UNIVERSITY OF CALIFORNIA, SAN DIEGO

A novel critical period for inhibitory plasticity in rat somatosensory cortex

A dissertation submitted in partial satisfaction of the

requirements for the degree Doctor of Philosophy

in

Neurosciences

by

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2011

The Dissertation of Renna J Stevens is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

Chair

University of California, San Diego

2011

This thesis is dedicated to my husband, Jason Wolfe, who has been everything to me. The depth of his love, tenacity of his devotion, and strength of his character are treasures, and truths unquestionable.

If at first you don’t succeed, try, try again.

~Anonymous

Table of Contents

List of Figures and Tables

Acknowledgments

There is not adequate space to express the whole of my gratitude to those who've helped me come this far in my scientific career. Everywhere that I've worked and studied I have had the blessing of countless friends and colleagues lend cherished advice and assistance. I must first thank all of the scientists that I worked with in the Benes and Raines labs at Harvard, as well as my outstanding neuroscience professors at Smith College, for preparing me for the challenge of graduate school. When I began at UCSD, I was amazed at the involvement and support on the part of the faculty for each graduate student. Every faculty member that I have turned to for help, particularly the individuals on my thesis committee, have always been there for me. I was also fortunate enough to enter amongst a class full of fantastic geniuses, each one eager to help out with a problem, or read papers together. I was equally astounded to encounter upperclassmen that would drop their own work to answer questions, or help solve technical problems. Only a few of these comrades include my former labmates DJ Brasier, Kevin and Vanessa Bender, as well as schoolmates Martha Bagnall, Will Barkis, and Aaron Blankenship. Justin Elstrott deserves a huge thanks for being such a spectacular labmate, as well as for his exceedingly patient help with *Igor*. Of course, where would I be without my awesome histology team: Jason Chung, Olivia Pourzia, and Susanna Fenstermacher? You guys are extraordinarily talented and helpful!

In addition to thoroughly enjoying his field of study, I fought hard to join Dan Feldman's lab because I had a suspicion that I wouldn't find a better advisor anywhere I looked. I turned out to be absolutely right. It mustn't be easy seeing one's graduate

student embark on, and terminate, at least 4 independent projects in as many years. Dan remained an unfailingly supportive, generous, optimistic, and technically helpful *mentor*, in the purest sense of the word, throughout the years and trials. Also worth noting is Dan's impressive humility, despite the mental warp speed at which he operates, and from this we can all learn. His office door is always open, and always welcoming. For all of this and more, I owe him a debt of gratitude that could not be adequately repaid.

I wish to convey my thanks to my family, but don't really know how. My mom, dad, and brother have had more faith in me than I've had in myself. Their love and undying support have been gifts without which I could never have even embarked on something like a PhD, and I couldn't ask for a more loving family to come home to. A special thanks goes to my nephew, who is unequivocally the coolest kid in the world. I've also had the blessing of acquiring two incredible sisters and parents-in-law this year, who deserve my most sincere thanks for welcoming me into their family with such open arms, and for their undying support and generosity.

In addition to his help with a few power tools, my husband Jason has given me the kind of inspiration, both as a scientist and a partner, that practically knocks you over. I truly believe that meeting him was one of the fateful reasons I wound up in the Feldman lab. In our last few years apart, his countless patient hours of listening to me complain over the phone about how I will never get through school, I think have helped me a lot to get through school. Jason miraculously makes me feel like I can truly accomplish anything, and he's nothing short of the best husband a man could be.

Well, I'm about to do it. I have to thank God (a lot). There, I did it.

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Awards

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ABSTRACT OF THE DISSERTATION

A novel critical period for inhibitory plasticity in somatosensory cortex

by

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Doctor of Philosophy in Neurosciences

University of California, San Diego, 2011

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The cerebral cortex encodes sensory information with astonishing precision, but it is also confronted with the impressive task of reworking and rewiring its physiology in the face of a changing environment. Hubel and Weisel first characterized the impact of sensory deprivation on the development of cortical response properties, but there is still much we do not know about which forms of cortical plasticity are induced with sensory deprivation, as well as which cell types and synapses mediate plasticity. While traditional models of cortical plasticity proposed Hebbian ("use it or lose it") rules in excitatory circuits as the primary substrate for cortical plasticity, recent advances to the classical model include an important role for non-Hebbian forms of plasticity, and show that inhibitory circuits are a major site of sensory plasticity. A precisely regulated balance between cortical excitation and inhibition is crucial for sensory processing and plasticity, but our understanding of inhibitory synapse development is lacking. Here we investigate the impact of sensory

experience on the development and function of inhibitory synapses in rat primary somatosensory cortex.

I deprived the D-row of rat whiskers (beginning on the 7th postnatal day, P7) in order to probe how experience guides inhibitory synapse development. I found that deprivation reduced inhibitory currents at P15 in layer (L) 4 and at P21 in L2/3. Evoked inhibition was also reduced at P15 in L4. This reduction in inhibition constitutes a homeostatic form of plasticity, as it would ultimately increase excitatory activity in response to sensory deprivation. Surprisingly, inhibitory currents recovered to control (spared) levels after this one-day period.

Our findings demonstrate that the development of inhibitory signaling in S1 during the first postnatal month occurs in a largely experience-*independent* fashion, but that sensory deprivation during this period causes a delayed and transient reduction in the efficacy of inhibitory signaling. Our results also reveal that these transient changes in mIPSC amplitude and frequency can be dissociated, meaning that they are mechanistically independent. These results add to the growing body of evidence that inhibitory circuits undergo homeostatic plasticity in response to sensory use and disuse in primary sensory cortex.

I. Introduction

The mammalian brain is remarkably capable of responding to changes in the sensory environment with alterations in cellular function. Indeed, the brain's ability to undergo such adaptive changes is responsible for no less than its feat of life-long learning and memory. One robust example of such brain plasticity is the case in which normal sensory input is altered. A wealth of research shows that sensory deprivation, especially when occurring during development, can lead to marked changes in the behavior of specific nerve cells and circuits in cortical regions of the brain that sub serve that particular sensory modality. While this field of neuroscience has come far in its study of the various cortical changes that can occur in the face of altered sensory input, our understanding of these changes is far from complete. It is valuable that we continue to pursue study of the conditions, parameters, and mechanisms with which the brain undergoes such adjustments in order to further our understanding of cortical plasticity and neurological disorders that are related to cortical development and plasticity, such as autism, epilepsy, and mental retardation.

Classic studies of sensory deprivation-induced plasticity began in the early 1960's with David Hubel and Torsten Wiesel's discovery of the effects of single-eye visual deprivation on ocular dominance columns in the primary visual cortex (V1) of kittens. Their work showed that, within a fixed window of time termed the "critical period", neurons would predominantly shift their ocular dominance preference to inputs from the non-deprived eye (Wiesel and Hubel, 1963), and provided the foundation for a model of cortical plasticity in which the representations of underused

(deprived) sensory inputs shrink, and those of overused (spared) inputs expand. This model of changes reflects classical *use it or lose it* "Hebbian" cortical plasticity, after the scientist Donald Hebb, who postulated that correlated firing between pre and postsynaptic neurons would lead to a strengthening of their connections (i.e. *neurons that fire together, wire together*, reviewed in Cooper 2005). Investigations have corroborated this model in other cortical systems such as audition and somatosensation. Selective exposure to particular sound frequencies during auditory development can lead to an expansion of isofrequency band within primary auditory cortex (A1) devoted to that frequency (Zhang et al. 2001). Somatosensory deprivation in the form of rodent whisker removal causes a robust weakening of the cortical representation of deprived inputs in the primary somatosensory cortex (S1), as well as a concomitant expansion of spared neighboring whisker input representations (Fox 1992, Diamond et al. 1993, Glazewski and Fox 1996).

This map plasticity reflects changes in individual cortical neuron receptive fields from altered sensory experience, and changes in neuronal receptive fields can represent a variety of changes at the cellular and synaptic level within both excitatory and inhibitory circuits. Studies probing these cellular and synaptic substrates have evidenced that Hebbian synaptic processes such as long-term synaptic potentiation and depression (LTP and LTD) and depression of excitatory feedforward circuitry likely contribute to this plasticity (Kirkwood and Bear 1994, Bear et al. 1987, Buonomano and Merzenich 1998, Rittenhouse et al. 1999, Allen et al. 2003). But in addition to Hebbian learning rules for excitatory plasticity, unfolding research over the last two decades has shown the situation to be much more complex than this single model, with

multiple forms of plasticity at many cellular and synaptic locations now known (Turrigiano and Nelson 2004, Hensch 2005, Feldman 2009, Fig. 1.1). As will be discussed below, studies have revealed an increasing montage of cellular and synaptic plasticity forms that can be recruited in response to altered sensory input, often simultaneously, and work in concert to produce intricate changes in cortical function. Our current understanding of these forms of plasticity is highly incomplete, and a paramount challenge that remains is to understand how, why, and when exactly which forms are employed.

Plasticity of cortical inhibitory circuitry

The relay of excitatory sensory information throughout cortical layers involves complex processing steps, where it is transformed and regulated by different types of GABAergic interneurons. Faithful sensory processing within cortical circuits relies on a delicate balance in both the relative timing (Pouille and Scanziani 2004, Gabernet et al. 2005, Wilent and Contreras 2005) and magnitude (Wehr and Zador 2003) of excitation and inhibition, and this balance sharpens sensory tuning and enforces spike timing precision. Two main classes of GABAergic cells include fast-spiking (FS) and regular-spiking (RS) interneurons, which mediate feedforward and feedback inhibitory signaling, in certain layers of the cortex (Fig. 1.2, Beierlein et al. 2003, Gabernet et al. 2005, Sun et al. 2006, Kapfer et al. 2007, Shao and Burkhalter 1996).

There has historically been a predominant focus on the experience-dependent plasticity of thalamocortical and intracortical excitatory circuitry (Sur and Leamey 2001), and until more recently, little was known about the impact of sensory

deprivation on cortical inhibition. Myriad activity-dependent changes that take place in cortical inhibitory circuitry have now been revealed in multiple sensory areas (Maffei and Turrigiano 2008, Sun 2007). For example, visual deprivation reduces GABAergic inhibition in V1 both during cortical development and in adult animals (Benevento et al. 1992, Morales et al. 2002, Chattopadhyaya et al. 2004). Additionally, a prolific series of experiments in rat visual cortex has revealed an impressive variety of changes in inhibitory circuit physiology following deprivation during early postnatal development. Depending on the particular inhibitory cell type, cortical layer, and time of deprivation relative to the critical period, inhibitory circuits were modified in different ways by visual deprivation (see discussion, Maffei et al. 2004, Maffei et al. 2006, Maffei et al. 2010).

In rodent somatosensory cortex, prolonged whisker deprivation (~3wk-3mo) is known to weaken inhibitory receptive fields in adult animals (Shoykhet et al. 2005, Lee et al. 2007). Also, many studies have shown that sensory manipulations alter GABA levels and GABA receptor expression in the adult animal: sensory deprivation reduces GABA function, and sensory training (e.g. appetitive conditioning or 24-hr whisker stimulation) increases GABA function (see Foeller and Feldman 2004 for review). However, there is less known about the impact of whisker deprivation on the *development* of inhibition in S1. A couple of different studies have reported a reduced number of L4 GABAergic synaptic terminals after two months of sensory deprivation (Micheva and Beaulieu 1995, Sadaka et al. 2003), and depressed inhibitory synapse strength in L4 after one month of whisker deprivation (Jiao et al. 2006, Sun 2009). However, these latter studies have serious technical shortcomings, leaving open the

question of whether or not inhibitory circuitry is susceptible to experience-dependent plasticity during S1 development.

Critical periods for sensory plasticity

A great deal of effort has been made to understand the factors that trigger sensory cortical plasticity, and those that influence its characteristics such as onset, duration, intracortical location, and sign. Not the least of such factors is the developmental stage of an animal when sensory experience is manipulated. Critical periods are discrete windows of time during which environmental manipulations can induce plasticity, and are well established for properties of many sensory systems. For example, the ability of visual circuitry to rewire during ocular dominance plasticity requires that visual deprivation take place during a developmental window beginning roughly at the third postnatal week (in rodents and kittens), and ending after approximately the fifth postnatal week, around postnatal day (P) 45 (Hubel and Weisel 1970, Fagiolini et al. 1994, Gordon and Stryker 1996). Similarly, tonotopic maps in auditory cortex are susceptible to dramatic refinement from selective exposure to particular frequencies during the time frame between P16 and P50 in rodents (Zhang et al. 2001, Chang and Merzenich 2003), but distinct critical periods also exist in A1 for different features of sound stimuli (Insanally et al. 2009).

In the rodent somatosensory system, sensory input from individual whiskers is represented in the cortex in corresponding individual cortical columns, marked by dense neuronal aggregates in L4 called “barrels”. This whisker-to-cortical column relay forms a faithful cortical map of the whiskers. Plasticity of this map involves

changes in receptive fields of neurons within a cortical column, as measured by single-unit recordings, in response to sensory manipulations. The classic critical period for sensory-deprivation whisker map plasticity in L4 was first described in Fox 1992, where whisker removal initiated only between P0-P5 resulted in altered whisker responses (measured >P30). However, other groups have provided evidence for the retention of L4 plasticity into adulthood under certain conditions (Diamond et al. 1993, Polley et al. 1999). In L2/3, P12-P14 has been shown to represent a critical period for plasticity of subthreshold (synaptic) pyramidal cell receptive field structure (Stern et al. 2001). However, deprivation-induced weakening of whisker responses in L2/3 are observed until P60, after which further deprivation plasticity proceeds by strengthening responses to spared whiskers (Glazewski and Fox 1996). These 'critical periods' are not absolute, because plasticity can occur with somewhat different sensory manipulations, at older ages (Glazewski and Fox 1996).

Major efforts have been made to understand what are the cues that regulate critical periods, as well as what is the role of sensory experience in this regulation process, and a growing body of data implicates the maturation of inhibitory circuitry in triggering the onset of critical periods. Some of the strongest evidence for this argument comes from studies showing that manipulations of normal GABAergic signaling during development can cause bidirectional shifts in the onset of ocular dominance plasticity in visual cortex (for review, see Hensch 2005, Hensch 2004). The onset of visual critical period plasticity can be triggered earlier than normal by prematurely enhancing inhibitory function with the application of benzodiazepines after eye-opening, or through over-expression of BDNF (which promotes the

maturation of inhibitory interneurons) (Huang et al. 1999, Hanover et al. 1999). Conversely, when inhibition is prevented from maturing normally, such as in GAD-65 knock-out mice, ocular dominance plasticity is also prevented until normal inhibition is pharmacologically restored (Hensch et al. 1998).

As described above, critical periods exist for overall receptive field plasticity, but whether this represents critical periods for plasticity of excitatory or inhibitory circuits, or both, is largely unknown. Since we know that inhibitory circuitry is a common site of sensory cortical plasticity, and the proper development of cortical inhibition is necessary for the regulation of critical periods, one important question involves the impact of early sensory deprivation on the development of cortical inhibitory signaling. Specifically, does sensory deprivation early in life disturb the development of inhibitory transmission? This issue has been addressed in V1, where evidence exists for a critical period for inhibitory circuit plasticity after early visual deprivation. Visual deprivation in the third, but not fifth postnatal week disturbed the maturation of perisomatic inhibitory synapses in visual cortical organotypic cultures (Chattopadhyaya et al. 2004), and deprivation before, but not after, the onset of the classic critical period caused a depression of inhibitory synapses in V1 (Maffei et al. 2004, Maffei et al. 2010). Are inhibitory cells a common locus for critical period plasticity in other sensory cortices as well? A major gap in our understanding involves whether plasticity of inhibitory circuits occurs during S1 development, and whether it is confined to a critical period.

Classic model for experience-dependent cortical development

The complex role of activity in the regulation of critical periods, and in cortical development in general, has been investigated for many years. The predominant model describing cortical development is well-supported from studies in visual cortex, and asserts that sensory deprivation retards or prevents the normal course of cellular and circuit changes that occur during cortical development. This model argues that sensory deprivation effectively freezes cortical circuitry in an immature state, and suggests that sensory experience plays a permissive role in cortical maturation (Blasdel and Pettigrew 1978, Fagiolini et al. 1994, Bartoletti et al. 2004, Mower 1991, Iwai et al. 2003). For instance, in P60 rats, multiple visual cortical tuning properties (eg. receptive field size, orientation and direction selectivity), as well as visual acuity, were prevented from developing normally by either dark rearing or single eye suturing, and resembled the quality of those functions at P19-21 (Fagiolini et al. 1994). On the synaptic level, dark rearing during the second and third postnatal weeks prevented the normal progressive decline in the amplitudes of spontaneous miniature excitatory currents (Desai et al, 2002). Similar findings have been reported in primary auditory cortex, where rearing rat pups in continuous white noise delayed the refinement of receptive fields, as well as the proper topographic representation of tones (Chang and Merzenich 2003).

Evidence also exists for this model in the development of inhibitory transmission in V1, where dark rearing was shown to retard or prevent the maturation of inhibitory signaling (Benevento et a. 1995, Morales et al. 2002, Gianfranceschi et al. 2003, Chattopadhyaya et al. 2004). However, studies performed during early development in visual cortex (before the critical period), found that, depending on the

precise time (either P14-16 or P18-20), sensory deprivation also induces multiple plasticity mechanisms at inhibitory synapses that can be expressed in opposing directions (for review see Maffei and Turrigiano 2008). This suggests that the early development of inhibitory transmission is more complex than the single experience-dependent model that sensory deprivation freezes cortical circuits in an immature state.

The major question addressed in the present thesis is whether this model accurately describes the functional development of inhibition in other cortices besides V1, particularly in somatosensory cortex. A recent study in mouse S1 found that whisker-deprivation during the second postnatal week caused a reduction in the thalamocortical activation of L4 FS interneurons, but no change in FS inhibitory synapses onto excitatory cells was found (Chittajallu and Isaac, 2010). However, a series of experiments in mouse S1 reported that continuous whisker deprivation from P7-P30 reduced both the strength and number of inhibitory synapses in L4 of deprived cortical columns (Jiao et al. 2006, Sun 2009). Importantly, brain slices recorded in these two studies were prepared using an inappropriate plane of section that calls into question whether recordings were made correctly from deprived columns. Regardless of whether or not those data are correct, it is important to know whether the same results would be obtained in the rat, in order to compare these findings with other plasticity studies (which are mostly from rat). The results presented here address this question, and establish whether early development of inhibitory signaling in rat barrel cortex proceeds in an activity-dependent manner.

Homeostatic plasticity in cortex

At the outset of this study, it was unclear whether whisker sensory deprivation during S1 development would drive a net weakening of inhibition, (which was found during "pre-critical" period deprivation in Maffei et al. 2004 and 2010), a net strengthening of inhibition (as shown during "post-critical" period deprivation in Maffei et al. 2004 and 2010), or no change. While strengthening of inhibition may act to suppress responses to the deprived whisker, thereby promoting Hebbian map plasticity, weakening of inhibition could act to enhance responses to deprived inputs. This would be a compensatory or homeostatic mechanism that may restore mean cortical activity in response to deprivation. The following section discusses the function, prevalence, and known mechanisms for such homeostatic plasticity in neocortex.

Although results from many studies support the existence of traditional Hebbian mechanisms for cortical plasticity (Rittenhouse et al. 1999, Heynan et al. 2003, Allen et al. 2003, for review, Cruikshank and Weinberger 1996, Feldman et al. 1999, Malenka and Bear 2004), it has been argued that if Hebbian forces (which are fundamentally positive-feedback in nature, such at LTP and LTD) were solely at play, excitatory neural circuits could be driven in a positive feedback loop ultimately to overexcitation, or alternatively, quiescence (Marder and Goiallard 2006, Turrigiano and Nelson 2004, Fig. 1.3A, B). Mounting evidence over the past decade has revealed a toolkit of plasticity mechanisms that effectively drive neuronal excitation in a direction counter to that expected from Hebbian forces, exerting the putatively re-

stabilizing "homeostatic" effect of maintaining a threshold level of cellular activity (one example of these mechanisms is synaptic scaling, Fig. 1.3C).

Seminal descriptions of this form of plasticity drew from experiments in cell culture preparations, where neurons pharmacologically prevented from spiking were found to increase the strength of their excitatory synaptic contacts (Ramakers et al. 1990, Turrigiano et al. 1998). More recent studies have found robust examples of homeostatic changes in both excitatory and inhibitory components of cortical circuitry in both *in vitro* and *in vivo* preparations following sensory deprivation. For example, counterintuitive from a traditional Hebbian standpoint, brief periods of monocular deprivation in rodents caused an increase in the amplitude of spontaneous miniature excitatory post-synaptic currents (mEPSCs) in monocular V1 corresponding to the closed eye (Desai et al. 2002), as well as an increased net excitatory drive and decreased net inhibitory drive in L4 of visual cortex (Maffei et al. 2004).

As summarized above, substantial evidence indicates that cortical map plasticity involves cellular plasticity both in excitatory and inhibitory circuits, as well as regulation of critical periods by inhibitory circuits. A key issue therefore is whether and how experience regulates the development of inhibitory circuits. Recent work in V1 and A1 suggests that early sensory deprivation can either strengthen or weaken inhibition, depending on age and other factors, but there is less data from S1 development. Furthermore, the general hypothesis that has guided studies on this topic is that sensory deprivation impairs or altogether freezes the development of cortical circuitry, including inhibition. There is strong evidence for this from V1, but less so from S1 (and the evidence that does exist comes predominantly from mouse).

Due to fundamental physiological and anatomical differences between S1 and V1, and potential species differences, it remains unclear whether the proper development of inhibitory synapses in rat S1 is retarded by early sensory deprivation, or subject to other forms of sensory plasticity (either Hebbian or homeostatic). The present study addresses these specific questions. I found that deprivation causes delayed, transient reduction in inhibitory function. This reduction in inhibition is homeostatic (negative feedback) in nature, and provides additional evidence that opposing homeostatic and Hebbian plasticity coexists during experience-dependent map plasticity. However, surprisingly, this transient expression of plasticity lasted only 1-2 days, leaving the overall development of inhibitory synapses in S1 intact by the end of the first postnatal month, despite continued sensory deprivation.

S1 as a model system for studying sensory deprivation plasticity

It is important to probe various systems in the study of sensory cortical plasticity so that we can learn about differences unique to each. An additional motivation for studying S1 is that we know more about cellular plasticity mechanisms in this region than any other sensory area. Understanding how inhibitory circuits are altered by deprivation can be integrated with our growing understanding of how excitatory circuits are altered, to generate a more complete view of plasticity.

Fortunately the rodent somatosensory system provides a very tractable model for studying the effects of sensory deprivation on cellular and circuit properties. In this system, somatosensory information garnered by the animal's whiskers is relayed first through the trigeminal brainstem nucleus, and then through the ventral posterior

medial nucleus of the thalamus, after which thalamocortical projections reach L4 of S1. The whiskers on the rats face are arrayed in a stereotypic way, organized into five distinct rows (A through E), and seven columns (arcs). The whisker map is faithfully preserved in cortical S1, with each individual whisker sending sensory input primarily to one "column" of cortical neurons (Fig. 1.4). This one-to-one mapping of whiskers to cortical columns makes the somatosensory system uniquely amenable to investigating the consequences of various paradigms of sensory deprivation. Specific whiskers can be removed, and the corresponding regions of cortex subsequently targeted for physiological or anatomical study, with the useful internal control of probing neighboring columns that received intact sensory information.

Conclusion

Our understanding of cortical plasticity has evolved tremendously in recent years, providing greater insight into the critical factors and conditions necessary for triggering plasticity, as well as the increasingly vast array of cellular and synaptic changes that can take place, and the multitude of cell types involved in such plastic changes. A major area of focus that remains is to better understand the variety of changes induced by sensory deprivation in S1. In the present study, we aimed to determine the impact of sensory deprivation on the early development and function of inhibitory circuitry in this region, and our results identify a novel transient expression of homeostatic inhibitory plasticity.

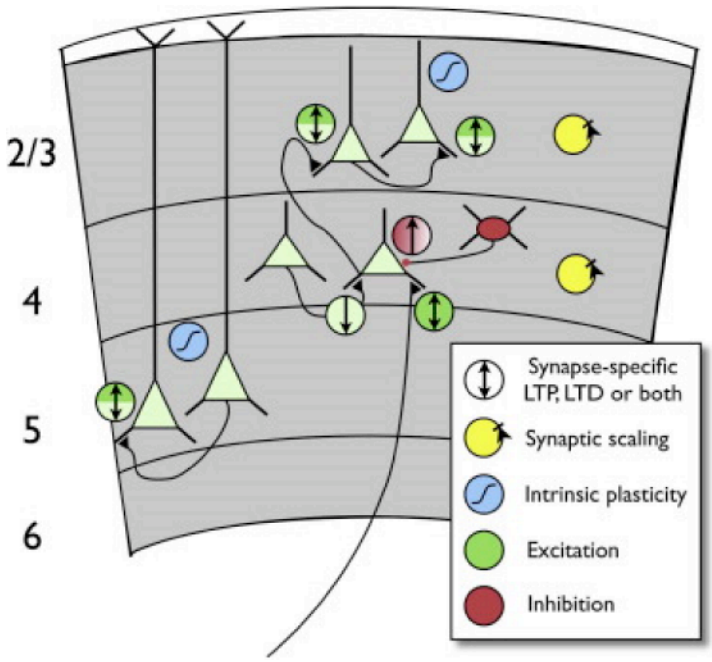

Fig 1.1: Multiple cellular and synaptic changes in excitatory and inhibitory circuitry mediate cortical plasticity. Three major forms of plasticity (long-term synaptic modification, synaptic scaling, and intrinsic plasticity) are illustrated in the various cortical layers, and work in tandem to generate complex plastic responses to sensory manipulations. (Figure from Nelson and Turrigiano 2008).

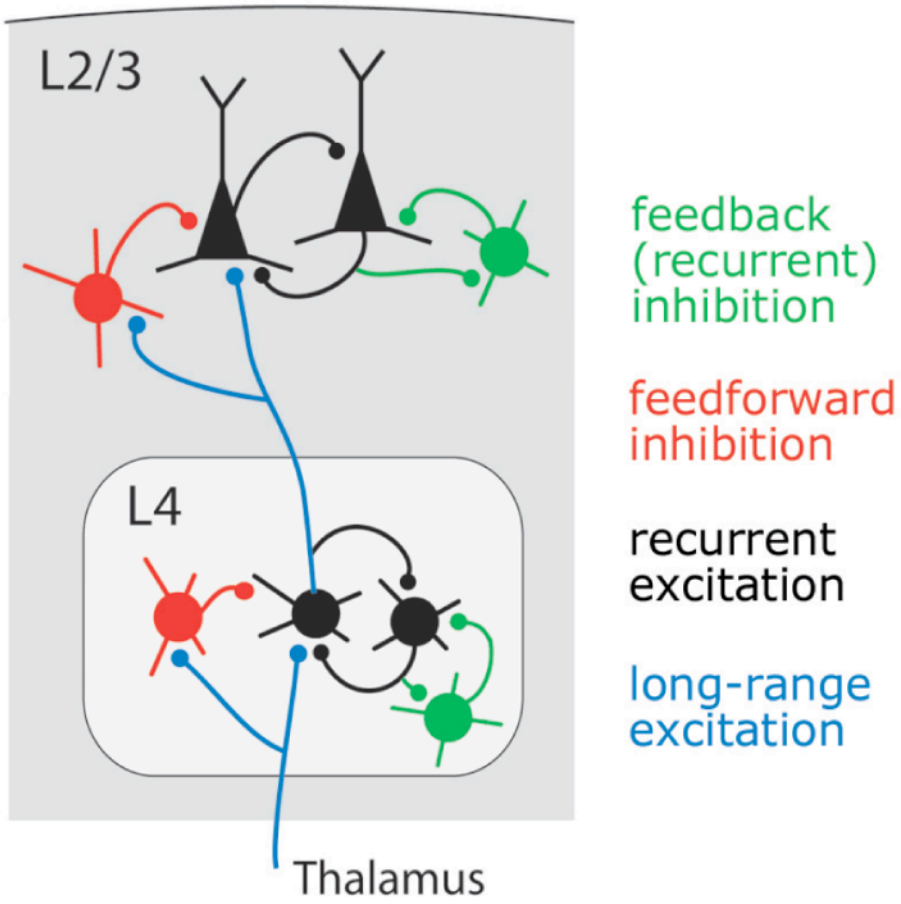

Fig 1.2: Major excitatory and inhibitory pathways in L4 and L2/3 of somatosensory cortex. Excitatory pathways from thalamus to L4 or L4 to L2/3 target both feedforward excitatory cells and feedforward inhibitory cells. In both layers, this excitatory signal is shaped by feedforward and feedback inhibition, as well as recurrent excitation.

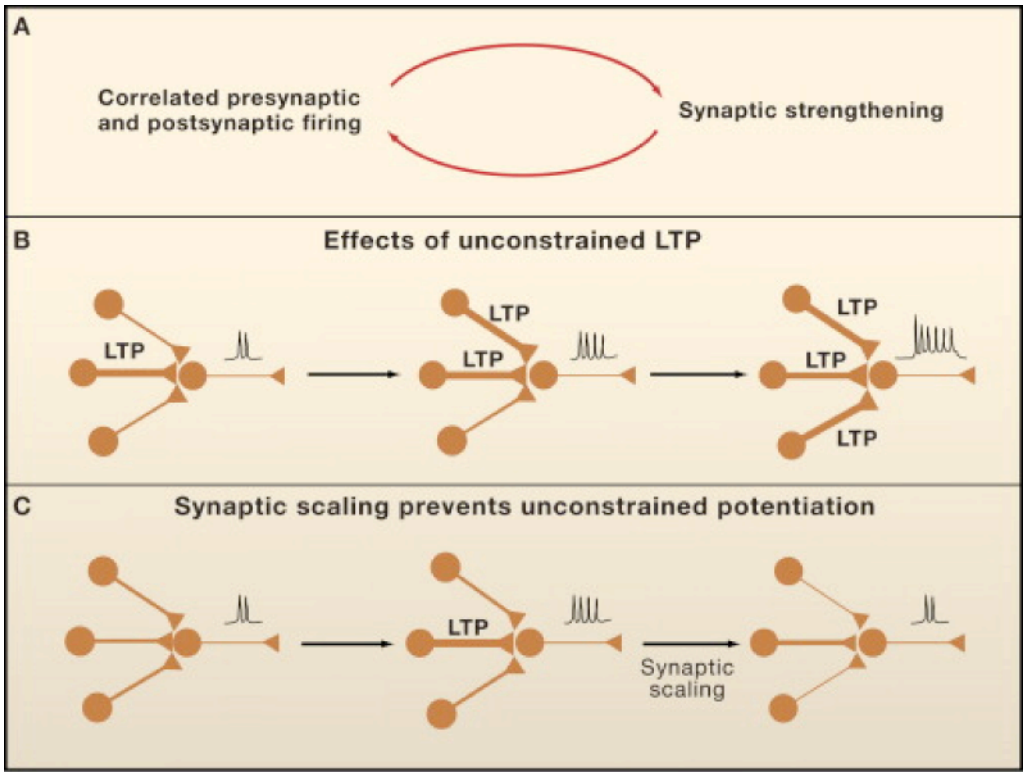

Fig 1.3: Homeostatic scaling of synaptic strength. A, Example of a positive feedback loop of correlated activity between pre- and postsynaptic neurons and synaptic strengthening, as in a traditional model of Hebbian plasticity. B, This positive feedback loop would eventually result in over-excitation from synaptic strengthening of all contacts onto a given target cell. C, Global homeostatic synaptic scaling can reduce the strength of all synapses onto a target cell, while preserving their relative weights. (Figure from Turrigiano 2008).

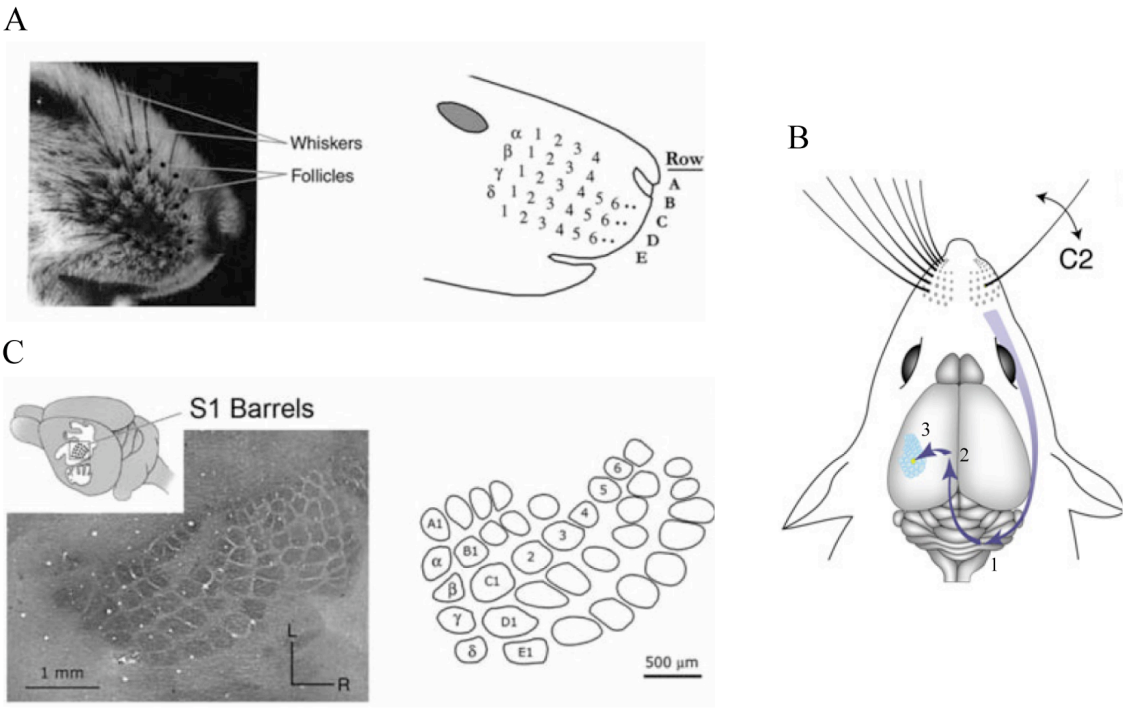

Fig 1.4: Somatosensory pathway from whiskers to S1 barrel cortex. A, Rat whiskers are arranged on the snout in a stereotypic map of rows and columns. B, Sensory information from the whiskers first synapses in the brainstem, and then in the thalamus, after which it is relayed to barrel cortex. C, The arrangement of cortical barrels in S1 faithfully preserves the map of whiskers on the face, with a one-to-one correspondence between the two. (Figure 1.4A,C from Bear et al. Text, 1.4B from Petersen 2007).

II. Effect of whisker deprivation on development of inhibitory synapses in rat somatosensory cortex

Abstract

Manipulating sensory input causes plastic changes in neuronal physiology throughout mammalian cortex. Such plasticity is often restricted to finite "critical periods". Our understanding of the necessary conditions and substrates for neocortical plasticity has vastly increased in recent years, but the particular circuits and synapses that undergo plastic changes, as well as the rules that govern which mechanisms of plasticity will be recruited, and when, are still poorly characterized. In the present study we investigated the impact of continuous whisker deprivation on the development of inhibitory circuitry in rat S1.

We first studied the normal developmental course of spontaneous miniature inhibitory currents (mIPSCs) onto excitatory cells in layers 4 and 2/3, and found that mIPSC frequency dramatically increases within the first postnatal month in both layers, and that mIPSC amplitude increases in L2/3, but undergoes no overall net change in L4 during this period. We found that inhibitory currents are reduced in both layers of cortical columns deprived of sensory input relative to sensory-spared columns, but that this reduction only persists for a brief window of approximately 1-2 days during development. Beginning at P7, 8 days of continuous whisker deprivation transiently reduced mIPSC frequency and amplitude, as well as evoked IPSC amplitude, at the age P15 in L4. Whisker deprivation also reduced mIPSC amplitude

in L2/3, but this effect was delayed by about one week after L4 (P21). Inhibitory responses recovered to control levels in both L4 and L2/3 after P15 and P21, respectively, despite continued whisker removal. When the onset of whisker deprivation was delayed by 5 days, beginning at P12, mIPSC amplitudes were reduced at approximately the same age, but the reduction in mIPSC frequency was delayed by 5 days, now observed at P20. Our results reveal a novel and surprising form of cortical plasticity, in which sensory deprivation causes a delayed and transient depression of inhibitory signaling in S1 that rapidly recovers to control levels after 1-2 days.

Introduction

Since the early work of Torsten Weisel and David Hubel expounding the effects of visual deprivation in the cat visual cortex, the importance of intact sensory activity for the proper development of cortical circuits has been well documented. Their work, as well as that of many others, has reinforced the Hebbian "use it or lose it" plasticity rule in sensory cortex: excitatory feedforward circuits that are deprived of activity during a critical period may weaken in their ability to effectively transmit sensory information.

Only more recently have studies brought to light the increasing complexity of the situation. We have developed an appreciation for the variety of different forms of plasticity that can be expressed, as well as different cell-types and circuits that are capable of changing their physiology in parallel with canonical feedforward excitatory pathways. For instance, visual or somatosensory deprivation not only alters inhibitory pathways in addition to excitatory ones, but can also induce different forms of

plasticity in different subclasses of inhibitory interneurons (Maffei et al. 2004, Jiao et al. 2006, for review see Sun 2007 and Maffei and Turrigiano 2008). The elucidation of compensatory "homeostatic" mechanisms recruited in sensory cortex, which act counter to positive feedback "use it or lose it" changes, further argues that a simple Hebbian model of excitatory neocortical plasticity is only one piece of the puzzle (Nelson and Turrigiano 2008).

In addition to these findings, we have gained a much deeper understanding of the factors that trigger and regulate sensory cortical plasticity, such as critical periods, the role of activity and competition between circuits, and the importance of cortical inhibition. However, we are still left with a highly incomplete picture of cortical plasticity, and remain poorly equipped to make predictions about where, when, and what forms of plasticity will emerge given particular paradigms of sensory manipulation. One major question that remains concerns how sensory experience shapes the early development and function of cortical inhibitory circuitry.

Classically, results from many studies have yielded a model in which normal sensory drive is permissive for the proper development of neocortical synapses and circuits, and evidence for this has been shown for inhibitory circuitry (Morales et al. 2002). Support for this model of cortical synaptic development primarily comes from studies in V1 and a common prediction is that this phenomenon would translate to other cortical areas. However, recent studies in V1 have also found that early visual deprivation can induce different forms of plasticity, depression as well as enhancement, of the very same inhibitory synapses depending on the precise timing of sensory blockade (for review see Maffei and Turrigiano 2008). The question of what

effect early sensory deprivation might have on the development and function of inhibitory synapses in S1 has not been answered, and the activity dependence of synapse development in this cortical region is unclear. A recent set of studies aimed to address this issue in S1. Continuous whisker deprivation beginning at an early age, P7, was reported to reduce the efficacy of inhibitory circuitry in mouse barrel cortex, through both physiological and anatomical changes in L4, when inhibitory synapses in spared and deprived cortical columns were compared 3 weeks later at P30 (Jiao et al. 2006, Sun 2009). However, data from these studies were gathered from slices cut in a plane of section expected not to yield the appropriate complement of barrel rows for isolating spared VS deprived columns, calling the results into question. If their data *are* correct, it is also critical to know whether similar results would be obtained in the rat, in order to relevantly place the findings within a larger context of plasticity studies (which are mostly from rat). Furthermore, these studies did not probe cortical circuits earlier than P30, nor look for changes in L2/3, leaving open the question of how sensory deprivation affects the normal development of inhibitory signaling in S1.

We wanted to determine the impact of sensory deprivation on the development of inhibitory circuitry within both the thalamocortical input layer 4 and layer 2/3 of primary somatosensory cortex. In order to do this, we first characterized the developmental profile of synaptic inhibition by recording mIPSCs from excitatory cells in rodent S1 between the end of the first postnatal week (P7, the age when GABA receptors switch from passing inward excitatory current to outward inhibitory current due to changes in the Cl^- gradient), until the end of the first postnatal month (P30) (Blue and Parnavelas 1983, Luhmann and Prince 1991). To test whether

experience impacts this development, we compared mIPSCs from cortical columns whose principal whiskers had been removed with those from control columns that received intact sensory input (from within the same brain slice). Sensory deprivation beginning at P7 induced a transient reduction of mIPSC amplitude and frequency in both layers 4 and 2/3, at P15 and P21, respectively. Evoked monosynaptic IPSCs were similarly reduced at P15 in L4. Surprisingly, in both layers, the reduction in spontaneous and evoked IPSCs lasted only 1-2 days before recovering to control levels. Delaying the onset of whisker plucking by 5 days (so that sensory deprivation began at P12) caused mIPSC amplitudes to be reduced at approximately the same time (at P16), but delayed the reduction in mIPSC frequency by 5 days, until P20. These effects were again short-lived, with mIPSC amplitude and frequency returning to control levels within one day. These findings reveal that whisker deprivation can cause a transient reduction in inhibitory synaptic transmission, limited to a brief 1-2 day critical window in L4 and L2/3 of rat S1.

Methods

Slice preparation

Long-Evans rats aged P7-30 were decapitated under isoflurane anesthesia, and the brain was quickly removed and placed in ice cold oxygenated Ringer's solution (containing in mM: NaCl 119, NaHCO3 26.2, D-(+)-Glucose 11, MgSO4 1.3, KCl 2.5, NaH2PO4 1.0, CaCl2 2.5). Slices (350 μm thickness) of the left cortical hemisphere were cut 45^0 towards coronal from the sagittal plane. In this "across-row" plane of section, slices containing the posteromedial barrel subfield (PMBSF) include

one barrel column from each of five whisker barrel rows, A through E (Fig. 2.1, Finnerty and Connors 1999, Allen et al. 2003). After cutting, slices were incubated at 32 ^{0}C for 30 min, and then maintained at room temperature until recording, between one and seven hours later.

Electrophysiological Recording and IPSC Analysis

Whisker barrels in cortical layer 4 were visualized by transillumination under a 10X objective, which allowed identification of whisker-related columns and cortical layers. Using infrared differential interference contrast light microscopy (IR-DIC) at 40X magnification, pyramidal-shaped neurons in L2/3 and L4 were selected for whole-cell voltage-clamp recording (Fig. 2.1). Recordings were made using a Multiclamp 700B amplifier (Axon Instruments, Union City, CA), low-pass filtered at 2 kHz, and sampled at 5 kHz using a 12-bit data acquisition board (National Instruments, Austin, TX). Electrophysiological data were collected using custom acquisition routines in Igor Pro (Wavemetrics, Lake Oswego, OR). Recordings were performed at 30-31^{0}C in oxygenated Ringer's solution. Recording pipette resistances in the bath were 2-4 MΩ. Inhibitory postsynaptic currents (IPSCs) were measured in voltage clamp at 0 mV, using Cs+ based internal solution (containing in mM: D-gluconic acid 108, Cesium OH 108, HEPES 20, TEACl 5, NaCl 2.8, EGTA 0.4, supplemented with GTP 0.3, ATP 4.0, and phosphocreatine 10.0). Cells were recorded with series resistance between 10 MΩ and 20 MΩ, and were discarded if series or

input resistance changed more than 15% during the course of an experiment, or if holding current necessary to achieve Vhold = -70 mV was greater than 200 pA.

All experiments were performed during bath superfusion of excitatory blockers D-AP5 50 μM and NBQX 10 μM. Certain experiments also included one or more of the following agents: TTX 500 nM, saclofen 100 μM, and gabazine 1.5 μM, picrotoxin 100 μM (all from Tocris Biosciences, Ellisville, MO). Spontaneous miniature IPSCs (mIPSCs) were recorded in the presence of excitatory blockers and TTX, and mean peak-to-peak recording noise was approximately 14 pA (range from 10 – 18 pA). mIPSC recordings were imported into Axograph X (AxoGraph Scientific, Sydney Australia) for analysis, and approximately 500 mIPSC events (peak amplitude 8.0-250 pA) were analyzed per cell. All mIPSC analysis was performed blind to the animal's sensory experience.

Evoked IPSCs were studied in L4 using minimal stimulation techniques. A two-prong bipolar extracellular stimulating electrode, tip space ~100 μm, was placed ~200 μm deep into the slice. The stimulating electrode was inserted at the bottom of the desired barrel, perpendicular to the pial surface. Evoked IPSCs were measured from excitatory cells in blockers of AMPA, NMDA, and $GABA_B$ receptors. Stimulation intensity was adjusted to achieve a failure rate of approximately 50% for postsynaptic IPSCs. For each cell, 50 sweeps were collected. In a first-pass analysis, all evoked IPSCs greater than a threshold amplitude of 8 pA were analyzed (Fig. 2.13A). In a second method of analysis, the net response amplitude (evoked – baseline) was measured for each sweep in the experiment. A histogram of noise amplitudes was generated from all responses with net amplitude < 0 mV, plus their

absolute values (i.e., mirror replicating the measured responses above zero). The resulting noise histogram was fit with a Gaussian, which was then plotted on top of the full distribution of response amplitudes. Response amplitudes from the full distribution that were contained within the "noise" Gaussian were discarded. The remaining data points were considered successes, and their average was calculated, yielding a measure of IPSC "potency" (Isaac et al. 1996).

Histological recovery of L4 neurons

A subset of neurons in cortical L4 was filled with biocytin (Invitrogen, 0.25% (w/v) in internal). After recording, slices were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer. Slices were re-sectioned at 100 μm on a freezing microtome, reacted with streptavidin-fluorescein 0.2% (Vector Labs, Burlingame, CA) in 0.1 M PB for 4 days, mounted and coverslipped with vectashield. Recovered cells were examined for the presence or absence of dendritic spines (indication of excitatory versus inhibitory cells, respectively, Fig. 2.2).

Whisker Deprivation

To test how whisker deprivation altered inhibitory synapse development, a subset of rats were deprived (plucked) of D1-D6 and gamma whiskers on the right side of the snout (Fig. 2.1). Plucking began at either age P7 or P12, and continued on alternating days until the animal was sacrificed for slice recording. Plucking was performed under transient isoflurane anesthesia (3% in 2L/min oxygen).

Results

To measure mIPSCs in excitatory L4 and L2/3 neurons, we made whole-cell recordings from neurons with pyramid-shaped somata. In L2/3, these neurons are known to be pyramidal (excitatory) cells (Feldman 2000). In L4, we tested whether recorded neurons were excitatory by histological recovery of a subset of neurons (n=58) filled with biocytin during recording. Of these neurons, 54 exhibited dendritic spines (and were either spiny stellate or pyramidal cells), indicating 93% accuracy in targeting excitatory neurons (Fig. 2.2). mIPSCs were measured in voltage clamp in the presence of NBQX (10 μM), APV (50 μM), and TTX (500 nM). mIPSCs were evident as spontaneous inward currents at 0 mV. mIPSCs were identified with a template matching algorithm, using a 8.0 pA detection threshold (minimum amplitude) (AxoGraph Scientific, Sydney Australia). Background noise was ~ 15 pA (peak-to-peak). In 6 cells, mIPSCs reversed at approximately -70 mV, close to the chloride reversal potential (-68 mV for these solutions, example experiment shown in Fig. 2.3A). In two cells, mIPSCs were completely and reversibly blocked by gabazine (1.5 μM) and by picrotoxin (100 μM), indicating that currents were generated from $GABA_A$ receptors (Fig. 2.3B).

Development of mIPSCs in S1

We first investigated the developmental profile of spontaneous miniature IPSCs in layers 4 and 2/3 of the primary somatosensory cortex of rats aged between P7 and P30. The frequency of mIPSCs in cortical L4 was extremely low at P7 (P7 interevent interval [IEI] 7.4 ± 1.6 s, Fig. 2.4 and Table 2.1). mIPSC frequency

increased with age after P7 (ANOVA, $p< 0.0001$), with a 10-fold increase by P12 (P12 IEI: 371 ± 41 ms, Fisher's PLSD $p<0.0001$, Fig. 2.4C). mIPSC frequency reached a plateau and did not change significantly after P22 (P22 IEI: 90 ± 20 ms). The developmental increase in mIPSC frequency can also be observed in cumulative probability profiles of inter-event intervals (Fig. 2.3B).

Average mIPSC amplitude in L4 exhibited two distinct phases of development between P7 and P30, with a significant effect of age on amplitude (ANOVA, $p< 0.02$). There was an initial significant increase between P7 and P18 (P7: 24.6 ± 1.7 pA, P18: 35 ± 1.8 pA, $p<0.0001$ Fisher's PLSD, Fig. 2.5 and Table 2.1). Amplitude then decreased between P18 and P22 (P22: 28.1 ± 2.3 pA , $p< 0.001$, Fisher's PLSD) without further change through P30 (Fig. 2.5C). As a result of this biphasic development, there was no significant difference in mIPSC amplitude between P7 and P30(Fig. 2.5C).

In L2/3, mIPSC frequency developed with a similar pattern as in L4, increasing dramatically after P7, and overall increasing significantly with age (ANOVA, $p< 0.0001$, Fig. 2.6A). Interevent interval was significantly greater at P12 than P7 (P7: 3.7 ± 4.7 s, P12: 544 ± 96 ms, $p<0.0001$, Fisher's PLSD, Fig. 2.6B and Table 2.1), and significantly greater at P22 than at P12 (P22: 110 +/- 9 ms, $p<0.05$). There was no further significant change after P22. In contrast with L4, mIPSC amplitude in L2/3 significantly increased with age (ANOVA, $p< 0.02$). This increase took place between P7 and P18 ($p<0.001$, Fig. 2.7), and amplitude showed no further change between P18 and P30.

The data reported above are from "B" whisker columns from rats in which the "D" row whiskers were removed to study plasticity (see next section). Cells within the "B" barrel received normal intact sensory input, and are separated from the deprived "D" barrel by a spacing of two cortical columns, limiting the potential for plasticity of spared responses.

Whisker deprivation reduced mIPSC efficacy

We next investigated the impact of D-row whisker deprivation on normal development of spontaneous mIPSCs, beginning with L4. D-row whiskers were deprived continuously by plucking beginning at P7, and the effect of deprivation was assessed by comparing mIPSCs in deprived "D" vs. spared "B" columns. Measurements at P15 (after 8 continuous days of deprivation) showed a 16% reduction in mIPSC amplitude in deprived vs. spared columns (spared: 31.3 ± 0.9 pA, deprived: 26.9 ± 0.9 pA, Fig. 2.8, $p<0.01$, unpaired t-test). Thus, cumulative probability curves for mIPSC amplitude were shifted leftward in deprived columns compared to spared columns in 6 different litters (Fig. 2.8C).

To our surprise, this effect of deprivation was completely confined to P15, with no effect of deprivation at earlier or later ages. Cumulative histograms of mIPSC amplitude showed no differences between deprived and control curves at P14 or P16 (Fig. 2.9B). Correspondingly, composite average mIPSCs were not different between spared and deprived columns at P14 or P16 (Fig. 2.9A). Mean mIPSC amplitude for spared and deprived mIPSCs are plotted for all ages studied between P12 and P30

(Fig. 2.9C). This plot shows that continuous whisker deprivation significantly reduced mIPSC amplitude only at P15.

mIPSC frequency in L4 was also reduced in deprived vs. spared columns at P15. Figure 2.10B shows that deprivation significantly increased mean IEI by 17% compared to control spared mean IEI ($p<0.05$). As with mIPSC amplitude, this effect was only observed at P15, with no significant effect on IEI at P14 or P16 (Fig. 2.10A and C).

To test whether a similar brief time window for plasticity occurred in L2/3, we measured mIPSCs from L2/3 pyramidal cells in spared and deprived columns. We found that whisker deprivation also significantly impacted mIPSCs in L2/3. D-row whisker deprivation began at P7 and continued until recording, as for the L4 experiments. Measuring at P21 (after 14 days of deprivation), we observed a leftward shift in the cumulative probability histogram for mIPSC amplitude in deprived vs. spared columns (Fig. 2.11A). At this age, deprivation significantly reduced mean mIPSC amplitude by 14% (Fig. 2.11B). As in L2/3, this effect was limited to measurements at (or near) P21, with no significant difference in mIPSC amplitude between spared and deprived columns at P18 or at P22 (Fig. 2.11C). Deprivation produced a small but nonsignificant trend to reduce mIPSC frequency, as evidenced by the rightward shift in the cumulative probability curve for IEI in deprived vs. spared columns at P21 ($p<0.2$, unpaired t-test) (Fig. 2.12A). Thus, deprivation beginning at P7 caused a delayed, transient decrease in mIPSC amplitude, and to a lesser degree mIPSC frequency, at P15 in L4, and at P21 in L2/3.

Whisker deprivation reduced evoked inhibitory transmission in L4

To test whether deprivation-induced changes in mIPSCs also affected evoked synaptic transmission, we recorded extracellular evoked IPSCs from pyramid-shaped (presumed excitatory) cells in L4 using minimal stimulation. Biocytin reconstruction confirmed that 93% of recovered neurons had spines and were therefore excitatory (see above). The stimulating electrode was placed within the same L4 barrel as the recorded neuron. IPSCs were recorded in the presence of APV 50 μM, NBQX 10 μM, and saclofen 100 μM. The stimulation intensity was adjusted to produce a 50% failure rate for IPSCs in the recorded neuron (Fig. 2.13A, see methods). D-row deprivation beginning at P7 caused a reduction in the mean amplitude of evoked IPSCs measured at P15. When evoked responses considered "successes" (IPSCs larger than a threshold amplitude of 8 pA) were averaged, IPSC amplitude was reduced by approximately 30% in deprived vs. spared columns (Fig. 2.13C, $p<0.01$). In a second method of analysis (see methods), IPSC potency was comparably reduced (Fig. 2.13D, $p< 0.01$). This reduction was accompanied by more than a doubling of paired pulse ratio (PPR, 25 ms interval), from 0.99 ± 0.1 in recordings from spared columns, to 2.4 ± 0.6 in recordings from deprived columns (Fig. 2.14, $p<0.05$). Consistent with a brief, transient period of susceptibility to deprivation, neither mean evoked IPSC amplitude nor PPR differed between spared and deprived columns at P17 (Fig. 2.15).

Changes in mIPSC amplitude and frequency were dissociated with shorter deprivation

To determine whether deprivation altered mIPSCs at a specific age (P15 in L4, P21 in L2/3) or at a specific duration of deprivation (8 days in L4, 14 days in L2/3),

we tested the effects of D-row deprivation starting at P12. In L4, deprivation from P12 reduced mIPSC amplitude in deprived vs. spared columns at P16. This is illustrated by the leftward shift of the cumulative probability curve for mIPSC amplitude (Fig. 2.15B), and the mean amplitude plot in Fig. 2.15C & D ($p<0.05$).

Unexpectedly, deprivation beginning at P12 did not alter mIPSC frequency at P16 but instead caused mIPSC frequency to decrease (mean IEI to increase) at P20, after 8 days of derivation (Fig. 2.17, $p<0.02$). This is five days later than when plucking began at P7. Thus, delayed onset of deprivation dissociated plasticity of mIPSC amplitude and frequency, indicating they represent distinct cellular plasticity mechanisms. Deprivation effects on mIPSC amplitude were absent at P15 and 19 and deprivation effects on mIPSC frequency were absent at P19 and 21 (Figs. 2.16D and 2.17D). Thus, deprivation effects were still limited to brief time periods, after which they recovered to control levels.

Discussion

Development of mIPSCs in S1

Our results show that mIPSC frequency in L2/3 is low at P7, increases sharply during the second postnatal week, and shows a further small increase between P14 and P30 (Fig. 2.6). This is consistent with results from L2/3 of mouse barrel cortex, where a strong increase in mIPSC frequency was observed between P6 and P16, and a less pronounced enhancement continued between P16 and P30 (Kobayashi et a. 2008). In mouse, electron microscopy results showed an increase in the density of symmetrical synapses between P6 and P30 (DeFelipe et al. 1997). Additionally, quantal analysis

revealed an increase in release sites between P6 and P30, and CV analysis indicated increased release probability between P6 and P12 (Kobayashi et a. 2008). Increased mIPSC frequency can probably be explained by a developmental increase in inhibitory synapse number, and may also reflect changes in release probability. Thus, our results show similar development of mIPSC frequency between rats and mice, and suggest that the initial strong increase in mIPSC frequency between P7 and P14 may reflect a developmental increase in release probability as well as inhibitory synapse number.

Average mIPSC amplitude increased steadily between P7 and P18, and then reached plateau after P18 (Fig. 2.7). This result is in contrast with that found in the mouse, where mIPSC amplitudes recorded from L2/3 pyramidal neurons were reduced between P7 and P18, and then increased between P20 and P30 (Kobayashi et a. 2008). This direct comparison reveals a novel species-specific difference in the development of mIPSC amplitude in L2/3 of S1.

In L4, we found that mIPSC frequency develops similarly to L2/3 (Fig. 2.4 and Table 2.1). mIPSCs were less frequent in L4 than in L2/3 at P7, but the reverse was true at P30, which is consistent with reports of higher spontaneous IPSC frequency in L4 than L2/3 of the adult rat (Salin and Prince 1996). The amplitude of mIPSCs in L4 exhibited two distinct phases of development: an initial increase between P7 and P18, followed by a subsequent decrease between P18 and P30, such that mIPSC amplitudes are not significantly different between P7 and P30 (Fig 2.5). Thus, the general trend for mIPSC amplitude development is different between L4 and L2/3, suggesting different mechanisms for inhibitory synapse development between these layers. It is

not clear whether the changes in mIPSC amplitude arise from pre or postsynaptic changes, and further experiments would be necessary to determine this.

Effects of sensory deprivation on inhibitory currents

Our results show that early somatosensory deprivation causes inhibitory synapses in L4 and L2/3 to transiently weaken. When animals were continually deprived of D-row whisker input from P7, mIPSCs recorded from L4 excitatory cells in deprived "D" barrels were transiently reduced in both amplitude and frequency compared with those recorded in spared "B" barrels (Fig. 2.8). The onset of this effect was delayed 8 days after the onset of whisker deprivation, observed at P15, and rapidly offset after P15 (Fig. 2.9). Correspondingly, minimal-evoked IPSCs recorded from excitatory neurons also weakened at age P15, after 8 days of sensory deprivation (Fig. 2.13). This weakening was accompanied by a concomitant increase in PPR (Fig. 2.14). We anticipated a possible reduction in inhibitory transmission from sensory deprivation in light of reports of experience-driven inhibitory plasticity in V1 and mouse S1 (Sun 2009, Maffei and Turrigiano 2008), and our results provide further evidence that the early and brief critical period for plasticity of whisker responses in L4 (P0-P5, Fox 1992) does not hold for inhibitory plasticity. The development of cortical inhibitory circuitry is delayed and protracted compared to that of excitatory circuitry (Micheva Beaulieu 1997), and this might render inhibitory circuits plastic longer during development than excitatory circuits.

We found similar effects of whisker deprivation in L4 also in L2/3: sensory deprivation transiently reduced the amplitude of mIPSCs (a non-significant reduction

in mIPSC frequency was also observed, Fig. 2.11 and Fig. 2.12). However, the onset of this plasticity was delayed by approximately one week, now observed at P21, compared with P15 in L4. Another study investigating experience dependent inhibitory plasticity in S1 found that two months of whisker deprivation reduced the number of GABAergic synapses in L4 but not in L2/3 (Micheva and Beaulieu 1995). In contrast, our findings suggest a similar capacity between L4 and L2/3 for inhibitory plasticity, but the difference in animal age and deprivation duration between these studies could account for this discrepancy.

We found that L4 deprivation-induced plasticity of inhibitory synapses precedes L2/3 plasticity in S1. Plastic responses to sensory deprivation in cortex are highly layer-dependent, and many lines of evidence support a sequential expression of plasticity from L4 to L2/3. Monocular deprivation between P14 and P16 enhanced spontaneous excitatory activity in L4 (with no change in L2/3), and only deprivation approximately one week later at P21-P23 caused enhancement in L2/3 (Desai et al. 2002, Maffei et al. 2004). LTP and LTD in the visual cortex are also expressed sequentially: they become uninducible in L4 shortly after eye opening, but persist in L2/3 into adulthood (Dudek and Friedlander 1996). Critical periods for visual and somatosensory plasticity in L2/3 also typically outlast those for L4, often extending into adulthood (Daw et al. 1992, Diamond et al. 1993, Glazewski and Fox 1996), and evidence suggests that plasticity of subthreshold (synaptic) receptive field structure after whisker deprivation also proceeds from L4 to L2/3 (Stern et al. 2001). Additionally, it was recently shown that GABAergic synapse maturation in visual cortex is delayed by one week in L2/3 compared to L4 (Jiang et al. 2010). Our results

provide evidence that this developmental shift in plasticity from L4 to L2/3 may be true for cortical inhibition as well as excitation in S1, and may be related to the developmental delay in the maturation of inhibitory circuitry.

Inhibitory plasticity represents a homeostatic response to whisker deprivation

Our finding that sensory deprivation causes a transient reduction in the strength of inhibitory signaling in cortical layers 4 and 2/3 demonstrates a novel form of homeostatic plasticity in S1. A traditional Hebbian response to sensory deprivation would lead to a reduction in excitatory drive from input-deprived circuits, but a depression of inhibitory signaling would effectively enhance excitatory drive in these layers. Thus, weakened inhibition can be considered a homeostatic response to whisker derivation.

This weakening of inhibitory synapses is consistent with *in vivo* studies of inhibitory plasticity in S1, where 40 days of whisker trimming led to weakened inhibitory receptive fields in L4 (Shoyket et al. 2005, Lee et al. 2007), as well as *ex vivo* studies where whisker plucking reduced the number of inhibitory synapses in L4 after approximately two months of age (Micheva and Beaulieu 1995, Sadaka et al. 2003), whisker overstimulation in adult mice increased the density of inhibitory synapses onto dendritic spines in L4 after 24 hrs (Knott et al. 2002), and early whisker trimming reduced thalamocortical recruitment of FS interneurons in L4 during the second postnatal week (Chittajallu and Isaac 2010). Our finding that deprivation reduces inhibitory synapse strength is also consistent with reports that deprivation reduced GABA immunoreactivity in V1 (Benevento et al. 1996, Gordon et al. 1997),

as well as results from binocular V1, where early "pre-critical" period monocular deprivation (before P21) reduced the amplitude of mIPCS in L4 (Maffei et al. 2010), and from monocular V1 where pre-critical deprivation depressed spontaneous IPSCs as well as reduced the strength of FS → Pyr cell synapses (Maffei et al. 2004). Similar to our findings, this last study also showed an increase in PPR at inhibitory synapses. Although less well-studied, inhibitory plasticity in L2/3 of V1 has been reported to follow the same trend of a reduction of inhibitory synapse strength after visual deprivation beginning from birth and continuing through the critical period. This consistency suggests that an early homeostatic downregulation of inhibitory synapses might be a common response to sensory deprivation across sensory cortices.

Interestingly, however, visual deprivation impacts monocular zone L4 inhibitory synapses in opposite directions depending on the precise time of deprivation relative to the critical period. Visual deprivation from P18-P21 (during the classic critical period) caused a potentiation of the very same inhibitory synapses that were weakened when deprivation occurred only a few days earlier (Maffei et al. 2006). Similar results were obtained when the effects of deprivation were probed in V1 binocular zone: before P21, MD caused a reduction in mIPSC amplitude and after P21, MD increased mIPSC amplitude (Maffei et al. 2010). In contrast, we found no deprivation-induced increase in L4 mIPSCs around P21-P22. One explanation for this discrepancy could be the fact that our experimental procedure involved continuous sensory deprivation rather than a brief period of 2-3 days. Alternatively, this contrast could be due to area-specific dynamics of critical period regulation, since S1 and V1

critical periods are very different, and no such critical period in S1 has been shown around this P21 age.

Brief critical window for the expression of inhibitory plasticity

In both layers 4 and 2/3, the reduction in mIPSC amplitude and frequency by whisker deprivation had two unusual characteristics: first, it was delayed, with mIPSCs developing normally for 8 days after deprivation onset, prior to weakening; and second, it was transient, lasting only approximately one day, after which mIPSC amplitude and frequency returned to normal levels despite the fact that deprivation continued uninterrupted. Changes in evoked IPSCs at P15 due to sensory deprivation were also absent two days later, at P17 (Fig. 2.15).

We know of no other example of such transient deprivation-induced plasticity. If sensory input is restored and circuits briefly recover for a matter or hours or days after some period of deprivation, it might be expected to observe a recovery from certain forms of experience-dependent plasticity, and this has been shown for inhibitory plasticity in both S1 and V1 (Knott et al. 2002, Morales et al. 2002). But in our experiments where whisker deprivation was ongoing, we were surprised to find such a rapid onset and cessation of plasticity. This novel finding could represent one of two functional possibilities: 1st) the molecular processes that bring about these changes are short-lived, and terminate after approximately one day, or 2nd) there is an independent mechanism that actively restores inhibition to control levels, which may reflect a form of metaplasticity. Further experiments would be necessary to determine which of these scenarios is accurate.

Dissociation of plasticity of mIPSC amplitude and frequency

Our finding that 8 days of continuous sensory deprivation weakened mIPSCs in L4 of rat S1 at age P15 led us to inquire whether this form of plasticity was somehow somehow restricted to a specific critical age of P15. This could be true if unique properties of L4 microcircuitry at this precise developmental time point rendered inhibitory synapses plastic. An alternative explanation is that the reduction in mIPSCs occurred after a specific duration of deprivation (here, 8 days), which is necessary to produce plasticity, regardless of the age of the animal. A third possibility is that some combination of the above two scenarios is true, or that a critical period for S1 inhibitory plasticity might exist that would prevent plasticity from occurring with deprivation after some critical age. In order to determine which of these possibilities is true, we delayed the onset of whisker plucking by 5 days, beginning at P12 instead of P7.

Results showed that when whisker deprivation began at P12, there remained a significant attenuation of mIPSC amplitude, and this effect occurred one day later than mIPSC plasticity from P7 deprivation (now observed at P16, Fig. 2.16). Surprisingly, at this age when mIPSC amplitude was depressed by deprivation, we found no change in mIPSC frequency. Instead, we found that mIPSC frequency was significantly reduced by deprivation at P20 (with no concomitant change in mIPSC amplitude, Fig. 2.17). Thus, these two different measures of synaptic function, mIPSC amplitude and frequency, were dissociated when whisker deprivation began at P12, and thus are regulated independently by experience.

Our results support the possibility that the transient whisker-induced reduction of mIPSC amplitude may somehow be unique to the developmental stage of the L4 cortical circuit at P15-16, while in contrast the reduction in mIPSC frequency may require a full 8 days of deprivation (regardless of the age of the animal), but both effects are still marked by rapid offset. In L4, it could be that P15 marks a stage within an unknown critical period in S1, or marks a certain developmental transition important for the regulation of inhibitory synapse strength.

Possible mechanisms for plasticity

Whisker deprivation reduced miniature and evoked IPSC amplitude, as well as mIPSC frequency, and increased PPR at L4 inhibitory synapses. The dissociation of changes in mIPSC frequency and amplitude when sensory deprivation began at P12 suggests mechanistic independence between the two, but more experiments are necessary to determine their precise mechanisms. In classic models of neurotransmission, a reduction in synaptic efficacy may be caused by changes in the number of synaptic contacts (N), the probability of release at each synaptic contact (Pr), or the postsynaptic response to one vesicle of transmitter (quantal amplitude, Q), which could represent changes in postsynaptic receptor number or function, or presynaptic vesicle content. Early visual deprivation reduces the connection probability between RSNP interneurons and pyramidal neurons by more than half in L4 of V1, suggesting a reduction in the number of inhibitory synaptic contacts between the two (Maffei et al. 2004). In S1, two months of whisker deprivation beginning from birth has also been shown to decrease the number of GABAergic

synaptic contacts onto excitatory dendritic spines in L4 by almost two-thirds (Micheva and Beaulieu 1995).

The change we observed in evoked IPSC PPR suggests a possible reduction in Pr of inhibitory synapses with sensory deprivation in S1 (Zucker and Regehr 2002). Changes in mIPSC frequency may provide additional evidence for a change in Pr, but could also result from a decrease in the number of synaptic contacts, N. Lastly, it is possible that the reduction in miniature and evoked IPSC amplitude are due to changes in postsynaptic receptors, or perhaps, changes in Q. Because our evoked currents were measured under conditions of minimal stimulation, it is more likely that the reduction in IPSC amplitude is due to postsynaptic changes in receptor content or dynamics.

Another mechanism for inhibitory synaptic plasticity found in the hippocampus involves reduced strength of inhibitory synapses as a result of changes in Cl- transporter activity (and thus changes in ECl, Woodin et al. 2003). In that study, plasticity was induced by repetitive postsynaptic spiking and voltage-gated calcium influx. It is unlikely that a similar mechanism could account for the changes in mIPSCs under our conditions because postsynaptic cells were voltage-clamped at 0 mV, hampering the activation of voltage-gated channels.

Our interpretations of potential synaptic mechanisms of plasticity are muddied by the fact that our recordings represent activity from a heterogeneous population of inhibitory synapses, individual classes of which may be uniquely regulated in response to sensory deprivation. An example of this has been shown for inhibitory plasticity in L4 of V1 after early sensory deprivation: the strength of synapses onto pyramidal cells from FS interneurons was reduced, whereas synapses from RS interneurons were

actually potentiated more than two-fold (Maffei et al. 2004). Further experiments will be necessary to determine which class(es) of inhibitory synapses are modified during our whisker deprivation paradigm.

Inhibitory synapse development proceeds largely normally during whisker deprivation

The prominent model of cortical circuit and synapse development asserts that development is largely activity-dependent, so that depriving sensory input effectively freezes cortical circuitry in an underdeveloped state. Most evidence for this theory comes from studies of visual cortical development, and V1 critical periods (Blasdel and Pettigrew 1978, Fagiolini et al. 1994, Bartoletti et al. 2004, Mower 1991, Iwai et al. 2003, Chang and Merzenich 2003, Desai et al. 2002, Benevento et al. 1995, Morales et al. 2002, Gianfranceschi et al. 2003, Chattopadhyaya et al. 2004, Chittajallu and Isaac 2010, see Chapter I for description). Our results suggest that this is not the case for inhibitory synapses in rat S1. Indeed, with the exception of the brief ~1 day periods of transient inhibitory weakening, sustained whisker deprivation does not alter mIPSC amplitude or frequency over the first postnatal month, up to P30. Our findings represent some contrast with what has been reported during development of mouse barrel cortex. In one recent study, the activity of FS interneurons in L4 of mouse S1 was regulated by sensory experience, because whisker trimming reduced the strength of excitatory thalamocortical input onto FS cells (Chittajallu and Isaac 2010). However, importantly, FS inhibitory synapses were unaffected by deprivation in this study. In another series of experiments, D-row whisker deprivation beginning at P7 was found to induce multiple mechanisms of inhibitory synapse weakening in L4

(measured at P30). These included a reduction in parvalbumin expression, mIPSC amplitude, and both unitary and evoked IPSC amplitude, as well as intrinsic plasticity of FS interneurons, and anatomical barrel shrinkage (Jiao et al. 2006, Sun 2009).

Why were deprivation-induced changes in inhibitory synapses at the end of the first postnatal month not found in the present study? One possibility is species-specific differences in plasticity between mouse and rat. Another possibility is that their studies were performed in barrel slices that were not made in the proper plane of section for isolating different whisker barrel rows, which would cause mis-identification of spared and deprived barrel columns. A final explanation for our discrepancy is that their studies were performed in GAD67-GFP heterozygous mice (to allow targeted recording of interneurons vs. excitatory neurons). This is a GFP knockin into the GAD67 locus, and heterozygotes express only 61% of normal levels of GABA at birth (84% at 6-7 weeks of age, Tamamaki et al. 2003). It is possible that inhibitory synapse plasticity occurs differently in these animals due to reduced GABA transmission than in wildtype animals. Thus, the reported effects in these papers may represent an artifact. Results from our study suggest that, with the exception of brief periods of plasticity, inhibitory transmission in L4 and L2/3 of rat S1 develops normally in the absence of sensory experience.

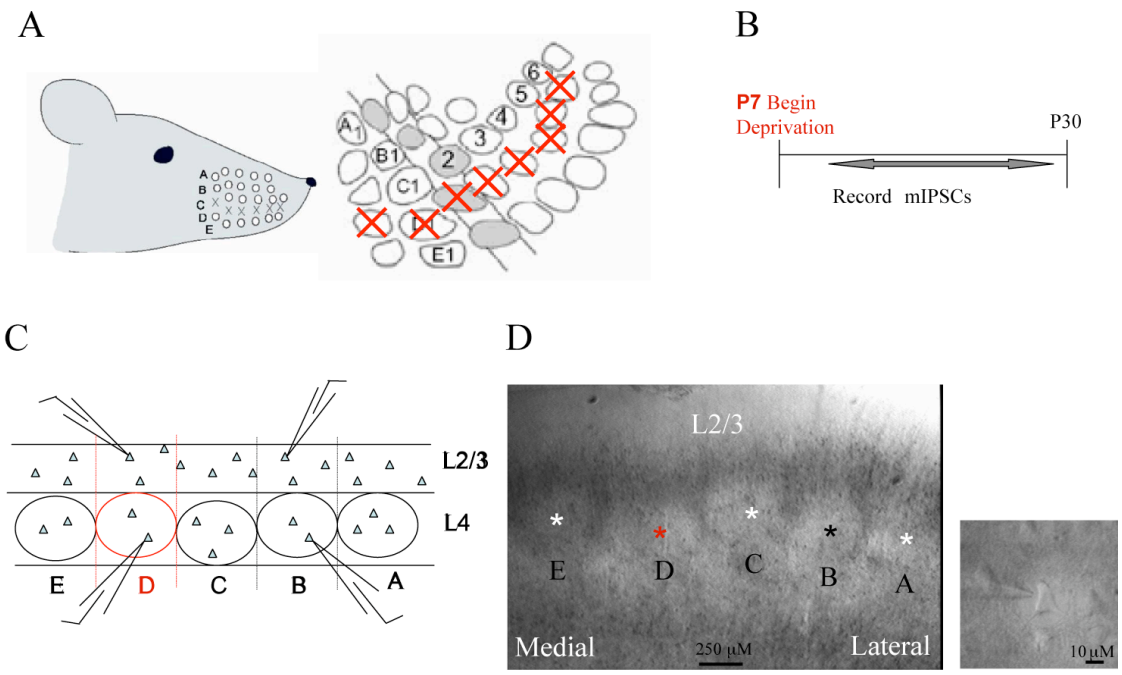

Fig 2.1: Whisker deprivation and S1 slice recording methods. A, Left, illustration of removal of D-row whiskers on the right side of the rat snout, including D-gamma. Right, schematic of corresponding barrels in S1 deprived of sensory input. Lines indicate plane of section during slice preparation. B, whisker plucking and recording timeline. C, Schematic of electrophysiological recording configuration from cells in both L2/3 and L4, in deprived "D" and spared "B" barrels. Red indicates the sensory input-deprived barrel. D, Left, IR-DIC image at 4X magnification showing the full complement of whisker barrels A through E. Right, IR-DIC image at 40X magnification showing recording pipette targeting pyramidal morphology somata.

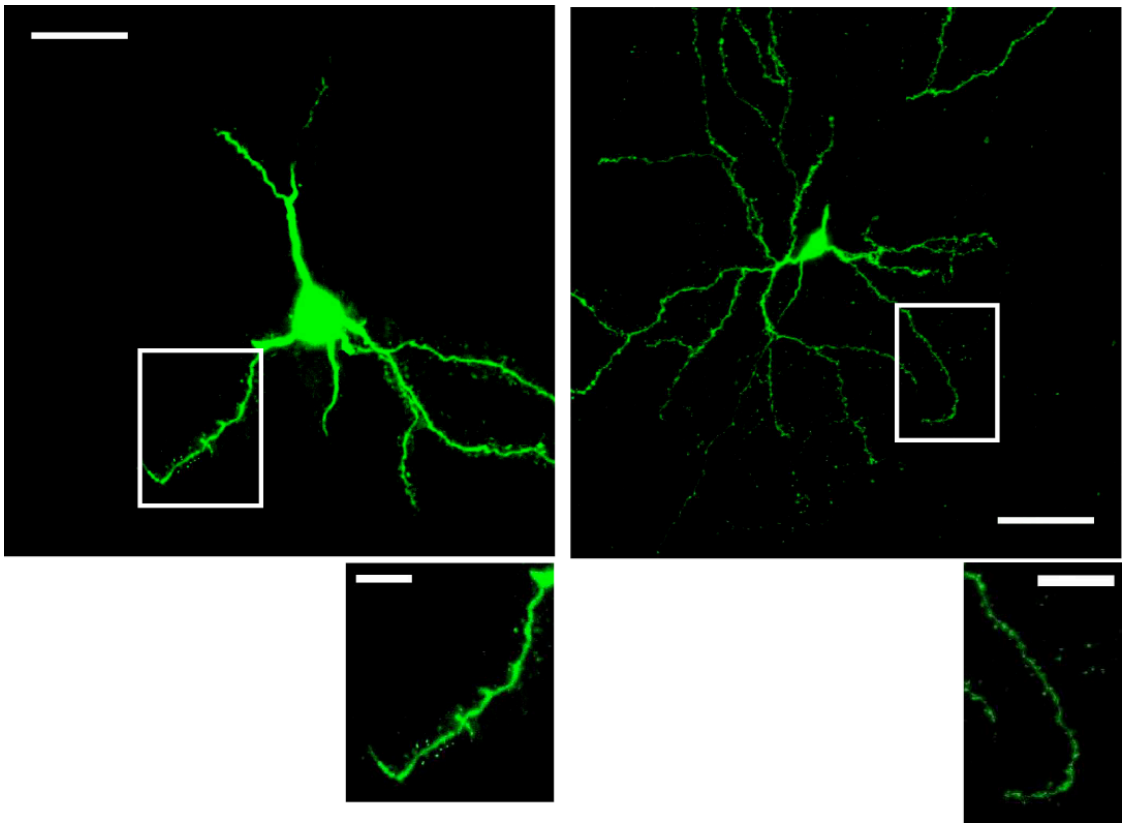

Fig 2.2: Data were collected primarily from spiny (excitatory) cells. Left, Example of L4 recovered pyramidal cell. Right, Example L4 recovered spiny stellate cell. Insets are expanded view of the area within white rectangles. Scale bars of the full panels represent 25 μm, and scale bars of the insets represent 10 μm.

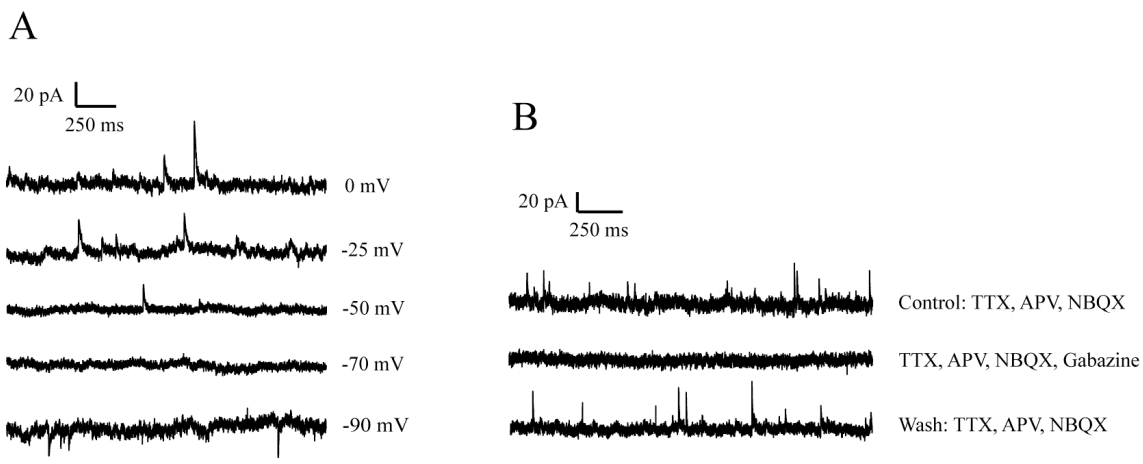

Fig 2.3: Miniature IPSC reversal and block by gabazine. A, Example traces of mIPSCs recorded at five different holding potentials from 0 mV to -90 mV. B, Example traces before (top), 3 minutes after application (middle), and 15 minutes after washout (bottom) of Gabazine 1.5 μM.

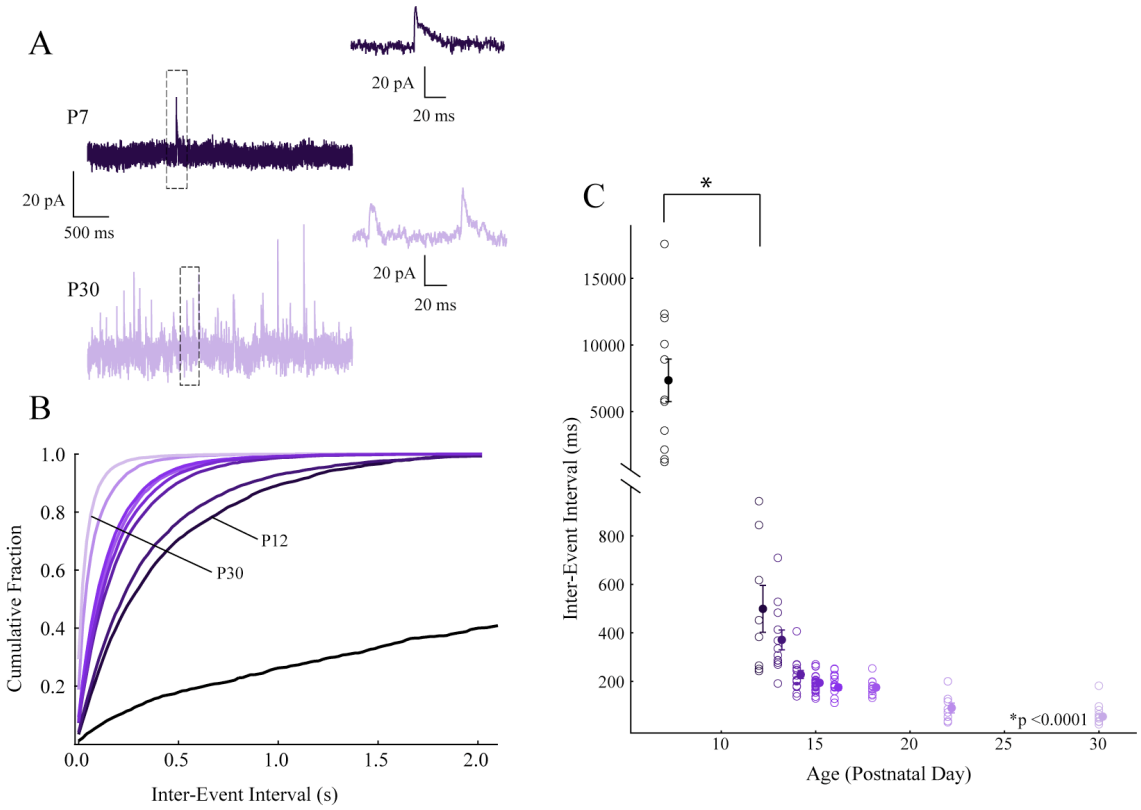

Fig 2.4: L4 mIPSC inter-event interval decreased between P7-P30. A, Example traces of mIPSCs collected at P7 (upper trace) and P30 (lower trace). Upper right insets are expanded views of area within dashed rectangles. B, Cumulative histogram of mIPSC IEI at 9 different ages from P7 to P30, color matched with mean IEI values plotted versus age in C. C, Mean mIPSC IEI plotted for 9 different ages, error bars are SEM. Asterisks indicate significance by Fisher's PLSD for single factor anova.

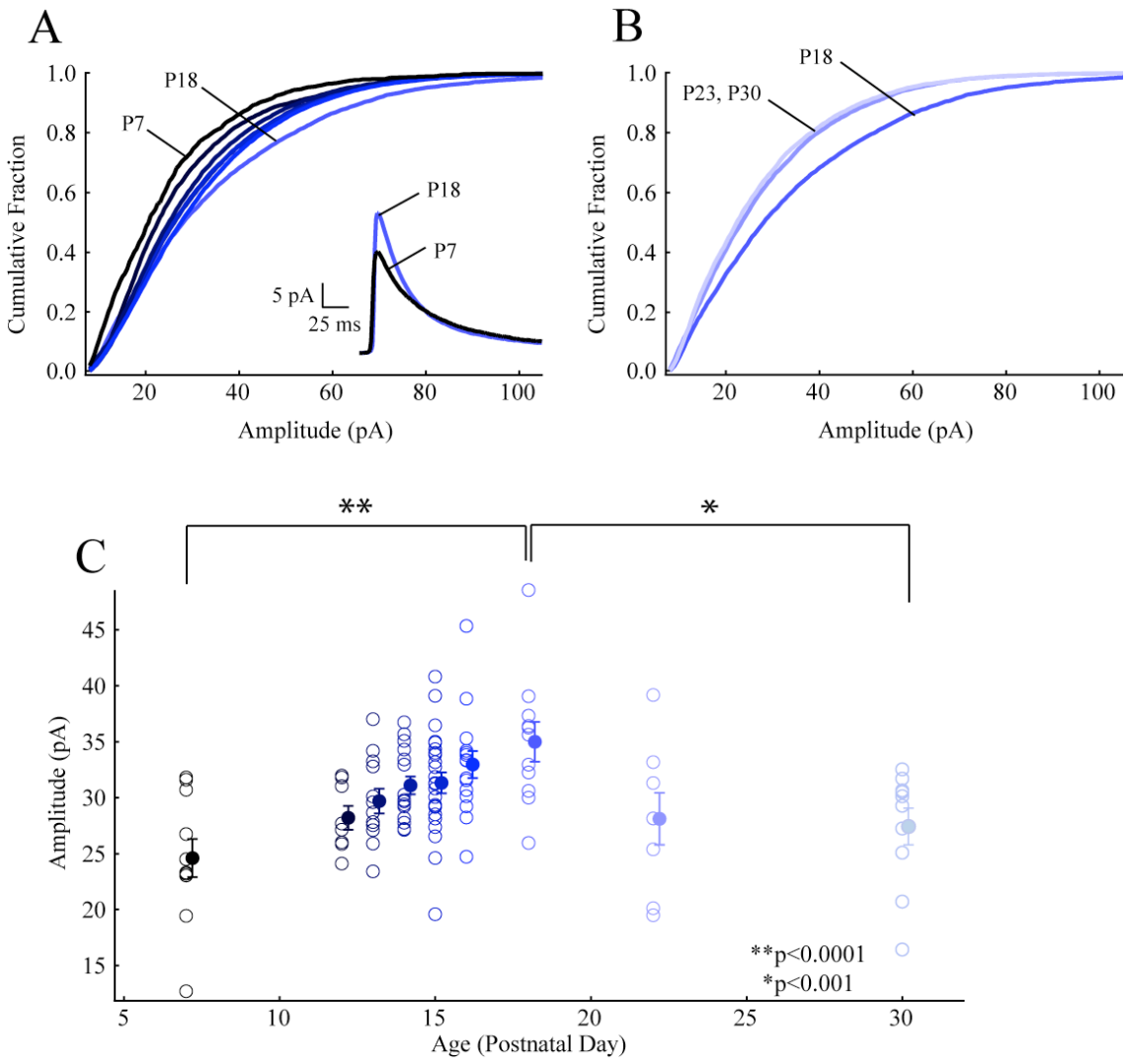

Fig 2.5: L4 mIPSC amplitude underwent biphasic development. A, Cumulative histogram of mIPSC amplitude at 7 different ages from P7 to P18. Inset, average composite mIPSC at P7 and P18. B, Cumulative histogram of mIPSC amplitude at 3 different ages from P18 to P30. C, Mean mIPSC amplitude plotted for 9 different ages, error bars are SEM. Asterisks indicate significance by Fisher's PLSD for single factor anova.

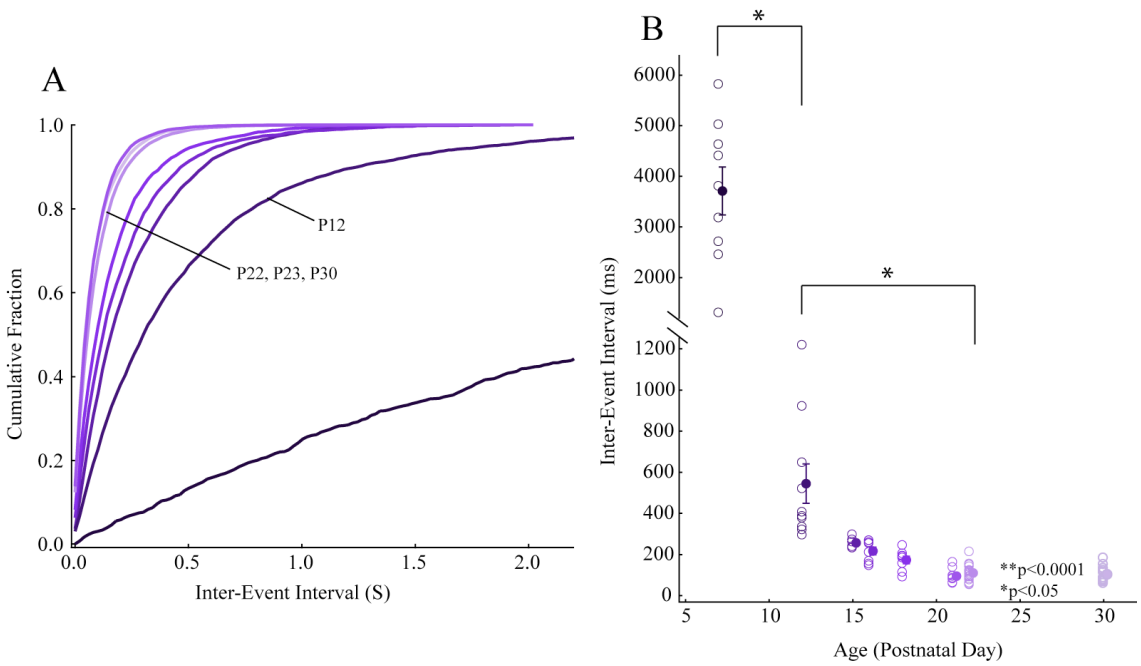

Fig 2.6: L2/3 mIPSC inter-event interval decreased between P7-P30. A, Cumulative histogram of mIPSC IEI at 8 different ages from P7 to P30. B, Mean mIPSC IEI plotted for 8 different ages, error bars are SEM. Asterisks indicate significance by Fisher's PLSD for single factor anova.

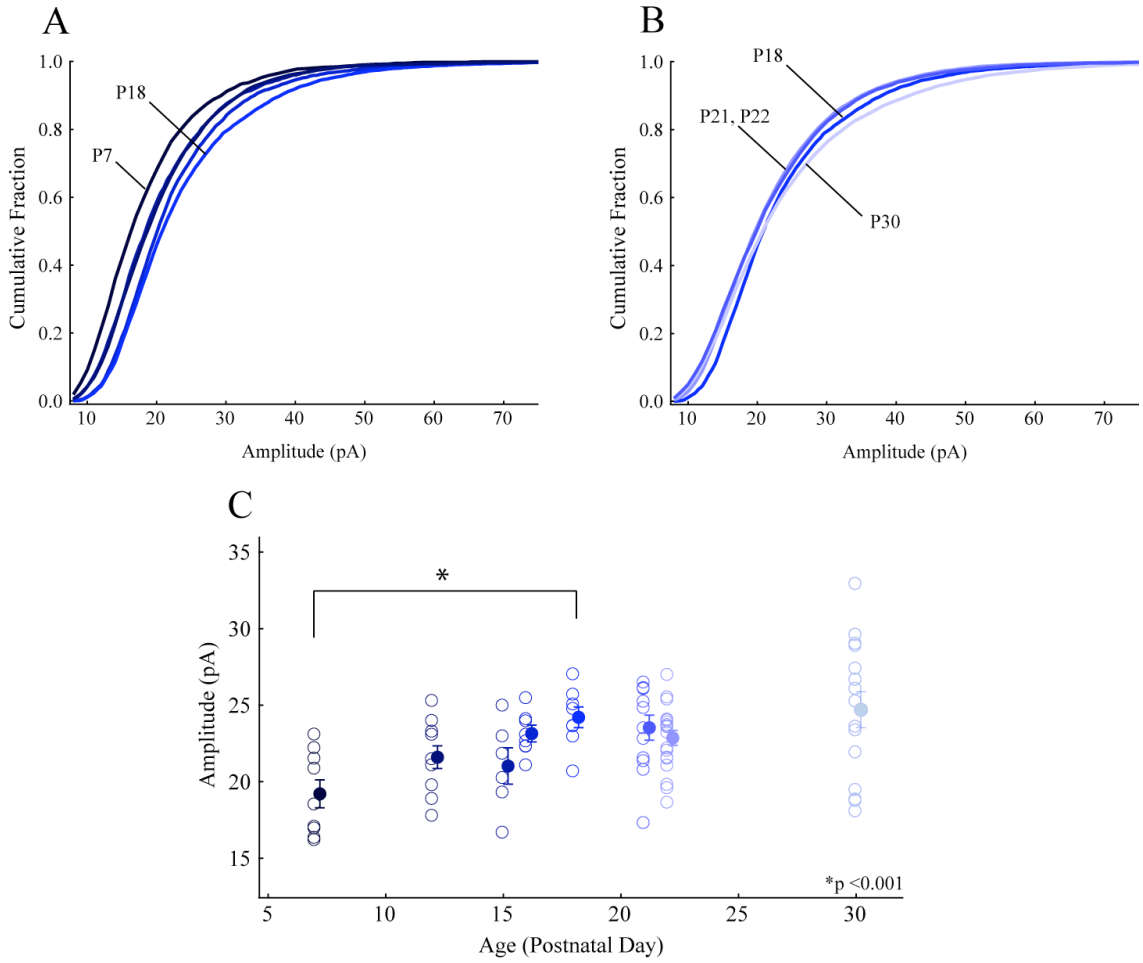

Fig 2.7: L2/3 mIPSC amplitude increased between P7-P30. A, Cumulative histogram of mIPSC amplitude at 5 different ages from P7 to P18. B, Cumulative histogram of mIPSC amplitude at 3 different ages from P18 to P30. C, Mean mIPSC amplitude plotted for 8 different ages, error bars are SEM. Asterisks indicate significance by Fisher's PLSD for single factor anova.

Table 2.1: Cellular and mIPSC properties in L4 and L2/3.

L4

AGE (P)	'n' (cells)	mIPSC IEI (ms)	mIPSC Amp (pA)	mIPSC 20%-80% Rise time (ms)	Cell Input Resistance (MΩ)
7	11	7351 ± 1594	24.6 ± 1.7	0.73 ± 0.09	591 ± 26
12	8	499 ± 97	28.2 +/- 1.1	0.63 ± 0.02	406 ± 14.8
13	12	371 ± 41	29.7 ± 1.1	0.58 ± 0.03	350 ± 37
14	15	228 ± 17	31.1 ± 3.3	0.53 ± 0.02	306 ± 35
15	24	194 ± 7	31.33 ± 0.9	0.65 ± 0.03	255 ± 14.8
16	15	175 ± 11	33 ± 1.2	0.66 ± 0.03	253 ± 19.4
18	11	176 ± 10	34.99 ± 1.8	0.72 ± 0.05	212 ± 19
22	8	90 ± 20	28.11 ± 2.3	0.77 ± 0.05	203 ± 22.2
30	10	55 ± 10	27.4 ± 1.4	1.1 ± 0.09	163 ± 18.3

L2/3

AGE (P)	'n' (cells)	mIPSC IEI (ms)	mIPSC Amp (pA)	mIPSC 20%-80% Rise time (ms)	Cell Input Resistance (MΩ)
7	9	3709 ± 474	19.2 ± 0.9	0.86 ± 0.08	566.7 ± 44
12	10	544 ± 96	21.6 ± 0.74	0.98 ± 0.06	369 ± 24.2
15	6	257 ± 10	21.0 ± 1.2	1.1 ± 0.1	342 ± 20.1
16	7	217 ± 19	23.1 ± 0.6	1.08 ± 0.04	238 ± 17.4
18	8	174 ± 18	24.2 ± 0.7	1.09 ± 0.05	225 ± 20.6
22	19	110 ± 9	22.9 ± 0.5	1.04 ± 0.06	171 ± 18.2
30	15	104 ± 9	24.7 ± 1.1	1.5 ± 0.1	135 ± 17

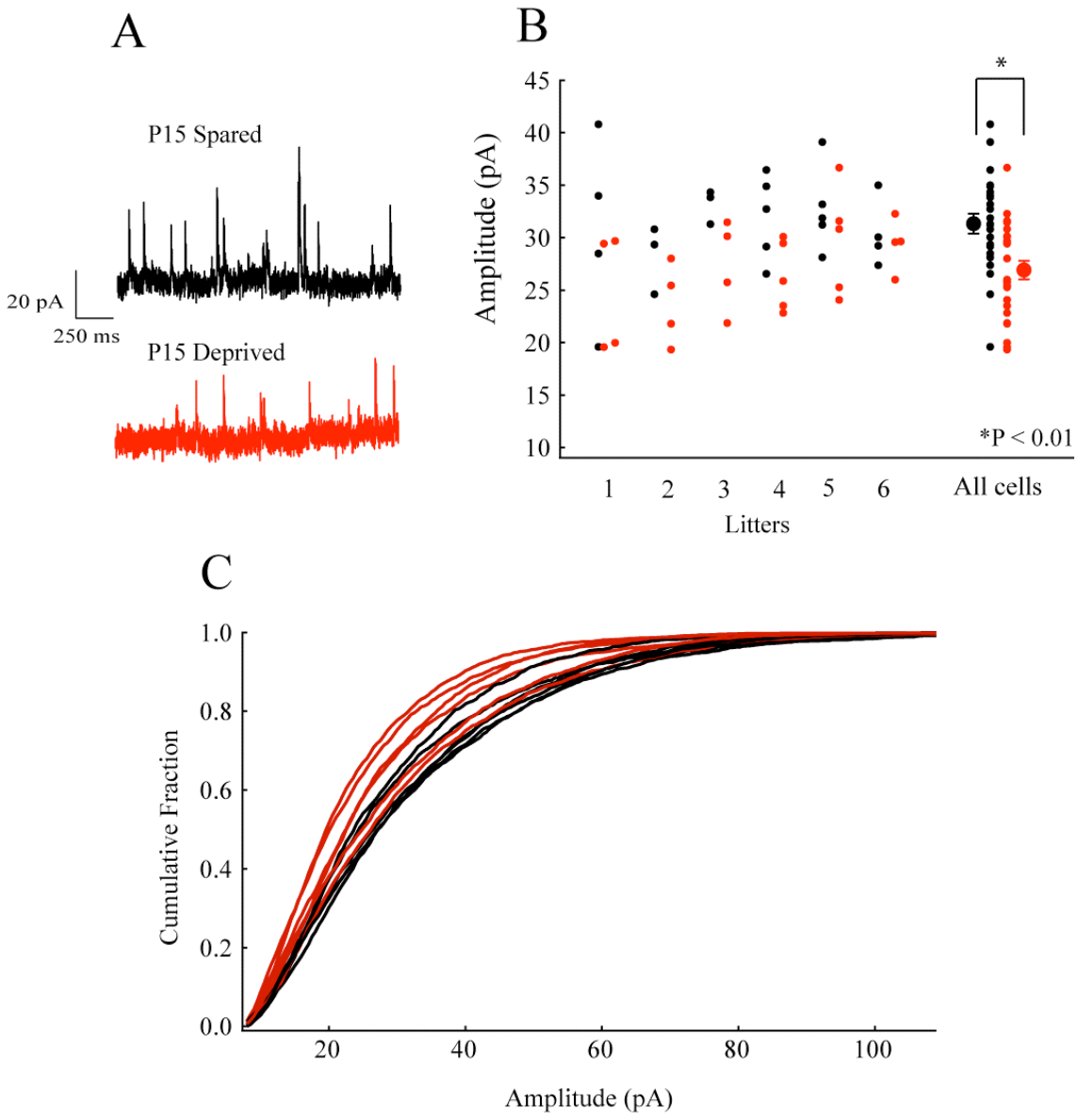

Fig 2.8: Eight days of D-row whisker deprivation reduced mIPSC amplitude in L4 (at P15). A, Example traces of mIPSCs recorded from a spared "B" barrel (black) and deprived "D" barrel (red). B, mIPSC amplitude from cells recorded from spared and deprived cortex across 6 different litters, and pooled together with means. C, Cumulative histogram of mIPSC amplitude recorded from spared and deprived cortex from 6 litters.

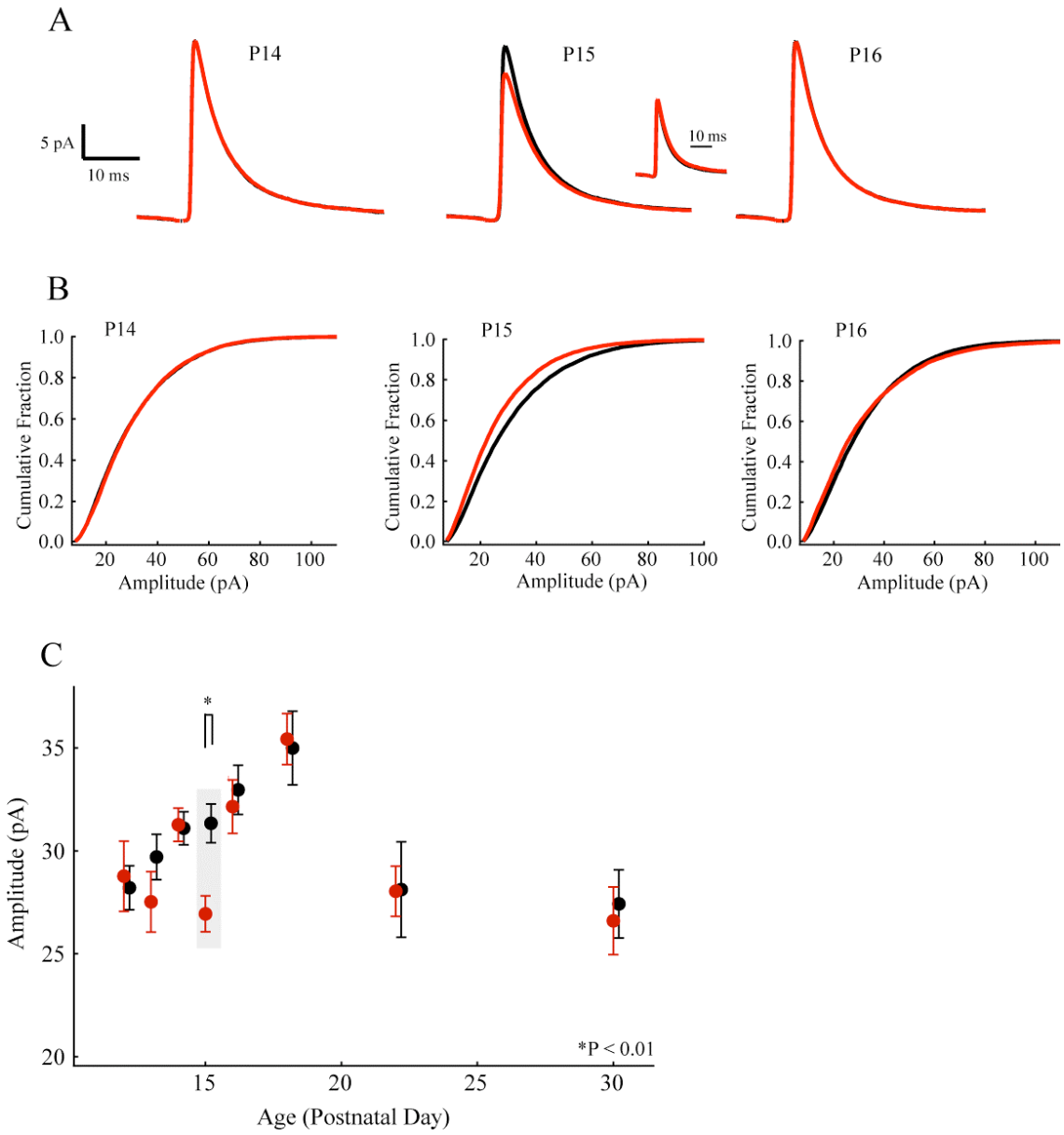

Fig 2.9: L4 mIPSC amplitude was unchanged by deprivation immediately before and after P15. A, Composite averages of mIPSCs from spared (black) and deprived (red) cortex at ages P14, P15, and P16. Inset, composite mIPSC from deprived cortex normalized to and overlaid onto that from spared. B, Cumulative histograms of mIPSC amplitude from spared and deprived cortex at P14, P15, and P16. C, Mean mIPSC amplitude from spared and deprived cortex at 8 different ages from P12 to P30.

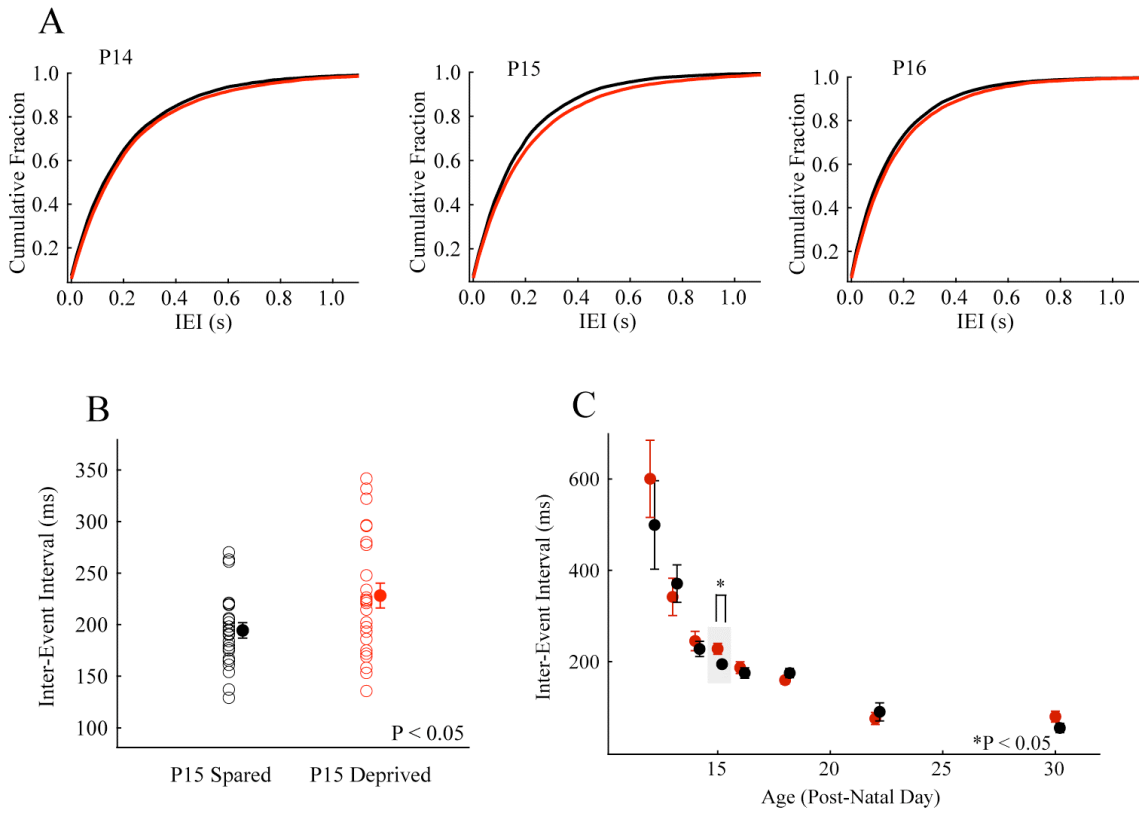

Fig 2.10: L4 mIPSC interevent interval was reduced after 8 days of deprivation, at P15. A, Cumulative histograms of mIPSC IEI from spared and deprived cortex at P14, P15, and P16. B, IEI values plotted, with means, from spared and deprived cortex at P15. C, Mean mIPSC IEI from spared and deprived cortex at 8 different ages from P12 to P30.

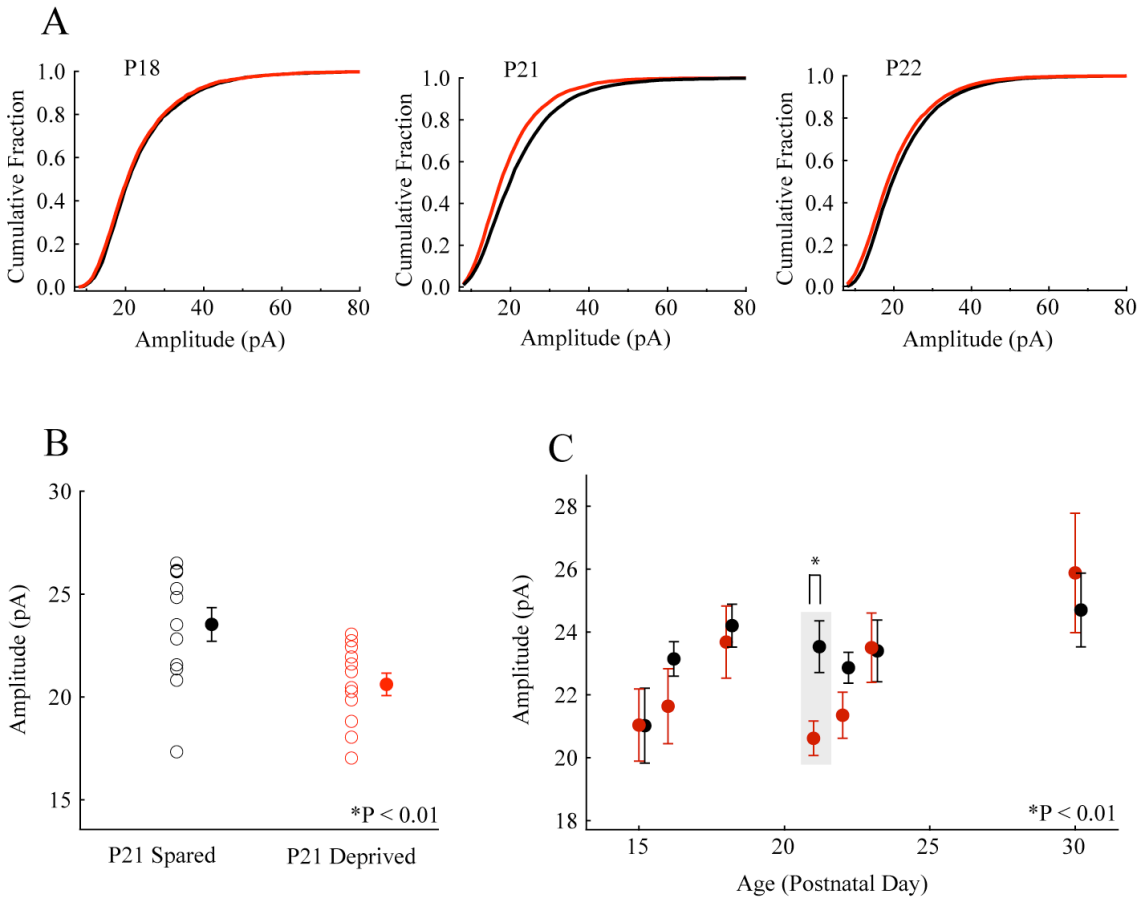

Fig 2.11: L2/3 mIPSC amplitude was reduced after 14 days of deprivation, at P21. A, Cumulative histograms of mIPSC amplitude from spared and deprived cortex at P18, P21, and P22. B, Amplitude values plotted, with means, from spared and deprived cortex at P21. C, Mean mIPSC amplitude from spared and deprived cortex at 7 different ages from P15 to P30.

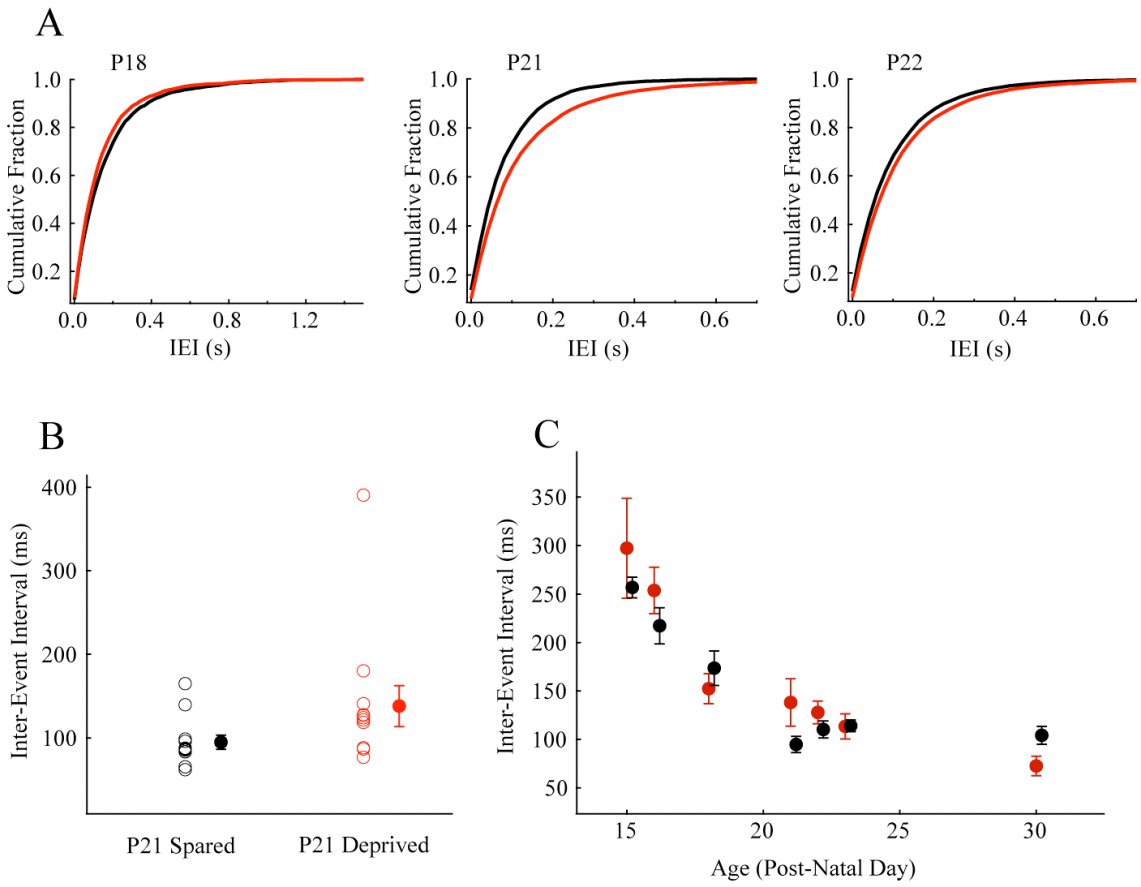

Fig 2.12: L2/3 mIPSC interevent interval was not significantly altered by deprivation. A, Cumulative histogram of mIPSC IEI from spared and deprived cortex at P18, P21, and P22. B, IEI values plotted, with means, from spared and deprived cortex at P15. C, Mean mIPSC IEI from spared and deprived cortex at 7 different ages from P15 to P30.

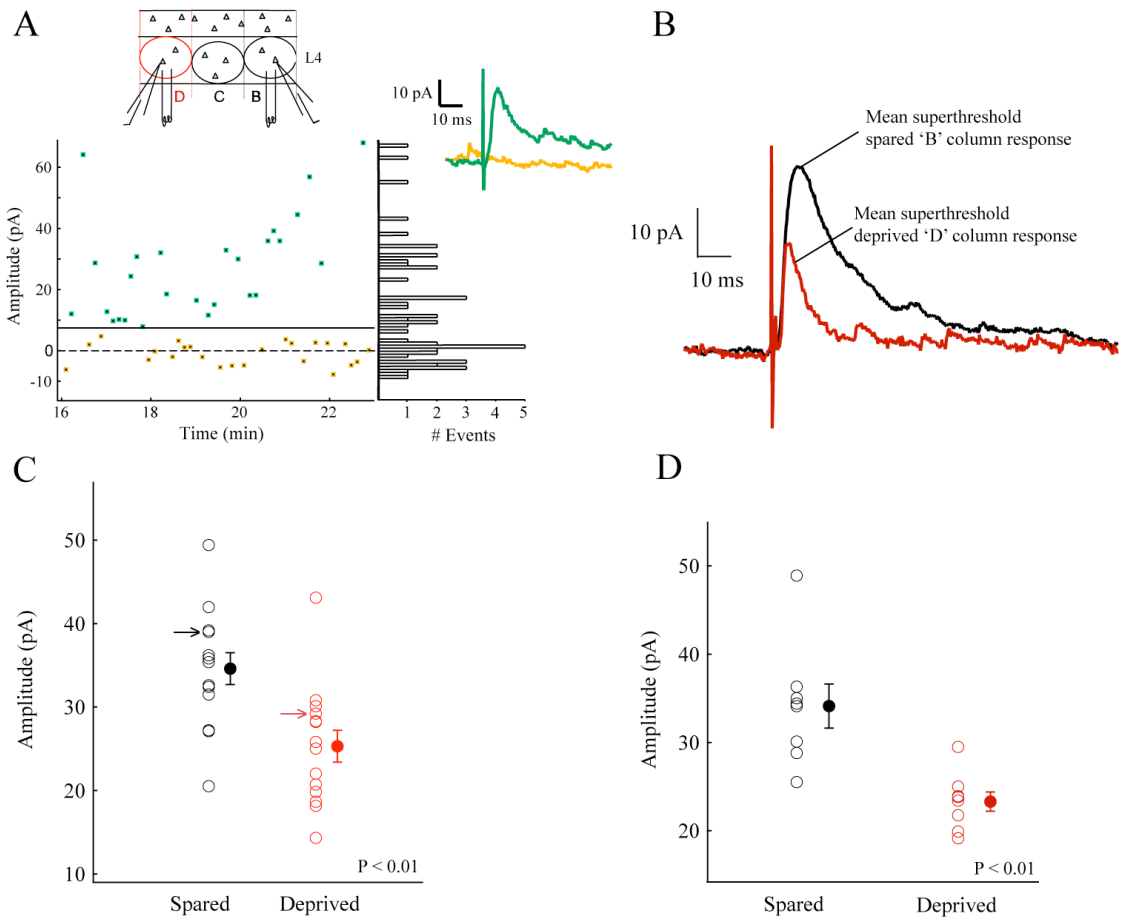

Fig 2.13: Evoked IPSCs in L4 were reduced after 8 days of deprivation, at P15. A, Example experiment recording "minimal-evoked IPSCs" (responses recorded at approximately 50% failure rate). Green points represent responses considered above IPSC threshold (8 pA) and orange points represent responses considered below IPSC threshold. Right axis, histogram of all responses in the experiment. Top left inset, schematic of stimulating and recording configuration in L4 spared and deprived barrels. Top right inset, average of super- and sub-threshold responses from the experiment shown. B, Example mean super-threshold IPSC recorded from a deprived "D" barrel (red) and spared "B" barrel (black) within the same slice overlaid on top of one another. C, Mean superthreshold IPSC recorded from spared and deprived cortex (arrows point to the examples shown in B. D, Mean IPSC potency from spared and deprived cortex, taken from a subset of cells in C, analyzed alternatively (see methods).

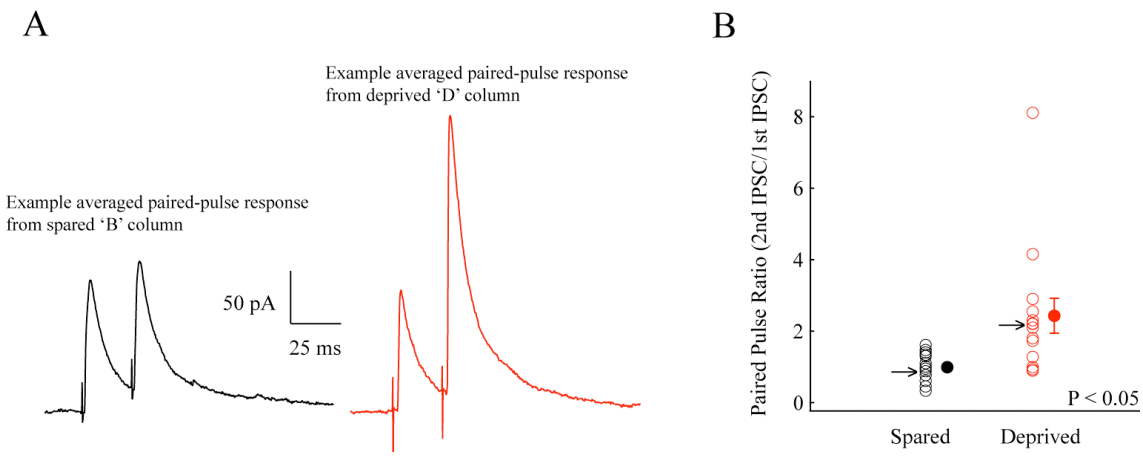

Fig 2.14: Paired-pulse ratio in L4 was increased after 8 days of deprivation, at P15. A, Example paired pulse experiments (average of 10 sweeps) recorded from a cell in the "B" barrel (left) and "D" barrel (right) of the same slice. B, Paired pulse ratios recorded from cells in spared "B" and deprived "D" barrels. Arrows indicate examples shown in A.

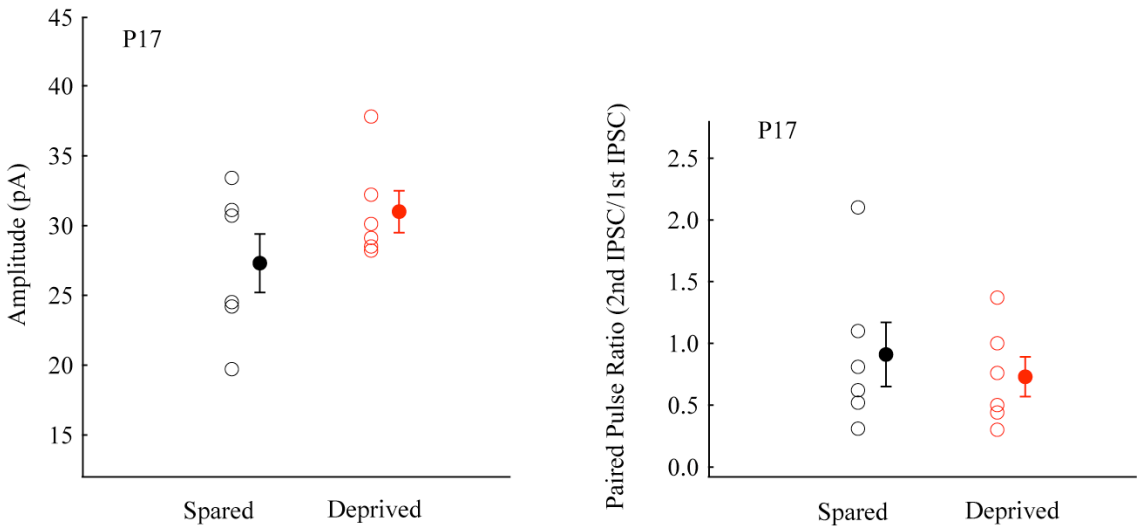

Fig 2.15: Neither evoked IPSC amplitude nor PPR was changed after 10 days of deprivation, at P17. Left, Mean IPSC potency from spared and deprived cortex. Right, Paired pulse ratios recorded from spared and deprived cortex.

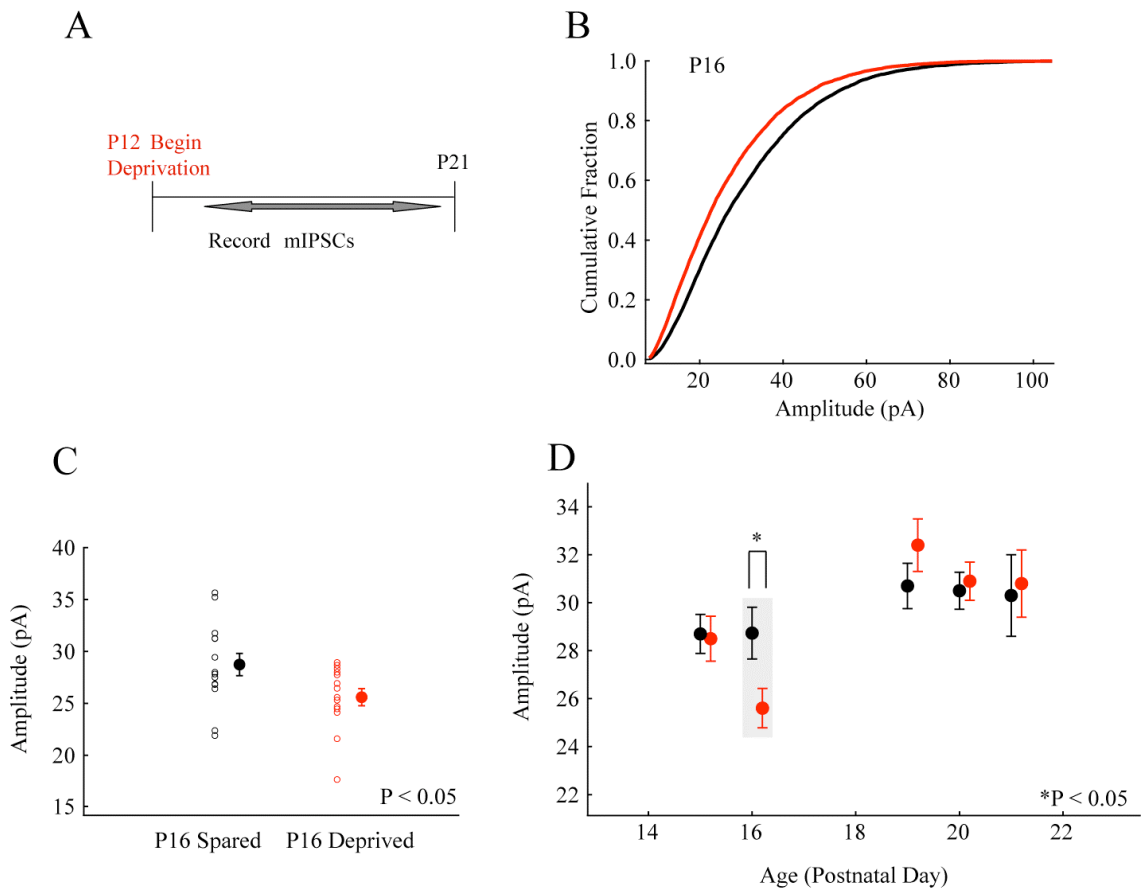

Fig 2.16: Delaying the onset of whisker deprivation by 5 days only slightly delayed the reduction of mIPSC amplitude. A, Altered timeline of whisker deprivation, now beginning P12. B, Cumulative histogram of mIPSC amplitude from spared and deprived cortex after 4 days of deprivation, at P16. C, mIPSC amplitude values, with means, from spared and deprived cortex at P16. D, Mean mIPSC amplitude from spared and deprived cortex at 5 different ages from P15 to P21.

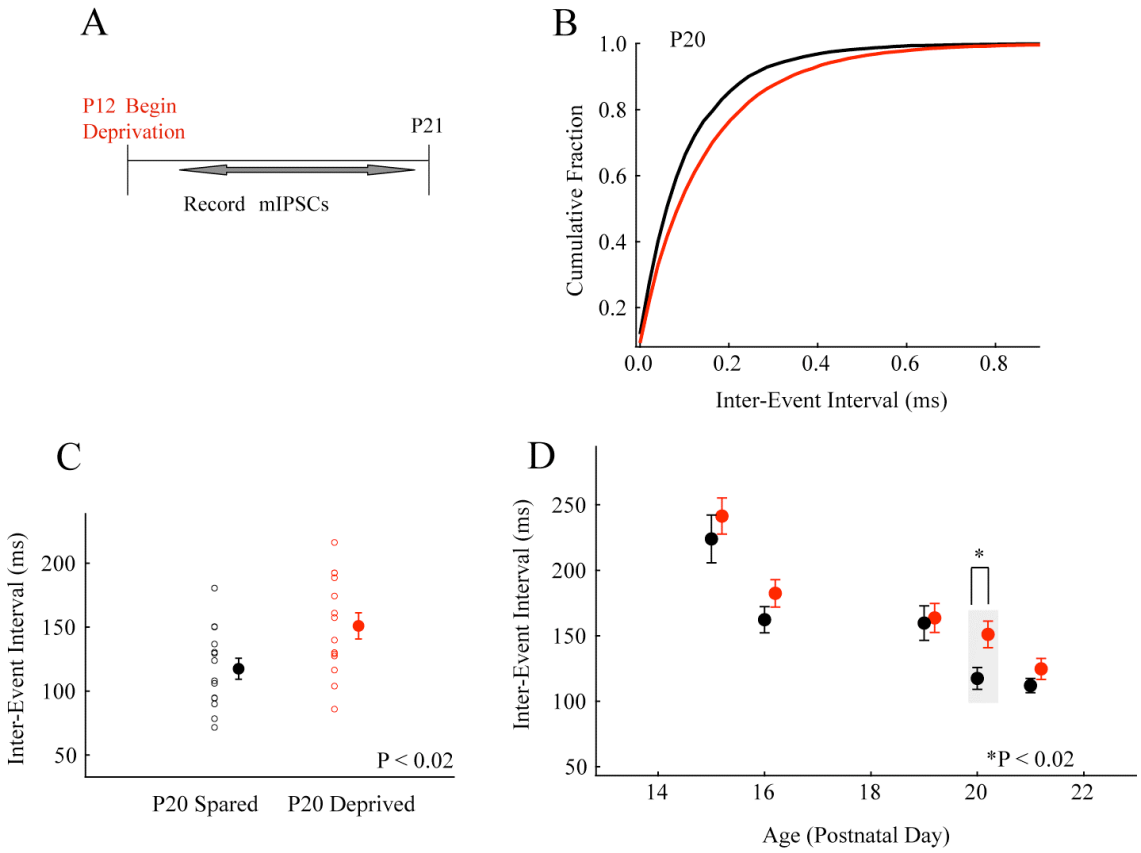

Fig 2.17: Delaying the onset of whisker deprivation by 5 days delayed the reduction of mIPSC frequency by 5 days. A, Altered timeline of whisker deprivation, now beginning at P12. B, Cumulative histogram of mIPSC IEI from spared and deprived cortex after 8 days of deprivation, at P20. C, mIPSC amplitude values, with means, from spared and deprived cortex at P16. D, Mean mIPSC IEI from spared and deprived cortex at 5 different ages from P15 to P21.

III. Conclusions and future directions

We found that sensory deprivation transiently reduces the efficacy of inhibitory signaling onto excitatory cells in developing rat S1, as measured by mIPSCs as well as evoked IPSCs. This effect is a homeostatic form of plasticity because it would act to enhance excitatory drive in response to sensory deprivation. The effects on inhibitory signaling were only observed during a brief critical window, lasting 1-2 days, after which responses recovered to control levels despite continued whisker deprivation. The decreases in mIPSC amplitude and frequency were teased apart in experiments where the onset of sensory deprivation was delayed, demonstrating that these two effects are mechanistically independent. We conclude that, with the exception of brief periods of plasticity, the development of inhibitory signaling in layers 2/3 and 4 proceeds in an experience-*independent* manner during the first postnatal month of rat S1. This finding is in contrast with what has been reported in rat visual cortex (Turrigiano and Maffei 2008) and mouse somatosensory cortex (Sun 2007, Sun 2009).

Targeting specific neuron classes

The present study targeted excitatory neurons in L4 and excitatory pyramidal neurons in L2/3 (93% of recovered cells were spiny, see methods). Unlike most cortical layers, L4 is unique in that it contains similar proportions of two morphologically separate classes of primary excitatory neurons: star pyramidal (SP) and spiny stellate (SS). In our hands, 16 out of 38 recovered cell bodies (42%) were

classified as SP cells, thus present results must be interpreted with respect to this target cell heterogeneity, and future experiments (such as paired recordings with post-hoc cell recovery) would be necessary to determine if there are target-cell specific differences in the expression of plasticity.

Different classes of interneurons have functionally unique roles in cortical processing, and can respond differently to early monocular visual deprivation. For example, synapses between fast-spiking interneurons and pyramidal cells in L4 underwent a decrease in unitary strength and increase in PPR, but unitary responses between regular-spiking interneurons and pyramidal cells were dramatically increased (Maffei et al. 2004). This intricate regulation of inhibitory plasticity in V1 necessitates further experiments in S1 to determine whether the plasticity we observed after whisker deprivation impacts inhibitory synapses in a subclass-specific manner. This can be accomplished with paired recordings between inhibitory and excitatory cells where physiological and morphological criteria are used to distinguish interneuron subclasses. Additionally, transgenic mice are available where certain classes of interneurons are labeled with fluorescent makers, allowing targeted recordings to those cell classes.

Investigating synaptic mechanisms

Further experiments will be necessary to determine the cellular and molecular mechanisms that underlie the changes we report in mIPSCs during normal development, and during whisker-deprivation plasticity. One interesting question is whether or not the changes observed during these two conditions share the same

mechanisms. For example, the dramatic developmental increase in mIPSC frequency after P7 may represent the increase in inhibitory synaptic contacts at this time (DeFelipe et al. 1997), but does the deprivation-induced reduction in mIPSC frequency at P15 represent a reversal of this change (i.e. a decrease in synaptic contacts), or a different synaptic change? Furthermore, is the normal reduction in L4 mIPSC amplitude between P18 and P22 mediated by the same mechanistic pathway as the deprivation-induced reduction of L4 mIPSC amplitude at P15?

Our finding that deprivation induces a very brief change in mIPSCs and evoked IPSCs begs the question of how this plasticity is transiently regulated. Specifically, does the recovery of mIPSC amplitude and frequency back to control levels after P15 in L4 and P21 in L2/3 represent a reversal of the mechanisms that underlie plasticity, or independent changes initiated to counter plasticity? In either of these cases, what triggers the rapid recovery of IPSCs? Layer-specific differences in the expression of deprivation induced plasticity have been shown in V1, (Crozier et al. 2008), thus it will also be important to determine whether mIPSC plasticity observed here in L4 and L2/3 share the same mechanisms.

Determining the role for competition in driving plasticity

In our experiments, physiological responses were compared between the deprived "D" row of cortical barrels, and the spared "B" row. Competitive interactions between inputs are known to be important for driving certain aspects of plasticity in S1, and may also be important for certain non-Hebbian forms of plasticity (Fox 2002, Bienenstock et al. 1982, Turrigiano and Nelson 2000). In our experiments, deprived

barrel columns are surrounded by spared ones, and it is uncertain whether cross-columnar connections between the two provide competition that is necessary for inducing the plasticity that we observe. A useful experiment to answer this question would be to perform full whisker removal on one set of animals, and leave one set unperturbed. With this paradigm, recordings can be made from sensory-deprived brain slices, and compared with those from sensory-spared brain slices. If no differences between these two conditions are measured, it would tell us that competitive interactions between spared and deprived columns are necessary for inducing plasticity.

Studying excitatory/inhibitory balance

It would be difficult to posit the functional outcome of the inhibitory (I) plasticity observed in this study for sensory processing without knowing how excitatory (E) transmission is regulated in conjunction. A delicate E/I balance in cortical circuits is critical during sensory processing and to avoid epilepsy (Chagnac-Amitai and Connors 1989, Tasker and Dudek 1991, Wehr and Zador 2003, Cossart et al. 2001), and so how this balance is maintained during cortical plasticity is an important subject of study.

Recent studies in V1 have shown that experience dependent synaptic changes in excitation are often accompanied by changes in inhibition. Pre-critical period visual deprivation in the rat enhanced excitatory synaptic transmission in L4, and simultaneously weakened FS-Pyr cell inhibitory synapses, amounting to a robust homeostatic response to sensory deprivation (Desai et al. 2002, Maffei et al. 2004).

Our study focused on only inhibitory synapses, and thus an important next step in investigating the changes we have observed is to examine the effect of whisker deprivation on miniature and evoked EPSCs in L4 and L2/3 of barrel cortex during the first postnatal month of development.

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