neurons. J Neurosci 23:2961-2970.
Scimemi A, Fine A, Kullmann DM, Rusakov DA (2004) NR2B-containing receptors mediate cross talk among hippocampal synapses. J Neurosci 24:4767-4777.
Shah MM, Hammond RS, Hoffman DA (2010) Dendritic ion channel trafficking and plasticity. Trends Neurosci.
Shatz CJ, Stryker MP (1978) Ocular dominance in layer IV of the cat's visual cortex and the effects of monocular deprivation. J Physiol 281:267-283.
Shema R, Sacktor TC, Dudai Y (2007) Rapid erasure of long-term memory associations in the cortex by an inhibitor of PKM zeta. Science 317:951-953.
Shen H, Moussawi K, Zhou W, Toda S, Kalivas PW (2011) Heroin relapse requires long-term potentiation-like plasticity mediated by NMDA2b-containing receptors. Proc Natl Acad Sci U S A 108:19407-19412.
Sheng M, Lee SH (2001) AMPA receptor trafficking and the control of synaptic transmission. Cell 105:825-828.
Sheng M, Cummings J, Roldan LA, Jan YN, Jan LY (1994) Changing subunit composition of heteromeric NMDA receptors during development of rat cortex. Nature 368:144-147.
Shepherd GM, Svoboda K (2005) Laminar and columnar organization of ascending excitatory projections to layer 2/3 pyramidal neurons in rat barrel cortex. J Neurosci 25:5670-5679.
Shepherd GM, Pologruto TA, Svoboda K (2003) Circuit analysis of experience-dependent plasticity in the developing rat barrel cortex. Neuron 38:277-289.
Shi SH, Hayashi Y, Petralia RS, Zaman SH, Wenthold RJ, Svoboda K, Malinow R (1999) Rapid spine delivery and redistribution of AMPA receptors after synaptic NMDA receptor activation. Science 284:1811-1816.
Shoykhet M, Land PW, Simons DJ (2005) Whisker trimming begun at birth or on postnatal day 12 affects excitatory and inhibitory receptive fields of layer IV barrel neurons. J Neurophysiol 94:3987-3995.
Siegel SJ, Brose N, Janssen WG, Gasic GP, Jahn R, Heinemann SF, Morrison JH (1994) Regional, cellular, and ultrastructural distribution of N-methyl-D-aspartate receptor subunit 1 in monkey hippocampus. Proc Natl Acad Sci U S A 91:564-568.
Silva AJ, Paylor R, Wehner JM, Tonegawa S (1992) Impaired spatial learning in alpha-calcium-calmodulin kinase II mutant mice. Science 257:206-211.

Simons DJ (1978) Response properties of vibrissa units in rat SI somatosensory neocortex. J Neurophysiol 41:798-820.
Simons DJ (1985) Temporal and spatial integration in the rat SI vibrissa cortex. J Neurophysiol 54:615-635.
Simons DJ, Land PW (1987) Early experience of tactile stimulation influences organization of somatic sensory cortex. Nature 326:694-697.
Sjostrom PJ, Turrigiano GG, Nelson SB (2003) Neocortical LTD via coincident activation of presynaptic NMDA and cannabinoid receptors. Neuron 39:641-654.
Smith GB, Heynen AJ, Bear MF (2009) Bidirectional synaptic mechanisms of ocular dominance plasticity in visual cortex. Philos Trans R Soc Lond B Biol Sci 364:357-367.
Southwell DG, Froemke RC, Alvarez-Buylla A, Stryker MP, Gandhi SP (2010) Cortical plasticity induced by inhibitory neuron transplantation. Science 327:1145-1148.
Sprengel R, Suchanek B, Amico C, Brusa R, Burnashev N, Rozov A, Hvalby O, Jensen V, Paulsen O, Andersen P, Kim JJ, Thompson RF, Sun W, Webster LC, Grant SG, Eilers J, Konnerth A, Li J, McNamara JO, Seeburg PH (1998) Importance of the intracellular domain of NR2 subunits for NMDA receptor function in vivo. Cell 92:279-289.
Standaert DG, Landwehrmeyer GB, Kerner JA, Penney JB, Jr., Young AB (1996) Expression of NMDAR2D glutamate receptor subunit mRNA in neurochemically identified interneurons in the rat neostriatum, neocortex and hippocampus. Brain Res Mol Brain Res 42:89-102.
Staubli U, Lynch G (1990) Stable depression of potentiated synaptic responses in the hippocampus with 1-5 Hz stimulation. Brain Res 513:113-118.
Staubli U, Chun D (1996) Factors regulating the reversibility of long-term potentiation. J Neurosci 16:853-860.
Stern EA, Maravall M, Svoboda K (2001) Rapid development and plasticity of layer 2/3 maps in rat barrel cortex in vivo. Neuron 31:305-315.
Stocca G, Vicini S (1998) Increased contribution of NR2A subunit to synaptic NMDA receptors in developing rat cortical neurons. J Physiol 507 (Pt 1):13-24.
Suarez LM, Solis JM (2006) Taurine potentiates presynaptic NMDA receptors in hippocampal Schaffer collateral axons. Eur J Neurosci 24:405-418.
Suarez LM, Suarez F, Del Olmo N, Ruiz M, Gonzalez-Escalada JR, Solis JM (2005) Presynaptic NMDA autoreceptors facilitate axon excitability: a new molecular target for the anticonvulsant gabapentin. Eur J Neurosci 21:197-209.
Sugiyama S, Di Nardo AA, Aizawa S, Matsuo I, Volovitch M, Prochiantz A, Hensch TK (2008) Experience-dependent transfer of Otx2 homeoprotein into the visual cortex activates postnatal plasticity. Cell 134:508-520.
Sun HY, Bartley AF, Dobrunz LE (2009) Calcium-permeable presynaptic kainate receptors involved in excitatory short-term facilitation onto somatostatin interneurons during natural stimulus patterns. J Neurophysiol 101:1043-1055.
Sun QQ, Huguenard JR, Prince DA (2006) Barrel cortex microcircuits: thalamocortical feedforward inhibition in spiny stellate cells is mediated by a small number of fast-spiking interneurons. J Neurosci 26:1219-1230.

Sutton MA, Ito HT, Cressy P, Kempf C, Woo JC, Schuman EM (2006) Miniature neurotransmission stabilizes synaptic function via tonic suppression of local dendritic protein synthesis. Cell 125:785-799.
Swadlow HA (2002) Thalamocortical control of feed-forward inhibition in awake somatosensory 'barrel' cortex. Philos Trans R Soc Lond B Biol Sci 357:1717-1727.
Swadlow HA (2003) Fast-spike interneurons and feedforward inhibition in awake sensory neocortex. Cereb Cortex 13:25-32.
Swanson GT, Kamboj SK, Cull-Candy SG (1997) Single-channel properties of recombinant AMPA receptors depend on RNA editing, splice variation, and subunit composition. J Neurosci 17:58-69.
Takahashi T, Svoboda K, Malinow R (2003) Experience strengthening transmission by driving AMPA receptors into synapses. Science 299:1585-1588.
Tang YP, Shimizu E, Dube GR, Rampon C, Kerchner GA, Zhuo M, Liu G, Tsien JZ (1999) Genetic enhancement of learning and memory in mice. Nature 401:63-69.
Thiagarajan TC, Lindskog M, Tsien RW (2005) Adaptation to synaptic inactivity in hippocampal neurons. Neuron 47:725-737.
Tononi G, Cirelli C (2003) Sleep and synaptic homeostasis: a hypothesis. Brain Res Bull 62:143-150.
Tovar KR, Westbrook GL (1999) The incorporation of NMDA receptors with a distinct subunit composition at nascent hippocampal synapses in vitro. J Neurosci 19:4180-4188.
Tovar KR, Westbrook GL (2002) Mobile NMDA receptors at hippocampal synapses. Neuron 34:255-264.
Tsuzuki K, Lambolez B, Rossier J, Ozawa S (2001) Absolute quantification of AMPA receptor subunit mRNAs in single hippocampal neurons. J Neurochem 77:1650-1659.
Van der Loos H, Woolsey TA (1973) Somatosensory cortex: structural alterations following early injury to sense organs. Science 179:395-398.
Veinante P, Deschenes M (1999) Single- and multi-whisker channels in the ascending projections from the principal trigeminal nucleus in the rat. J Neurosci 19:5085-5095.
Villarreal DM, Do V, Haddad E, Derrick BE (2002) NMDA receptor antagonists sustain LTP and spatial memory: active processes mediate LTP decay. Nat Neurosci 5:48-52.
Vyazovskiy VV, Cirelli C, Pfister-Genskow M, Faraguna U, Tononi G (2008) Molecular and electrophysiological evidence for net synaptic potentiation in wake and depression in sleep. Nat Neurosci 11:200-208.
Wagner JJ, Alger BE (1995) GABAergic and developmental influences on homosynaptic LTD and depotentiation in rat hippocampus. J Neurosci 15:1577-1586.
Walker HC, Lawrence JJ, McBain CJ (2002) Activation of kinetically distinct synaptic conductances on inhibitory interneurons by electrotonically overlapping afferents. Neuron 35:161-171.
Walker MP, Brakefield T, Hobson JA, Stickgold R (2003) Dissociable stages of human memory consolidation and reconsolidation. Nature 425:616-620.

Wallace H, Fox K (1999a) The effect of vibrissa deprivation pattern on the form of plasticity induced in rat barrel cortex. Somatosens Mot Res 16:122-138.
Wallace H, Fox K (1999b) Local cortical interactions determine the form of cortical plasticity. J Neurobiol 41:58-63.
Wang CC, Held RG, Chang SC, Yang L, Delpire E, Ghosh A, Hall BJ (2011) A critical role for GluN2B-containing NMDA receptors in cortical development and function. Neuron 72:789-805.
Washburn MS, Dingledine R (1996) Block of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors by polyamines and polyamine toxins. J Pharmacol Exp Ther 278:669-678.
Waxman EA, Lynch DR (2005) N-methyl-D-aspartate receptor subtypes: multiple roles in excitotoxicity and neurological disease. Neuroscientist 11:37-49.
Welker WI, Johnson JI, Jr., Pubols BH, Jr. (1964) Some Morphological and Physiological Characteristics of the Somatic Sensory System in Raccoons. Am Zool 4:75-94.
Wen JA, Barth AL (2011) Input-specific critical periods for experience-dependent plasticity in layer 2/3 pyramidal neurons. J Neurosci 31:4456-4465.
Wen JA, Barth AL (2012) Synaptic lability after experience-dependent plasticity is not mediated by calcium-permeable AMPARs. Front Mol Neurosci 5:15.
Wenthold RJ, Petralia RS, Blahos J, II, Niedzielski AS (1996) Evidence for multiple AMPA receptor complexes in hippocampal CA1/CA2 neurons. J Neurosci 16:1982-1989.
Whitlock JR, Heynen AJ, Shuler MG, Bear MF (2006) Learning induces long-term potentiation in the hippocampus. Science 313:1093-1097.
Wiesel TN, Hubel DH (1963) Single-Cell Responses in Striate Cortex of Kittens Deprived of Vision in One Eye. J Neurophysiol 26:1003-1017.
Wiesel TN, Hubel DH (1965) Comparison of the effects of unilateral and bilateral eye closure on cortical unit responses in kittens. J Neurophysiol 28:1029-1040.
Wiltgen BJ, Royle GA, Gray EE, Abdipranoto A, Thangthaeng N, Jacobs N, Saab F, Tonegawa S, Heinemann SF, O'Dell TJ, Fanselow MS, Vissel B (2010) A role for calcium-permeable AMPA receptors in synaptic plasticity and learning. PLoS One 5.
Wisden W, Seeburg PH (1993) Mammalian ionotropic glutamate receptors. Curr Opin Neurobiol 3:291-298.
Wong HK, Liu XB, Matos MF, Chan SF, Perez-Otano I, Boysen M, Cui J, Nakanishi N, Trimmer JS, Jones EG, Lipton SA, Sucher NJ (2002) Temporal and regional expression of NMDA receptor subunit NR3A in the mammalian brain. J Comp Neurol 450:303-317.
Woolsey TA, Van der Loos H (1970) The structural organization of layer IV in the somatosensory region (SI) of mouse cerebral cortex. The description of a cortical field composed of discrete cytoarchitectonic units. Brain Res 17:205-242.
Wright N, Glazewski S, Hardingham N, Phillips K, Pervolaraki E, Fox K (2008) Laminar analysis of the role of GluR1 in experience-dependent and synaptic depression in barrel cortex. Nat Neurosci 11:1140-1142.
Wu G, Malinow R, Cline HT (1996) Maturation of a central glutamatergic synapse. Science 274:972-976.

Xu-Friedman MA, Regehr WG (1999) Presynaptic strontium dynamics and synaptic transmission. Biophys J 76:2029-2042.
Xu L, Anwyl R, Rowan MJ (1998) Spatial exploration induces a persistent reversal of long-term potentiation in rat hippocampus. Nature 394:891-894.
Yang J, Woodhall GL, Jones RS (2006) Tonic facilitation of glutamate release by presynaptic NR2B-containing NMDA receptors is increased in the entorhinal cortex of chronically epileptic rats. J Neurosci 26:406-410.
Yang Y, Wang XB, Zhou Q (2010) Perisynaptic GluR2-lacking AMPA receptors control the reversibility of synaptic and spines modifications. Proc Natl Acad Sci U S A 107:11999-12004.
Yashiro K, Philpot BD (2008) Regulation of NMDA receptor subunit expression and its implications for LTD, LTP, and metaplasticity. Neuropharmacology 55:1081-1094.
Yazaki-Sugiyama Y, Kang S, Cateau H, Fukai T, Hensch TK (2009) Bidirectional plasticity in fast-spiking GABA circuits by visual experience. Nature 462:218-221.
Zhao MG, Toyoda H, Lee YS, Wu LJ, Ko SW, Zhang XH, Jia Y, Shum F, Xu H, Li BM, Kaang BK, Zhuo M (2005) Roles of NMDA NR2B subtype receptor in prefrontal long-term potentiation and contextual fear memory. Neuron 47:859-872.
Zhao Y, Rubio M, Tzounopoulos T (2011) Mechanisms underlying input-specific expression of endocannabinoid-mediated synaptic plasticity in the dorsal cochlear nucleus. Hear Res 279:67-73.
Zhou Q, Tao HW, Poo MM (2003) Reversal and stabilization of synaptic modifications in a developing visual system. Science 300:1953-1957.
Zhu JJ (2009) Activity level-dependent synapse-specific AMPA receptor trafficking regulates transmission kinetics. J Neurosci 29:6320-6335.
Zhu JJ, Esteban JA, Hayashi Y, Malinow R (2000) Postnatal synaptic potentiation: delivery of GluR4-containing AMPA receptors by spontaneous activity. Nat Neurosci 3:1098-1106.
Zhuo M, Zhang W, Son H, Mansuy I, Sobel RA, Seidman J, Kandel ER (1999) A selective role of calcineurin aalpha in synaptic depotentiation in hippocampus. Proc Natl Acad Sci U S A 96:4650-4655.
Zilberter M, Holmgren C, Shemer I, Silberberg G, Grillner S, Harkany T, Zilberter Y (2009) Input specificity and dependence of spike timing-dependent plasticity on preceding postsynaptic activity at unitary connections between neocortical layer 2/3 pyramidal cells. Cereb Cortex 19:2308-2320.