FUNCTIONAL REORGANIZATION OF BARREL CORTEX FOLLOWING ATYPICAL SENSORY REARING EXPERIENCES: THE EFFECT ON CORTICAL SPIKE SYNCHRONY

By

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Dedicated to my parents

who has not spent a moment of their life

without praying for my success

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CHAPTER I

INTRODUCTION

Functional reorganization following sensory deprivation in rat barrel cortex

Altered sensory experience has been shown to have numerous effects on cortical single cell and network properties. One popular method to study the effects of altered sensory experience on cortex has been to study the effects of reduced activity from the periphery by depriving a sensory system of the normal patterns of activity during postnatal development. The consequence of visual sensory deprivation has been seminally studied by Wiesel and Hubel (1963) in kitten visual cortex. They identified a clear postnatal “critical period” during which abnormal sensory experience, such as closing the eyelids over one eye, produced profound changes in the anatomy and physiology of visual cortex: the open eye developed an abnormally dominant drive on almost all cortical neurons and the inputs to cortical neurons from the closed eye were reduced in efficacy and/or rendered ineffective. Thereafter, a number of studies have reported the long lasting impact of sensory deprivation on the magnitude, timing and other cortical circuit properties in a sensory system. The effects of sensory deprivation on cortical activity and perception are particularly severe in humans affected by long-term sensory deprivation, especially when the deprivation occurs during the critical period of the deprived sensory modality (Sharma et al. 2002, Fine et al. 2003).

The rat whisker system and its cortical representation called the posteromedial barrel field cortex or simply barrel cortex has been a good model for studying the

functional reorganization produced by sensory deprivation. In addition to the smaller sensory hairs on their nose and around their mouth, rats have approximately 26-30 large mystacial whiskers or vibrissae on each side of their face, which are highly specialized as sinus hairs innervated by roughly 200 primary sensory axons. These whiskers are as arranged in 5 horizontal rows, with the corresponding whiskers in each row given a number from posterior to anterior and referenced as arcs (Fig. 1-1). The axons innervating the whiskers form extraordinary localized fascicles and terminals in their trajectory to cortex, with precise topographic clustering in the thalamic (VPM and POm) and brainstem (Trigeminal nuclei) relay stations (Belford and Killackey 1979; Arvidsson 1982; Land and Simons 1985) as well as in the posteromedial barrel subfield in S1 cortex (Woolsey and Van der Loos 1970) (Fig. 1-1B). In each of these stations including barrel cortex, there are specialized clusters of neurons that have been reported to respond predominantly to a single whisker. Such clusters of neurons in cortex were first described and named ‘barrels’ by Woolsey and Van der Loos in 1970. These barrel-like structural columns are surrounded by septal columns, which have strikingly different connections in the rat brain (Kim and Ebner, 1999). These structures can be readily identified by several histological markers like cytochrome oxidase (Wong-Riley and Welt 1980; Jain et al. 2003) (Fig. 1-1B).

Several features render the barrel cortex a good model for brain research. The fact that the peripheral whisker system can be easily manipulated to alter activity levels is one of the prime reasons for using this system as a model to study cortical reorganization following either developmental or adult alterations in sensory activity. Thus, several approaches have been adopted to create a whisker deprived model. The

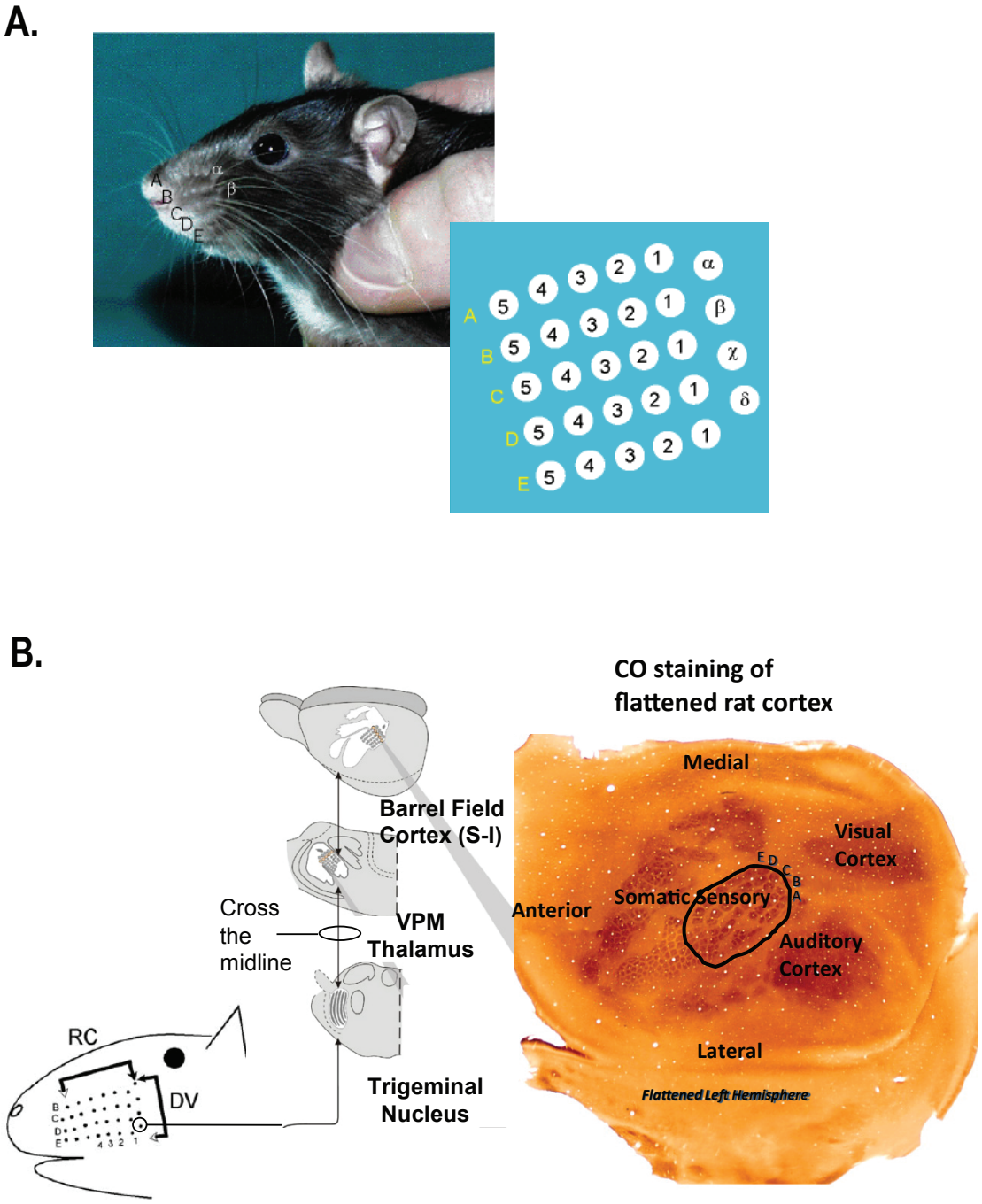

Figure 1-1: The Rat Whisker system

A. Organization of whiskers on the rat's face. Large whiskers are organized in dorsoventrally into five rows (A - E), rostrocaudally into five arcs (1 - 5). Rows are straddled caudally by another set of 4 large whiskers (α - δ). B. Whisker to S1 cortex pathway in the rat. Each whisker is innervated by trigeminal nerve fibers that terminate in the trigeminal nucleus of the brain stem. Fibers from the trigeminal nucleus cross the midline and project to the Ventral Postero Medial (VPM) nucleus and also to the posterior nucleus (POm, not shown). VPM fibers project to the barrel subfield of the S1 cortex, primarily in layer IV barrels giving rise to the barrel like structures in layer IV cortex. Such barrel structures (circled area) can be visible by cytochrome oxidase staining of flattened tangential sections through the layer IV as shown to the right (Jain et al. 2003).

whiskers could be non-invasively trimmed, slightly damaged by plucking, seriously disrupted by cauterizing the plucked whisker follicles, or cutting the infraorbital nerve before it spreads out to innervate the whisker pad. All of these manipulations reduce neural activity arising from a few or all whiskers on one or both sides of the face and have been shown to affect many aspects of cortical function. In this thesis I focus on the effect of non-traumatic reduction in neural activity using trimming of all whiskers on either one or both sides of the face. However, the literature with which my results are compared have employed many combinations of whisker manipulations. While some have reported effects of removing one row or one arc of whiskers, there are also reports that have studied the effect of removing a single whisker or all but 1 whisker on the development of barrel cortex. In adult rodents, a marked reduction of metabolic activity in the deprived barrels was observed following the non-traumatic removal of whiskers as early as 1970 (Durham and Woolsey 1978). Traumatic damage to the whisker pad in neonatal rats, in turn, was shown to alter both the anatomical arrangement of the barrels and the physiologically determined somatotopic representation of the sensory periphery if the damage occurred prior to postnatal day 4 (Simons et al. 1984). Non-traumatic procedures like whisker trimming starting from the day of birth and spanning the entire developmental critical period have been also shown to alter single cell neurophysiology of the deprived barrels when compared to the spared barrels in the same hemisphere (Simons and Land 1987; Fox 1992). Whisker trimming led to enlarged receptive fields, reduced directional preferences (angular tuning), increased responsiveness and altered spike timing patterns in the deprived barrel neurons when compared to those in the spared barrels. Interestingly, selective whisker trimming also

affected barrel neurons related to the spared whiskers which showed altered response properties such as smaller ON/OFF ratios compared to normal controls with all whiskers intact (Simons and Land 1987). Experience-dependent plasticity is also prominent in layer 2/3 of the barrel cortex. Whisker deprivation started before P14 disrupts receptive field structure in layer 2/3 neurons. Unlike normal animals, receptive fields of the deprived animals had small amplitude centers and large amplitude and broad surrounds with whiskers three or more follicles away producing peaks of excitation (Stern et al., 2001). Moreover, neonatal whisker damage has been shown to specifically alter the patterns of intracortical connections but not thalamocortical projections in barrel cortex (mouse: McCasland et al. 1992; Rat: Keller and Carlson 1999). Such experience dependent plasticity is now known to be dependent on NMDA receptor activation (Rema et al. 1998) and endocannabinoid signaling (Li et al. 2009). There is also substantial evidence that such plastic changes observed with sensory deprivation are dependent on a critical time window, which seems to be longer for supragranular layers than granular layers of barrel cortex (Fox 1992; Stein et al. 2003). In contrast, whisker deprivation has a limited effect on subcortical regions. There is some evidence that whisker trimming enhances short-latency responses in the ventral posterior medial (VPM) thalamic nucleus, reduces spontaneous activity (Dolan and Cahusac 2007), and reduces the discrimination of tactile stimuli by thalamic ensembles in adult rats (Nicolelis et al. 1997). However, whisker trimming started at birth has also been shown to have no effect on response properties of deprived VPM neurons in young adult rats (Simons and Land 1994; Fox et al 2002). This indicates that the thalamic relay nucleus for the

whisker system is less susceptible to activity-based changes caused by sensory deprivation.

Low levels of sensory activity produced by whisker trimming has also been shown to have deleterious effects targeted on inhibitory inter-neurons in rat barrel cortex (Akhtar and Land, 1991; Micheva and Beaulieu, 1995a,b). This effect is implicated in disrupting the excitatory-inhibitory balance necessary for normal sensory processing. Neonatal whisker trimming has been shown to alter the development of both excitatory and inhibitory receptive fields of barrel neurons, which can impair cortical integration of sensory information arising from multiple whiskers (Shoykhet et al. 2005). The balance between excitation and inhibition was also reported to be altered in deprived as well as non-deprived barrels when a single row of whisker was trimmed in young newborn rats (Marik et al. 2010). Fast spiking interneurons were shown to be selectively suppressed after sensory deprivation (Sun 2009) leading to enhanced LTD in cortical inhibitory networks (Sun and Zhang 2011). Also in the whisker trimmed animals the feed forward connection between layer IV and neurons in the superficial layers II/III is changed in a fundamental way. In normal animals, the flow of intracortical excitation upon striking a single vibrissae has been described as being transmitted within a column from a layer IV barrel, corresponding to the stimulated whisker, to the superficial layers above the same barrel with a few ms delay, before spreading horizontally to the septal column and the neighboring barrel columns (Armstrong-James et al., 1992; Kim and Ebner, 1999; Lubke et al. 2000; Petersen and Sakmann, 2000; Brecht and Sakmann, 2002). However, with reduced peripheral activity there was LTD-like depression at intracortical excitatory synapses between cortical layer IV and L-II/III pyramidal neurons, thereby

impairing the intracortical relay of deprived inputs from layer IV to layer II/III in barrel cortex at maturity (Rema et al. 2003; Allen et al. 2003). Thus active sensory experience seems to be important for the development of the initial intracortical network properties, and since whisker trimming has such specific deleterious effects on inhibitory interneurons, it can be implicated in disrupting the excitatory-inhibitory balance necessary for normal sensory processing. In fact, it has been shown recently that sensory experience governs strengthening of the thalamocortical synapses to the feedforward interneurons and down-regulation of the thalamocortical synapses onto excitatory neurons (Chittajallu and Isaac 2010) and is therefore important for the development of the excitation/inhibition balance in barrel cortex.

Strictly anatomically, the whisker-specific map in barrel cortex as well as in the subcortical regions (Henderson et al. 1992) is maintained in whisker trimmed animals, suggesting that sensory experience has a limited role in the formation of the map, but is critical for refinement of thalamocortical circuits. However, there are certain subtle anatomical changes that are observed with deprivation in barrel cortex. Unilateral whisker trimming leads to an increased number of dendritic spines in the deprived half of the cortex (Vees et al. 1998) with an increase of inhibitory synapses onto dendritic spines (Micheva and Beaulieu 1995a), in addition to reducing the protrusive motility of the spines (Lendvai et al. 2000). However, with bilateral whisker trimming there is a significant decrease in density of dendritic spines and spine head diameter. Such disruptions of spine development with bilateral whisker trimming were reported to be only in layers I and II-III but not in L-IV (Briner et al. 2010). Trimming of some whiskers also leads to a reduction of overall length of axonal branches in L-II/III, with the

reductions more conspicuous for the axons oriented towards the deprived barrels (Bruno et al. 2009). Moreover, the density of the VPM, and not the POm axons was also reduced in the deprived columns. However, the axonal changes were reversible upon regrowth of the trimmed whiskers which may indicate a form of continued anatomical plasticity in barrel cortex beyond the critical period (Wimmer et al. 2010).

Tactile behavior is also significantly affected by whisker trimming. Adults in whom whiskers have been removed show alterations in the behavior of bringing the snout into contact with surfaces of interest (Vincent 1912; Meyer & Meyer 1992). Animals in which whiskers are plucked shortly after birth also exhibit pronounced behavioral compensations as adults (Gustafson & Felbain-Keramidas, 1977; Volgyi et al. 1993) and also difficulty in nipple attachment and huddling (Sullivan et al. 2003). Rats with bilateral whisker clipping after birth show deficits in tactile acuity after the whiskers are allowed to regrow (Carvell and Simons 1996). These rats, who can distinguish between a rough and a smooth texture, fail to distinguish between two differently graded rough textures that normal animals can distinguish readily. On the other hand, rats and mice show a remarkable ability to perform behavioral tasks, like a gap-cross problem or object localization, even with a single whisker intact (Hutson and Masterton 1986; Harris et al. 1999; Mehta et al. 2007; O'Connor et al 2010). Also, behavioral deficits with unilateral deprivation are rarely reported. The neurophysiological basis for such deficits were unclear until recently Popescu and Ebner (2010) carried out careful experiments in teasing out single neuron response property deficits in bilateral and unilateral deprived animals. The study showed that developmental unilateral deprivation leads to a strong shift toward sensory processing in the sepal circuit instead of in the cortical barrel circuit

confirming previous *in vitro* results in brain slices from unilaterally deprived animals from only P-9 to P-14 (Shepherd et al. 2003). There is an increase responsiveness of the septal neurons during both the short and long latency responses. With bilateral deprivation there was a striking overall reduction in response magnitude and variable and longer onset-latency of responses (Fig. 1-2). These results indicated that the deficits associated with bilateral deprivation are more profound than those found after unilateral whisker trimming. With these studies to date as background, I looked at how neuronal network properties could be affected with sensory deprivation by studying the effect on cortical spike synchrony after bilateral and unilateral whisker trimming. Also, given that the unilateral single cell responses were uniquely different from the ones with bilateral trimming, we hypothesized that such differences would also be striking in the network properties.

Cross-sensory influence in primary sensory cortex

Multisensory interactions that generate convergence of information from different sensory modalities greatly enhance the perceptual sensitivity of an animal (Stein and Meredith 1990; 1993). Psychophysical phenomena in humans such as the “Parchment-Skin Illusion” (Jousmaki and Hari 1998) show how the somesthetic perceptions in humans for rough and smooth surfaces are influenced by associated sounds. These multisensory interactions highlight an extensive cross-talk between somatosensory and auditory sensory processing that predicts a significant somesthetic-auditory interaction during exploratory behavior. Other psychophysical experiments have shown that tactile stimuli can enhance a subject’s ability to perform in a visual reaction time task (Bauer et

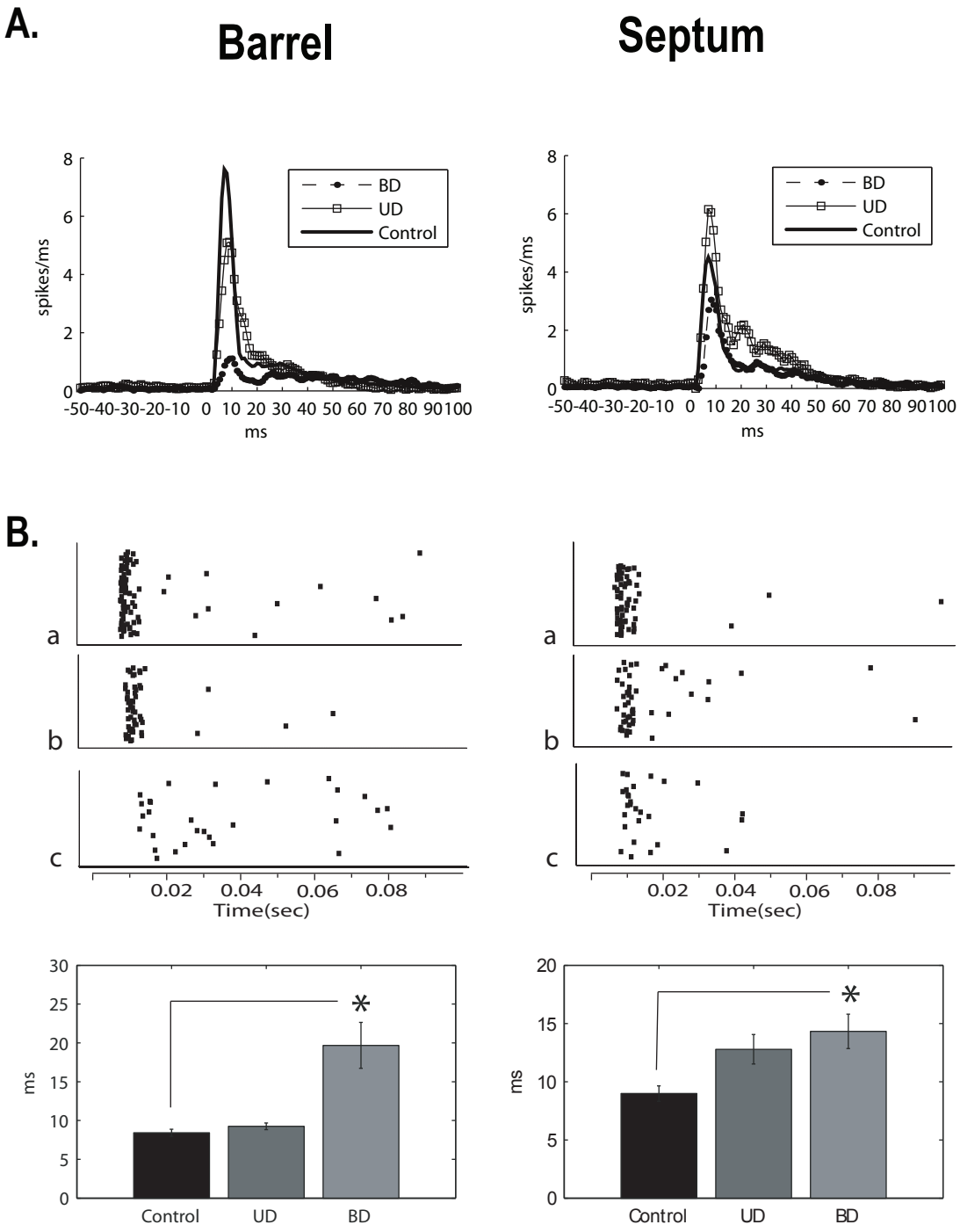

Figure 1-2: The effect of unilateral (UD) and bilateral (BD) whisker trimming on magnitude and onset latency of single cells

A. Population PSTHs in response to a whisker stimulus in layer IV barrels (left column) and septa (right column). When compared to the normal (CON), there is a significant reduction in the magnitude of response only in BD rats in both barrels and septa, whereas, with UD there is a significant increase in the response magnitude of the septa, but not in the barrels. B. Single cell first spike raster plots and population mean onset-latency bar graphs for CON, BD and UD neurons in layer IV barrels (left column) and septa (right column). With BD there is an increase in mean onset-latency for both the barrel and septum neurons. However, in UD rats no such significant increase was observed in either the barrels or the septa neurons. (Popescu and Ebner 2010)

al. 2009) indicating the presence of somesthetic-visual interactions in humans. There is also evidence of multisensory interactions between the auditory and visual modalities as shown by an improvement in visual contrast detection by a simultaneous sound (Lippert et al. 2007). Even olfaction has been shown to modulate word and face processing in humans (Walla 2008). Brainstem structures, particularly the superior colliculus (Meredith and Stein 1983; see Stein and Meredith 1993 for a review) and the Dorsal Cochlear nucleus (Shore et al. 2000; Zhou and Shore 2004; Dehmel et al. 2008) have long been identified as putative structures mediating many such multisensory interactions.

Several neocortical areas have been shown to process information from more than one sensory modality in primates (See Ghazanfar and Schroeder 2006 for a review). Association areas like the superior temporal sulcus (Hikosaka et al. 1988; Bruce et al. 1981; Schroeder and Foxe 2002), the caudomedial auditory 'belt' area in the superior temporal plane (Smiley et al. 2007), the lateral and ventral intraparietal sulci (Andersen et al. 1997; Duhamel et al. 1998; Bremmer et al. 2002), the frontal cortex including the prefrontal cortex (Fuster et al. 2000) and premotor cortex (Graziano et al. 1994,1999) in the primate brain have been shown to have strong multisensory convergence. Studies in cats also have shown specialized multisensory areas in the neocortex such as the ectosylvian and the sulprasylvian sulci (Wallace et al. 1992; Jiang et al. 2001). In rodents, the dysgranular zone between the barrel field and A1 cortex (Brett-Green et al. 2003) and the transitional zones between the different sensory areas in rodents (Wallace et al. 2004) show capabilities for integrating multisensory information.

Lower order sensory areas and the primary sensory areas have long been thought to respond to only one modality of sensory input and to be completely devoid of multisensory integration. Electrophysiological studies in the late 60's provided evidence for the possibility of visual cortex being responsive to non-visual acoustic or noxious stimuli (Murata 1965; Spinelli 1968; Bental 1968) but they were not taken seriously because of the large number of studies suggesting modality exclusivity, often without careful search for any multisensory influence. However, studies in the last two decades have generated a strong argument for the notion that neuronal responses of the lower order sensory areas including the primary sensory areas are significantly modulated by inputs from other sensory modalities in "normal" animals – a phenomenon that has been regarded as 'cross-modal' or 'cross-sensory' interactions (See Foxe and Schroeder 2005; Ghazanfar and Schroeder 2006; and Kayser and Logothetis 2007 for a review). Most of the evidence for such cross-sensory interactions has come from electrophysiological as well as anatomical studies performed in both awake and anesthetized primates, carnivores or rodents. Studies in ferrets have reported the presence of sub-threshold multisensory influences in visual area 21 which was previously considered to be an exclusively unimodal visual area. The multisensory influence was enhanced after disinhibition induced by GABA receptor antagonists. The authors predicted that the effect was mediated by corticocortical connections from A1 to area 21 (Allman et al. 2008). The primary auditory areas in ferrets including the anterior auditory field, anterior ventral field and A1 were shown to contain neurons responsive to only visual stimuli, and others responsive to paired auditory and visual stimuli (Bizley et al. 2006). This study also demonstrated the presence of anatomical connections arising

in V1 cortex and projecting directly to A1, to higher visual areas, and to other auditory areas in ferret (Bizley et al. 2006). Sub-threshold forms of multisensory interaction were demonstrated in cat S-IV (Dehner et al. 2004), in the FAES auditory field of cat (Meredith and Allman 2009), and in cat extrastriate visual cortex (Allman and Meredith 2007). Anatomical studies investigating the projections to and from the A1 cortex have revealed that many cortical non-auditory areas have reciprocal connections with the A1 cortex (See Budinger and Scheih 2009 for a review). For example, tract tracing studies in the Mongolian gerbil have shown that A1 projects to other non-auditory areas including the S1 cortex (Budinger et al. 2006). A thorough investigation in the marmoset showed the presence of indirect anatomical connections from the visual area FST to S1 via a relay in area MT, and a projection from several higher order somatosensory areas including S2 to A1 (Cappe and Barone 2005). Single unit recordings from anesthetized monkeys have shown that somatic and visual stimuli can strongly activate A1 as well as the well-documented caudal-medial belt area (Brosch et al. 2005). Recent local field potential (LFP) studies from awake behaving rhesus monkeys have shown that core area A-1 and the lateral belt area integrate facial (visual) and vocal (auditory) signals through enhancement and/or suppression of LFP amplitude (Ghazanfar et al. 2005). Both LFP's and single unit responses in primary and secondary auditory areas were also shown to be strongly modulated by visual (Kayser et al. 2008). These cross-sensory modulations by a visual stimulus were shown to increase the encoding of stimulus information carried by the neuronal responses in auditory cortex when alert monkeys were watching an audiovisual naturalistic stimulus. Importantly, such an enhancement was not observed when the visual stimulus did not match the sound

(Kayser et al. 2010). Recently Lakatos et al. (2007) examined the influence of somatosensory input in macaque A1 cortex. Event- related current source densities (CSD) showed a significant increase in amplitude in the supragranular layers of A1 to somatosensory stimulation, indicating a modulatory as opposed to a driving type of input from the somatosensory system. Moreover, a combined somatic sensory and auditory stimulus led to a super-additive multisensory interaction both in the CSD and in multi-unit activity in all layers of A1 (Fig. 1-3). Lakatos et al. postulated that somatosensory events reset the ambient oscillations in A1 and the phase of these reset oscillations determined the type of effect on the auditory responses (Lakatos et al. 2007).

Such cross-modal interactions have also been shown to be present in normal human cortex. Neuroimaging studies have shown activation of the primary auditory cortex by linguistic visual cues in the absence of auditory speech sounds (Calvert et al. 1997). Further, significant audio-tactile interactions occur in presumed unisensory structures such as S2, auditory belt area and posterior parietal cortex (Schurmann et al. 2006). Bauer et al. in 2009 showed an increase in gamma band MEG activity in occipital cortex in the presence of task-irrelevant tactile stimuli while subjects were performing a visual reaction time task. This increase in gamma was correlated with improved performance in the task, although the causal link has yet to be established (Bauer et al. 2009). All this evidence together strongly challenges the theory that primary sensory areas are purely unisensory.

The phenomenon of cross-modal/cross-sensory plasticity has been more readily proven. This phenomenon refers to the ability of a second sensory modality to take over

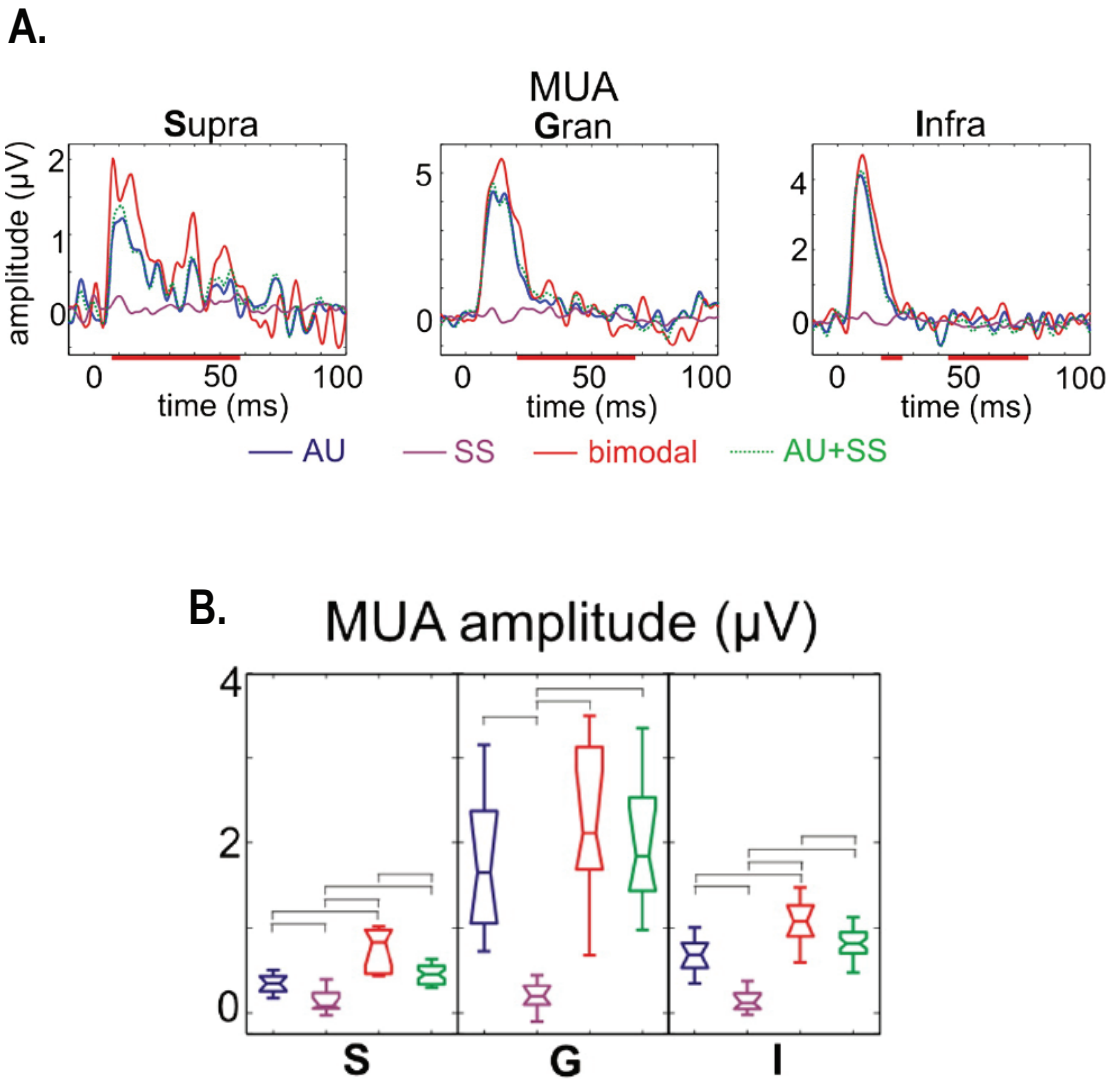

Figure 1-3. Somatosensory influences in macaque A1 cortex
A. Multiunit activity (MUA) of A1 supragranular (Supra), granular (Gran) and infragranular (Infra) neurons in response to auditory, somatosensory and bimodal stimuli, indicating super-additive response activity to the bimodal stimulus (red trace). Green dotted line shows the arithmetic sum of the unimodal responses. Red lines on the time axis denote time intervals where the averaged bimodal responses were significantly greater than the sum of the averaged unimodal responses. Strongest interactions were oberved in the suragranular layers of A1 cortex. B. Box plots show MUA amplitudes of the supragranular, granular and infragranular neurons (S, G, and I), averaged for the 15–60 ms time interval for the same conditions as in A. Brackets indicate the significant interactions (Lakatos et al. 2007).

or strongly modulate a weak or deprived modality (See Bavelier and Neville 2002 for review). Sensory deprivation of a single modality has been previously shown to affect the neurophysiological properties of neurons (Wiesel and Hubel 1963; Simons and Land 1987; Popescu and Ebner 2010) rendering it highly disorganized and inefficient. On the other hand, such deprived cortex has a remarkable capacity for showing cross-sensory plasticity or reorganization in response to the removal or modification of sensory inputs. For example, cross-sensory plasticity has been reported for visually deprived humans (See Collignon et al. 2009 for a review) and in higher order auditory areas after congenital auditory deprivation (See Kral 2007 for a review). Activation of visual areas including V1 cortex was observed in congenitally blind people while resting and reading Braille as measured by PET scanning (Sadato et al. 1996) or fMRI (Melzer et al. 2001). A PET study has shown an increase in activity in the occipital cortex of only blind individuals when they were performing Snellen orientation detection task by the tongue (Ptito et al. 2005). A subsequent PET study showed an increase in activation in the middle temporal area, cuneus and extrastriate area V3 while blind individuals were performing a motion discrimination task with their tongue, while normal subjects did not show any increase in activity in such areas (Ptito et al. 2009). Similarly, deaf individuals show activation of association auditory cortex, superior temporal gyrus (Nishimura et al. 1999; Petitto et al. 2000; MacSweeney et al. 2002), secondary auditory areas including BA 22, 42 and angular and supramarginal gyrus (Lambertz et al. 2005) while visually perceiving sign language. Finney et al. in 2001 and 2003 further showed that there is activation of primary, secondary and association auditory areas in response to moving dot patterns and moving gratings exclusively in deaf subjects.

More elaborate anatomical and electro-physiological evidence for cross-modal plasticity comes from the animal studies. For example, deafferentation of A1 cortex produced by aspiration of the inferior colliculus leads to a massive reorganization of A1, which becomes visually responsive (Sur et al. 1988, Pallas et al. 1990; Roe et al. 1992; See Sur et al. 1990 for review). Also, merely 16 days of deafness in ferrets makes the A1 to respond to somatosensory stimuli, which does not occur in normal ferrets (Allman et al. 2009). Complete visual deprivation produced by enucleation also leads to extensive reorganization of the visual areas. V1 cells begin to respond to auditory stimuli in enucleated hamsters and cats as well as in congenitally anopthalmic mice, and this multisensory responsiveness is accelerated by enriched environment rearing (Izraeli et al. 2002; Yaka et al. 2000; Piche et al. 2004). V1 cells also become responsive to tactile stimuli in enucleated rabbits (Newton et al. 2002). Moreover, bilateral enucleation in opossums reportedly leads to the emergence of a new multisensory area between area 17 and 18 which becomes responsive to auditory and somatosensory stimuli in addition to visual stimulus (Kahn and Krubitzer 2002). Anatomical studies in the enucleated and anopthalmic animals indicates that such complete visual deprivation leads to the emergence or retention of direct connections from the IC to the V1 (Laemle et al. 2006; Izraeli et al. 2002) and also corticocortical connections develop between somatosensory association areas and V1 cortex (Newton et al. 2002, Toldi et al. 1996). There is also evidence for cross-modal plasticity to occur in the subcortical multisensory structures such as the superior colliculus (Mundinano and Martinez-Milan 2009) and the DCN (Dehmel et al. 2008). Thus, the removal or modification of the sensory inputs can lead to gross reorganization of the brain, wherein,

higher order and lower order sensory areas including the primary sensory areas can be susceptible to take over by other modalities.

Although a great deal of evidence discussed above indicates the presence of cross-sensory interactions and cross-sensory plasticity in the primary auditory and primary visual cortex, to our knowledge, there is much less known about the primary somatosensory cortex. Zhou and Fuster in 2000 and 2004 have shown that areas 3a, 3b, 1 and 2 of an awake behaving monkey, can be activated by visual or auditory cues only when they are associated with a haptic discrimination task. Given this background to multisensory integration, I wanted to investigate whether neuronal responses in rat barrel cortex can be modulated or driven by a non-whisker auditory click stimulus. I also wanted to study whether increasing auditory activity by click stimuli in the rearing environment with or without decreasing activity in the somatosensory system by bilateral whisker trimming early in life would modify the expression of cross-modal properties in normal barrel cortex. Thus, the primary goal of this thesis was to see whether primary sensory S1 cortex can be shown to express multisensory properties, and how these properties might be related to the type of early sensory experience encountered during postnatal rearing.

Cortical spike synchrony: an introduction

Although the brain is made up of individual neurons, recent evidence suggests that the perception of a sensory experience requires the activity of an ensemble of neurons. It is possible the combined activity of a group of neurons in conjunction with each neuron's independent activity, creates a neural code that can be used to generate

all the information needed to generate a perception. Such a neural code can be based on instantaneous firing rates, integrated firing rates, mean inter-spike interval duration of a group of neurons and/or a cluster of neurons producing coincident spike firing (Eggermont 2001, 2006). The thought that neurons can indeed work together was pioneered in the early 1940s when Charles Sherrington hypothesized that communication between neurons involve some sort of a “population” code that might contain information not available from the individual responses of neurons (Sherrington 1941). In 1942, Lord Adrian showed that neurons in the olfactory bulb indeed show what he called coordinated activity. Many studies have followed, and although we do not completely understand how neurons encode information, there is a beginning consensus that neurons indeed are functionally dependent and information is better coded by a population of neurons rather than a single neuron (Casagrande et al. 2002; Shadlen and Newsome 1994).

One important constituent of a population code is synchronized neural activity in the brain. Synchronized neural activity can be defined as the activity of a group of neurons that has a specific temporal pattern irrespective of each neuron’s firing characteristics. Synchronized neural activity can be characterized by a variety of measures. Grossly, it can be classified into oscillatory synchrony and spike synchrony (Jermakowicz and Casagrande 2007). Oscillatory synchrony is a broader form of synchrony where large networks of neurons synchronize with each other. Oscillatory synchrony has been implicated in several cognitive functions, including feature binding and scene segmentation (Singer and Gray 1995), memory formation and recall (Tallon-Baudry and Bertrand 1999) and attention (Fries et al. 2001). Spike synchrony on the

other hand is the temporal correlation of spikes belonging to a group of neurons that are simultaneously recorded from a local circuit or from distant areas. Spike synchrony has been especially well studied in the visual system and appears to be extremely important, for encoding information in the visual areas. Spike synchrony is also widespread and is a common occurrence in other areas of the cortex as well. In this paper, I would review the incidence, importance and plasticity of spike synchrony in cortex with particular focus on the somatosensory areas.

Several terms are used interchangeably when describing spike synchrony such as spike correlations, spike coincidence, correlated discharge, and others. Correlation of neural activity can take many forms, e.g., it can refer to the detection of coincidences in the firing times of two neighboring nerve cells, or the detection of co-variation in the firing rates of those nerve cells. It can be the co-variation in the activity pattern of neuronal groups, but it can also be the co-variation in the postsynaptic activity caused by activity in a cell's many inputs (Eggermont 2007). The incidence of correlation can be measured in a number of ways. The most popular method has been the cross-correlogram (CCG). CCGs employ a cross correlation analysis between a pair of neurons and the result portrays changes in the probability of a target neuron discharge given that the reference neuron discharged at time zero. Time-locked discharges of a pair of neurons, called coincident events, appear as peaks or valleys in the CCG indicating possible excitatory or inhibitory interactions, respectively (Perkel et al. 1967; Gochin et al. 1989). A more specific method is the Joint-Peri- or Post-Stimulus-Time-Histograms (JPSTHs) that has good temporal resolution and allows one to observe the coincidence of spikes over time after a stimulus or a behavioral event (Gerstein et al.

1989). There are several other methods that have developed over time to calculate spike correlations between 2 neurons, such as conditional correlation analysis (Alloway and Roy 2002) and peri-event cross correlation analysis (Paiva et al. 2008) etc. Despite the method used, in a stimulus-based paradigm correlated neuronal activity can be produced by stimulus coordination or may occur randomly according to the rate of neuronal discharges. In order to correct such spurious correlations, several control steps are adopted one of which is the shift predictor (Gerstein and Perkel 1972).

Correlated neural firing can be measured in both single and “multiunit” groups of neurons. Many studies have deduced important conclusions from multiunit studies of neural synchrony. However, multiunit synchrony is controversial because evidence from neural modeling suggests that interpretation of neural correlations from multiunit recordings may be ambiguous as it is not a linear combination of correlations from the various single-unit pairs (Gerstein 2000). However, changes in single-unit correlation strengths will be accompanied by comparable changes in the correlation between multiunit activities (Eggermont 2000). Thus, multiunit synchrony under some conditions seems to be a good representational measure for neuronal spike synchrony, and it is easier because the waveforms do not have to be separated from individual neurons.

In the following sections, I would try to synthesize the different studies that employ the above measurements to quantify spike synchrony in the somatosensory area, in conjunction with the insights that has been gathered on neural synchrony from other cortical areas, in order to give a background on the role of spike synchrony in somesthetic or haptic information processing.

Incidence and importance of spike synchrony in somatosensory areas

Although the importance of coincident discharge in somatosensory processing has not been explored to a great extent like other cortical areas, especially the visual cortex, examples of spike synchrony reported in this area of cortex have given important insight into the functionality of synchrony. The literature for somatosensory synchrony is quite scattered and therefore in this section I would try to bring together all the evidence that support its functional role. The existence of neuronal coincidence in cortex was first hypothesized back in 1978 and 1987 by Edelman. This was extended to the somatosensory cortex in 1987 and 1990 where it was hypothesized that S1 cortex comprises radially oriented populations of neurons that share a common input, and that these inputs are shaped by coincident activity (Merzenich 1987; Merzenich et al. 1990). These radially oriented populations of neurons were referred to as functional units similar to “minicolumns” as defined by Mountcastle in 1957. The 2 basic hypothetical features of these functional units were elaborated as: 1. Strong coupling between neurons within the same functional unit as opposed to weak coupling between neurons of neighboring units and 2. Excitatory neurons in a group were positively coupled and all excitatory neurons in the same group share the same input. In other words, it was hypothesized that temporally coincident responses were necessary for assigning neuronal group memberships (Recanzone et al. 1992). This hypothesis was first tested in S1 cortex in 1993. It was found that there was a positive relationship between correlation strength and receptive field overlap between neurons. Interestingly, following intra-cortical micro-stimulation (ICMS), which reorganizes the receptive field (RF) properties of S1 neurons with distant neurons having more RF overlap (Recanzone

1992), there was an increase in correlated activity among the distant neurons that were uncorrelated before (Dinse 1993). This provides evidence that similarly grouped neurons have temporal coincidence of spikes and this coupling was a plastic property which can be modified in parallel with functional remodeling that were described previously (See Kaas 1991 for a review).

This finding led to a series of experiments trying to fully characterize the nature and more importantly the function of spike synchrony in somatosensory cortex. A subsequent study showed the presence of sharp neuronal spike synchrony in a single S1 barrel column among inhibitory interneurons of awake rabbits. The sharp synchrony observed here was not dependent on the activation of peripheral receptors as it was maintained even after locally anesthetizing the receptor periphery by lidocaine. In addition, the authors also showed that the sharp synchrony was not dependent on anesthesia as they found that administration of Brevital Sodium did not change the profile of synchronous discharge (Swadlow et al. 1998). It was hypothesized that the observed spike synchrony was probably induced by the divergent and convergent presynaptic thalamocortical network known to link VB barreloid neurons with the inhibitory interneurons (Swadlow 1995; Swadlow et al. 1998; White 1989). Functional synchronization between neurons is not only restricted within a single barrel. Simultaneous recording from neighboring barrels of isoflurane-anesthetized rats showed that neurons in different barrels are also weakly but significantly coupled under both spontaneous and stimulus-induced conditions (Zhang and Alloway 2004). Some characteristics of inter-barrel synchrony included, homogenous neural pairs with respect to its function (excitatory vs inhibitory) were more correlated during the response period

than heterogenous pairs, L-IV was least correlated both under spontaneous and stimulus-induced conditions compared to the supra and infra granular layers and were definitely weaker than the ones reported within barrel (Swadlow et al. 1998); and the infragranular neurons were maximally synchronized during the response period. This hinted at the possibility (Zhang and Alloway 2004; Zhang and Alloway 2005). Thus, in the barrel field cortex stimulus-induced synchrony is mostly observed between the 'output' layer inter-barrel neurons, whereas, 'synchrony at rest' is mostly prevalent between the 'input' layer neurons within a single barrel column. This could be due the horizontal connections in the infragranular and supragranular layers (Bernardo et al. 1990a,b; Chagnac-Amitai et al. 1990; Hoeflinger et al. 1995; Zhang and Deschenes 1997) that might mediate the intercolumnar synchrony between barrels in L-II/III and V/VI than the less horizontally connected L-IV neurons. Moreover, the magnitude and the temporal precision of inter-barrel correlated discharge was markedly influenced by the columnar configuration of barrel cortex neurons, such that neurons representing same row of whiskers had higher stimulus (both stationary and moving) induced correlations than pairs representing same arc of whisker or disparate arc or row of whiskers (Zhang and Alloway 2004). This could because there are more interconnections among barrel columns in the same row than among barrel columns in the same arc (Bernardo et al. 1990a,b; Petersen et al. 2003). The strength of neuronal synchrony was also dependent on stimulus orientation and the correlation coefficients were higher during row-directed stimulation than during arc-directed stimulation (Zhang and Alloway 2006). Such observations strongly indicate that synchronization pattern follows the anatomical connections in the barrel-field cortex.

Other examples studies have characterized synchrony in barrel cortex with the help of a different but comparable approach. Recently, Khatri et al. (2009) showed that in the cortical L-IV synchrony among neurons is independent of stimulus specific parameters like deflection angle of whiskers. Moreover, in cortex synchrony, calculated as response co-variation in this study, is reduced by 'whisker-deflection-induced sensory adaptation'. This adaptation-induced decorrelation of responses could enhance the impact of cells that continue to fire synchronously during ongoing stimulation associated with active touch (Khatri et al. 2009). By studying correlations between membrane-potential changes of neighboring neurons in supragranular layers of barrel cortex revealed exceptionally high correlation values of membrane-potential dynamics during quiet periods which robustly decreases during active whisking although the correlations remain significant (Poulet and Petersen 2008).

Neuronal synchronization is not only restricted to rat barrel cortex, but is also observed in the cat primary and secondary somatosensory cortex (Roy and Alloway 1999; Alloway et al. 2002). In cat S1 and S2 cortex synchronous activity declines with increased distance and decreased receptive field overlap and thereby supports the relation between synchrony and distance/RF overlap as shown earlier by Dinse et al. (1993) in rodent and primate S1. Thus, both the spontaneous and stimulus-induced synchronization rate and correlation coefficients were significantly affected at distances above 600 micrometer. However, the effect of distance on temporal variability was more apparent for spontaneous synchronizations (Roy and Alloway 1999; Alloway et al. 2002) which makes the point that 'sharp' synchrony at rest is more of a property of only closely spaced neurons, whereas, distant neurons can still be synchronized at minimal lag

probably due to the divergence of common inputs. In cats, however, the incidence of ‘synchrony at rest’ or spontaneous correlations are low but the proportion increased considerably with stationery/moving air-jet stimulation characterized by narrower crosscorrelogram peak-halfwidth and larger correlation coefficients than the ones observed at rest. Moreover, few neuron pairs that were synchronized by the moving air-jet showed directional preference of synchronization without showing corresponding changes in their firing rate (Roy and Alloway 1999).

Not only the local circuits of S1 and SII are synchronized, but neurons are also coupled between the primary and secondary areas. Studies show that by analyzing the temporal structure of stimulus induced neuronal responses recorded from the forepaw representation of the S1 and SII cortex, which are separated by at least 10 mm (Alloway and Burton 1985), long range synchronization within restricted time periods may occur between the two areas (Roy et al. 2001). The S1 and SII neurons were synchronized: (1) only if their RFs were highly similar and (2) when the neurons in the pair shared (overlapped) in more than half of their RFs. There was a vast majority of cells that did not show synchronization as they had non-overlapping RFs. Both thalamocortical and corticocortical interactions can play a role in mediating S1-SII synchrony. Several studies have suggested that cat S1 and SII receive parallel projections from overlapping parts of the VB nucleus of the thalamus and these thalamocortical projections also send collateral projections to both S1 and SII (Hand and Morrison, 1970; Saporta and Kruger, 1979; Spreafico et al., 1981; Burton and Kopf, 1984). There is also evidence for interconnections and interactions between corresponding representations in cat S1 and SII (Alloway and Burton 1985; Burton and Robinson 1987; Manzoni et al. 1990;

Schwark et al 1992) that can account for a corticocortical theory for SI-SII coupling. The functional significance of S1-SII synchronized activity can be attributed to facilitation of information processing and/or activation of the higher order sensorimotor areas of the cortex, receiving convergent inputs from SI and SII (Roy et al 2001). Long range synchronization was also reported between the primary motor cortex (M1) and the barrel field cortex, primarily the septum of rats (Chakrabarti et al. 2008). SI and MI activity was only infrequently synchronized in the absence of whisker stimulation, but whisker deflections caused a noticeable increase in the number of time-locked SI and MI events, with the frequency of stimulation having a considerable effect.

Examples of synchronous discharges in the somatosensory cortex are also prevalent in the primate somatosensory cortex. Studies of synchrony in primates have generated new ideas and insight on how synchrony might play an active role in somatosensory processing. In one of the studies, neurons were simultaneously recorded by a 100 electrode array from the supragranular layers of area 3b of anesthetized owl monkeys. A 0.5 sec long single site or dual site skin indentations were used as stimuli and JPSTHs were constructed to observe the level of synchrony between neurons recorded across the electrodes. Interestingly, correlated discharge was present both in neighboring and in distant neurons in area 3b. JPSTH analysis showed that the pairs were synchronized during the sustained response to skin indentation. However, the percentage and magnitude of coupled neurons decreased as the cortical distance increased (Fig. 1-4; Reed et al. 2008). This implicates that although spatial integration by area 3b neurons is very limited because of restricted RF size (DiCarlo et al. 1998; Pons et al 1987) the functional integration seems to be extended

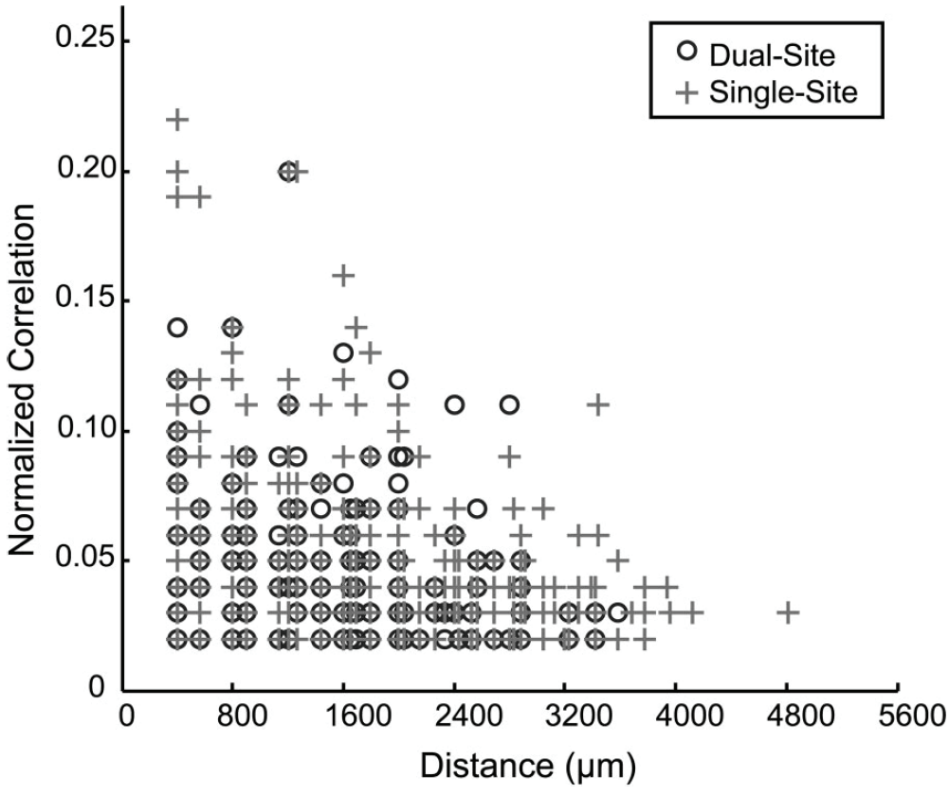

Figure 1-4. Relationship of spike synchrony with distance in area 3b of owl monkeys
A. Peak correlation magnitudes of a pair of neuron are plotted as a function of the distance between the correlated pair. The correlations were measured in response to non-adjacent site stimulations (Dual-Site) or single site control stimulations (Single-Site). There was a sharp decrease in the magnitude of correlation strength with increasing distance between the pairs in response to both the dual site and single site stimulations. However, neurons recorded from distant electrodes still had significant correlations (Reed et al. 2008).

over a larger distance (Reed et al. 2008). This study also suggests that RFs do not necessarily reflect the extent of integration that occurs in somatosensory cortex and supragranular neurons with dissimilar RF properties can be synchronized in the presence of stimulus for better integration of information. These results are consistent with the studies mentioned above, that show the presence of stimulus-induced synchrony between neurons of neighboring barrels in rat S1 cortex, with distinctly different receptive fields (Zhang and Alloway 2004, 2006).

Although the role of synchrony in somatosensory information processing is still debatable, few studies have directly correlated the presence or modulation of synchrony in somatosensory area with the perceptual processing of rodents and primates. In a very recent article Jadhav et al. (2009) have characterized that firing synchrony in rat barrel cortex is an important cue for the perception of surface texture by whiskers. When rats are trained to whisk on textured surfaces the whisking motion undergoes 'slip-stick' events' which have been hypothesized to convey information on surface properties (Wolfe et al. 2008). In this study, synchronous firing of simultaneously recorded neuron pairs in barrel cortex was observed to be significantly greater in the 20 ms window after slips than before and higher-acceleration slips drove a greater increase in response synchrony than lower acceleration slips. Because the statistics of slips vary with texture, it is possible that slip evoked synchrony along with the firing-rate may be cues for the texture. In support, it was found that synchronous firing in pairs of neurons showed significant differences with texture which was greater than the differences found in firing rate (Jadhav et al. 2009). This shows that stimulus-induced spike synchrony can be essential feature in the barrel cortex for extracting texture information. Another study

provides direct evidence that synchrony is a measure in the somatosensory area that can be modulated independently by attentional selection. Spike rates in SII have been previously shown to be influenced by selective attention (Hsiao et al. 1993). Steinmetz et al. (2000) investigated whether synchronous firing of neuron pairs in SII area of macaque cortex was also modulated with attention. In this study, three monkeys were trained in visual and tactile discrimination tasks and were also trained to switch their attentional focus between visual and tactile stimuli when cued. Distinct neurons were recorded by separate electrodes (400 microns apart) placed in the SII area and synchrony between the neuron pairs in a 50 ms time window, which is approximately the period of perceptual integration for tactile stimuli (Craig 1996). Majority of the SII neurons expectedly showed a significant change in the firing rate when the animal switched between the tactile and visual tasks (Figure). 66% of the neuron pairs had significant cross-correlogram peaks during the visual task, the tactile task or both. 17% per cent of the neuron pairs with significant cross-correlogram peaks also showed significant changes in synchrony between the visual and tactile tasks. 80% of those neurons responded with an increased synchrony and 20% responded with decreased synchrony when the monkey performed the tactile task. The rate of synchronous events was also higher during the tactile task than the visual task or due to chance (Fig. 1-5). The authors argue that only a small fraction of neuron pairs change their synchrony with attentional selection as only a subset of neurons in SII should be engaged in attending the stimulus while other neurons perform different perceptual roles. They also showed that probably the difficulty in a task or the amount of cognitive load might influence the percentage of neurons affected (Steinmetz et al. 2000; Roy et al. 2007). These studies

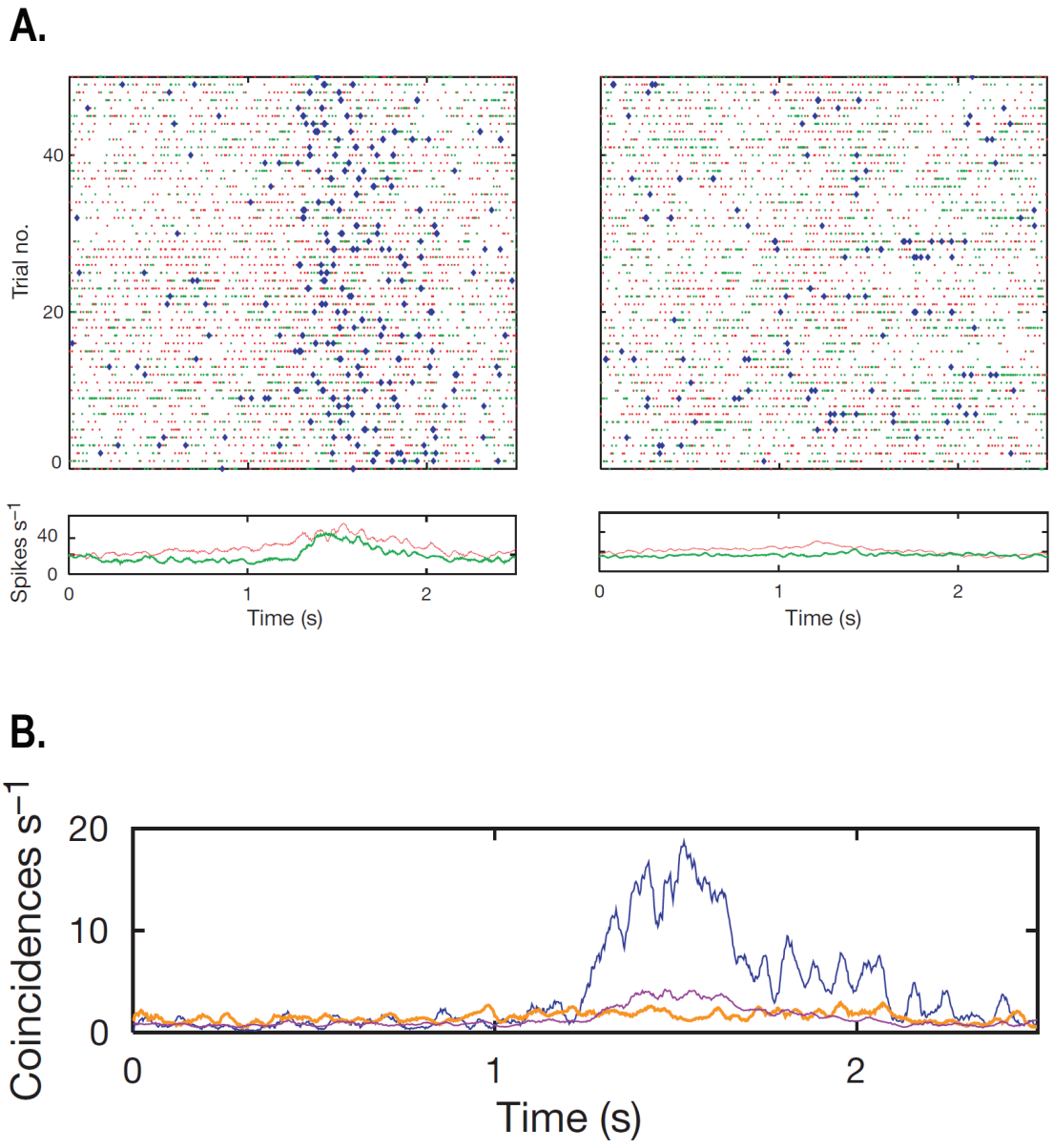

Figure 1-5. The effect of attention on spike synchrony between neurons of monkey S-II cortex

A. Raster plots and PSTHs of a typical neuron pair in S-II while a monkey performs a tactile task (left plots) and a visual task (right plots). Red and green dot represents the action potential of the neuron pair. The blue diamonds represent the synchronous events between the pair of neurons, defined as spikes from eachwithin 2.5 ms of each other. The number of synchronous events is much higher when attention is directed towards the tactile stimuli. B. Rates of synchronous events during the tactile task (blue curve), visual task (orange curve) and those expected by chance during the tactile task (violet curve) indicating the apparent increase in synchrony during the tactile task (Steinmetz et al. 2000).

implicate the importance of synchrony and it has been hypothesized that synchrony might increase the “loudness” of the message, making it easier for other neurons to listen to it above a noisy background (Salinas and Romo 2000). The finding and the idea fits perfectly with several computational models of attention (Crick and Koch 1990; Neibur and Koch 1994) and is in line with the findings in visual areas (De Oliveira et al. 1997; Fries et al. 2001). The mechanism by which synchrony can be induced is still a mystery but two candidates, namely, lateral coupling and common inputs, seems to be the most potent. The large descending feedback projections within the somatosensory system provide a strong anatomical basis for the latter mechanism (Niebur et al. 2002).

Somatosensory cortical neurons in awake primates also show significant trial-by-trial correlations in stimulus evoked spike counts even if the cells have dissimilar tuning properties and this correlated discharge was shown to increase the coding efficiency of neurons during a vibrotactile discrimination task (Romo et al. 2003). The SII circuitry generates two classes of neurons: some SII neurons show firing rate increases as a function of stimulus frequency, while others decrease their firing rate monotonically with increasing stimulus frequency (Salinas et al. 2000; Romo et al. 2002). When paired across classes these neurons typically show positive correlations during the stimulus. Zohary et al. in 1994 reported positive correlations in visual neurons between similarly tuned neurons and mathematically showed that positive correlations would seriously limit the beneficial effect of adding or averaging responses with similar tuning. However, this study in the primate somatosensory cortex shows that not all positive correlations are deleterious and positive coupling between dissimilarly tuned neurons produces a

signal that is better than would be obtained in the absence of correlations (Romo et al. 2003).

Synchrony was also viewed as a tool for modulating information transfer from the thalamus to the cerebral cortex (Alloway and Roy 2002). Bruno and Sakmann (2006) showed in a technically difficult study that synchronization of convergent thalamic inputs is enough for single neuron activation in rat somatosensory cortex. *In vivo* recordings showed that individual thalamocortical neuron inputs were extremely weak, and a single action potential was unable to drive cortical activity. However, strong sensory stimulation leads to near-synchronous thalamic activation, and subsequently to synchronous converging thalamocortical PSPs. Such an input pattern affords strong feed-forward excitation in the order of 10s of millivolts, which exceeds the size of net PSPs needed for threshold activations and observed normally for L-IV neurons. Thus it seems, unlike previously believed, recurrent excitatory connections in L-IV or cortical amplification is not required to activate cortical neurons during a sensory volley (Bruno and Sakmann 2006; Alonso 2006) leading to another important role of neuronal synchrony in thalamocortical relay of information (Fig. 1-6).

Other roles have also been loosely attributed to synchronous discharges in the somatosensory areas. Stimulation of left hypothalamus, unlike right hypothalamus, was followed by a significant increase in the number of correlated neuronal pairs in the rabbit neocortex including the somatosensory area (Pavlova et al. 1998). It was also shown that this re-arrangement of correlated activity in rabbit somatosensory areas also occurred under conditions of defensive motivation induced by stimulation of the medial hypothalamus and in conditions of natural food-related motivation (Pavlova 1996;

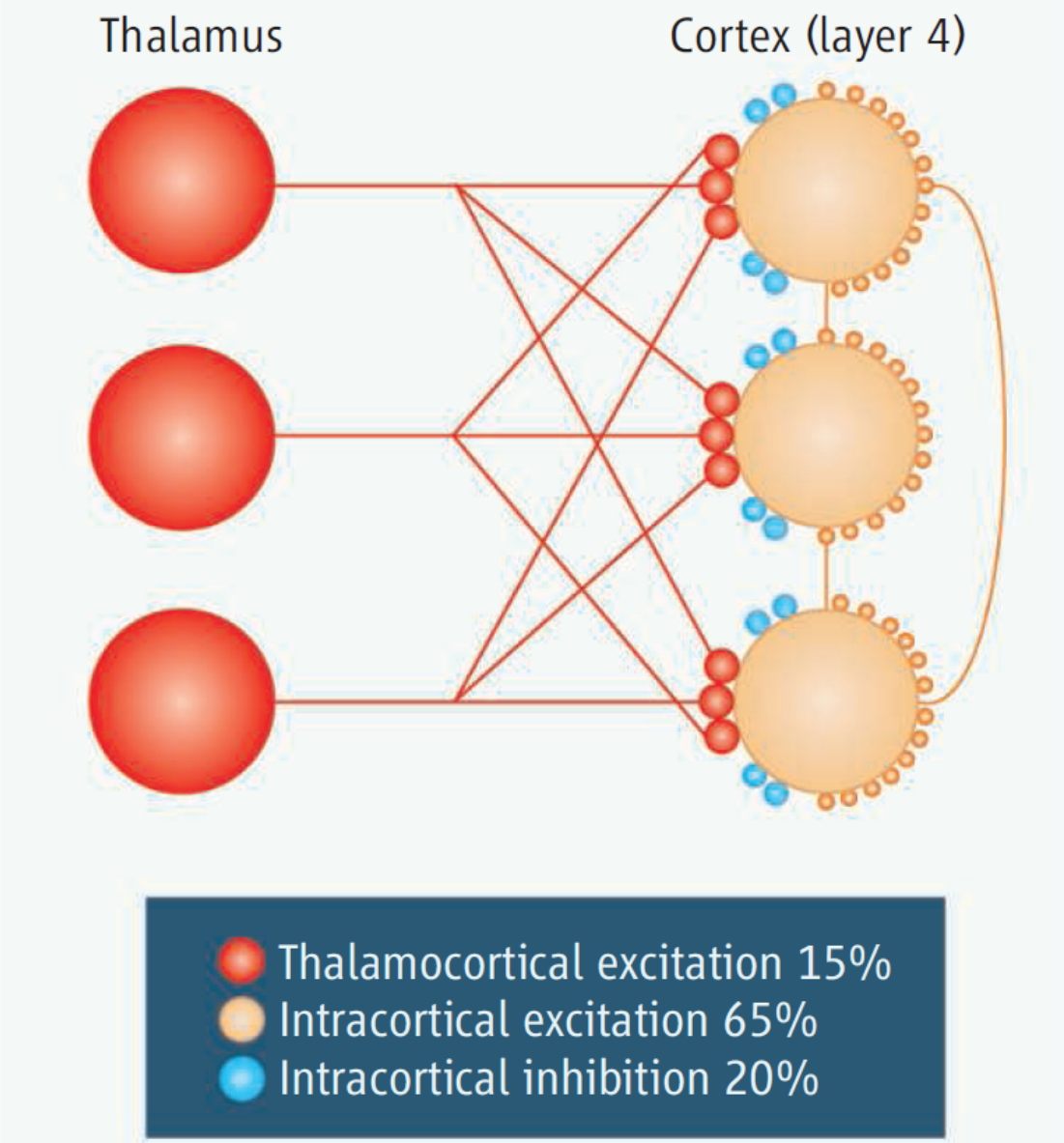

Figure 1-6. Role of synchrony in thalamocortical relay of information
The connections between the thalamus and a neuron in cortex are weak but it is shown (Bruno and Sakmann 2006) that they may become strong by being activated synchronously (Alonso 2006).

Pavlova et al. 1995). Neuronal correlations within S1 and between S1 and anterior cingulated cortex and medial dorsal thalamus have been shown to increase under conditions of expectations preceding painful stimuli suggesting that there is enhanced synchronization within the pain network (Wang et al. 2008).

The exact mechanism of spike synchrony is still a mystery, although a couple of studies hint at its possible explanation. One study shows that evoked transient network oscillations in rat somatosensory cortex is accompanied by an increase in extracellular K+ concentration and can be prevented by the K+ channel blocker quinidine (Berger et al. 2006). Blockade of AMPA, NMDA, GABAa and gap junctions also prevents evoked oscillations. Results from the study suggest that there is a mixed contribution of Glutamatergic, excitatory GABAergic, and transient gap junctions in the initiation and maintenance of the network oscillations (Berger et al. 2006). Another study also suggested that barrel cortical interneurons have abundant electrical synapses which can be responsible for their synchronized activity during the second and third postnatal week in rats. As evidence they showed that in Connexin-36 (a gap junction subunit) knock-out mice synchronous inhibitory activity was weaker and more spatially restricted (Deans et al. 2001). The fact that gap junction subunits like Connexin-36 has also been shown to have a developmental peak from 9-15PND (Liu and Jones 2003) similar to the peak observed for passive synchrony makes it an important candidate as a potent contributor towards the development of spontaneous synchrony among neurons. Moreover, the baseline levels of spontaneous synchrony in rat barrel cortex has also been shown to have a strong developmental peak around 9-15 PND after which it is reduced to normal adult levels (Ikemoze et al. 2008), hinting at a possible

developmental role of shaping synchrony between neighboring and functionally similar neurons.

Relevance of spike synchrony in other cortical areas

A discussion on cortical spike synchrony would be incomplete without mentioning the critical findings on synchrony in visual areas. The research on synchrony has been most extensive in the visual areas (For a review see Singer 1993; Engel et al. 1999, Usrey and Reid 1999; Jermakowicz and Casagrande 2007) and these investigations have given important insights into the perceptual role of synchrony which can be utilized to understand the role of synchrony in other cortical areas including the somatosensory area.

In both anesthetized and awake preparations spike synchrony has been locally observed within striate and extrastriate visual areas. Cross-correlation analysis in the cat primary visual cortex revealed that neurons are functionally coupled over horizontal distances of several millimeters and coupling tended to be enhanced between mini-columns of similar orientation. Moreover, the strength of correlation was observed to be facilitative between cells having the same eye preference (Ts'o et al. 1986). Positive interactions were also found between blob cells in the same or adjacent blobs when their receptive field and ocular dominance matched (Ts'o and Gilbert 1988). Striate cortex of squirrel monkeys was also shown to be synchronized when neurons had similar receptive field properties (Livingstone 1996). Like the striate areas, in extrastriate areas such as the caudal superior temporal sulcus of the macaque monkeys, adjacent neurons as well as spatially separated neurons were found to be synchronized (Kreiter

and Singer 1992). The synchronous discharges were not only internally generated, but there was evidence that showed that they were dependent on specific properties of the stimulus as well (Gray and Viana Di Prisco 1997). As in somatosensory cortex, it is hypothesized that spontaneous local synchronized responses are primarily generated by local interactions within the respective structures (Engel et al 1991; Munk et al. 1995), whereas externally-induced synchrony is presumably mostly due to feedforward signal flow from the periphery (Engel et al. 1999). It was also suggested that oscillatory neuronal firing in the visual areas (area 17 and 18) in response to a visual stimulus may also be a general mechanism by which activity patterns in spatially separate regions of the cortex are temporally coordinated (Gray and Singer 1989) and this temporal coordination can in turn provide a mechanism for the formation of neuronal assemblies in the visual cortex (Engel et al 1990). Recently, it was found that spike synchrony in primate primary visual cortex was strongly correlated between stimulus-induced and spontaneous conditions and was greater when the cells within a pair had overlapping receptive fields and preferred similar orientations rather than non-overlapping receptive fields and different orientations. This finding suggests that spike-time correlations in visual cortex present in evoked activity are generated by mechanisms common to those operating in spontaneous conditions, or at least they can be linked (Jermakowicz et al. 2009).

There are two general hypotheses regarding its role in visual cortex: the temporal binding hypothesis and the stimulus feature extraction hypothesis (Jermakowicz and Casagrande 2007). According to the temporal binding hypothesis, transient and precise synchronization of neuronal discharges can lead to dynamic binding of information

which in turn may be crucial for generating functionally efficacious representational states and for selection of behaviorally relevant information (Engel et al. 1999). Thus, synchrony as an additional coding dimension may allow the dissociation of the binding code from the feature code signaled by firing rates (Engel et al. 1999) and for selection of assemblies for further processing (Singer and Gray 1995; Singer et al. 1997). There are several studies in favor of this hypothesis including studies that show stimulus-specific synchronization of spatially distributed neuronal responses may provide a physiological mechanism for scene segmentation (Engel et al. 1991); distinguishing component vs pattern motion (Engel et al. 1998; Castelo-Branco et al. 2000; Thiele and Stoner 2003), buildup of phenomenal states and selection of visual information for access to awareness (Fries et al. 1997) and attentional mechanisms (Crick and Koch 1990; Fries et al. 2001; Tiesinga et al. 2004; Taylor et al. 2005; Womelsdorf et al. 2006). However, many studies have indicated that correlated firing between cells is not significantly different depending on whether the neurons are encoding features of the same or different objects (Lamme and Spekreijse 1998; Dakin and Bex 2002; Roelfsema et al. 2004; Palanca and DeAngelis 2005; van der Togt et al. 2006; Dong et al. 2008), thus providing evidence against the temporal binding hypothesis.

The stimulus feature extraction hypothesis suggests that stimulus-induced neuronal synchronization, in conjunction with changes in firing rate, serves to provide perceptually relevant but exclusive information about the stimulus. Support for this hypothesis comes from the presence of synchrony between V1 neurons having similar orientation preference (Gray et al. 1989; Kohn and Smith 2005), dependence of synchrony in visual areas on neuronal on the configuration of stimuli (Engel et al. 1990;

Kreiter and Singer 1992) and the fact that spike synchrony reflect Gestalt criteria of continuity, vicinity and common motion of stimuli (Gray et al. 1989; Engel et al. 1991). Recently it has been shown that synchronized activity of neurons in V1 leads to substantial improvements in encoding information to distinguish fine angle changes over information encoded by firing rate. Moreover, increasing the number of neurons in the synchronized assembly increases the amount of information coded (Samonds et al 2004; Samonds et al. 2006), thus providing evidence for the stimulus feature extraction hypothesis.

Information processing in auditory cortex has to be very fast and so it is extremely temporally dependent because of features like binaural interaction. Correlation and connectivity may ensure efficient propagation and preservation of the temporal precision of neural firing downstream along the neural pathway (Kimpo et al. 2003; Kistler and Gerstner 2002; Reyes 2003). Thus, neuronal spike synchrony perhaps can be more useful and effective as a neural code in auditory cortex than the other cortical areas. In spite of these benefits there have been relatively few studies assessing correlated neural activity in auditory cortex.

Pioneering studies in the cat auditory cortex indicated the presence of neural co-discharge, most of which was attributed to shared input to the neurons as only 5% of the correlated discharge was interpreted as functional interaction due to direct connections (Dickson and Gerstein 1974; Frostig et al. 1983). Later, it was shown that although cat auditory cortical neurons showed effective spontaneous correlations, which were significantly modulated after stimulated inputs (Espinosa and Gerstein 1988) depending on location and/or movement of the sound stimulus hinting that neuronal

synchrony in auditory cortex might encode sound location and movement (Ahissar 1992). Like somatosensory and visual cortex, even in A1 strength of correlation fell with increasing distance (Eggermont 1992, 1994, 2000), which was more shallower for A1 as compared to other areas such as the posterior auditory field of cats (Eggermont 2006, 2007). Also, like other cortical areas correlation strength of auditory cortical neurons depend on the RF properties of the paired neurons. The correlation strength was associated with the similarity in spectro-temporal receptive fields, binaural interactions and in temporal response properties such as response onset, offset and the temporal pattern of the response (Brosch and Schreiner 1999; Eggermont 2006). This suggests that synchronized activity provides means to evaluate the functional organization of auditory cortex (Brosch and Schreiner 1999).

Similar to other sensory areas, the role of synchrony in auditory cortex has not been well resolved. However, there are a few studies that indicate the synchrony is indeed necessary for neuronal encoding of acoustic information. As mentioned above, synchrony in auditory cortex was first shown to be linked with sound location and movement (Ahissar et al. 1992). In a later study on anesthetized marmosets, it was shown that populations of neurons in the primary auditory cortex can coordinate the relative timing of their action potentials such that spikes occur closer together in time during continuous tone stimuli even though their firing rates do not change. This indicates that population coding based on relative spike timing can independently signal stimulus features (deCharms and Merzenich 1996). In another study (Tomita and Eggermont 2005) results from the recorded neurons of the cat auditory cortex showed that stimulation reduced the correlation in background activity, and as a result, the

signal-to-noise ratio of correlated activity in response to the stimulus was enhanced. Neuronal synchrony was also shown to be used solely by sustained firing neurons in posterior ectosylvian gyrus to discriminate temporal envelope alterations and time reversions of cat vocalizations (Gourevitch and Eggermont 2007).

Coincident neuronal discharges are also evident in other cortical areas. Synchronization is a well known phenomenon in the olfactory system (For a review see Laurent 1996). First noted in the insect olfactory bulb (Adrian 1950; Rall and Shepherd 1968; Freeman 1972), it is now known that even in vertebrates like zebrafish (Friedrich et al. 2004) and rodent (Christie et al. 2005; Hayar et al. 2005) olfactory bulb, synchrony is a common occurrence. The external tufted (Hayar et al. 2005) cells and mitral cells (Christie et al. 2005) in the rat olfactory bulb show synchronized activity in the presence of odors or artificial stimulation (Schoppa 2006). Studies of synchrony in the olfactory system have been especially important for teasing out the mechanisms governing synchronous discharges. It was first suggested by studying the olfactory system that gap junctions play an important role in the correlated spiking between mitral cells as Connexin-36 knock-out mice did not have synchronized mitral cell firing (Christie et al. 2005). Patch clamp recordings from the mitral cells and subsequent analysis of coupling potentials combined with dendritic sectioning showed that mitral cell synchrony could also be driven by inhibitory IPSPs imposed by granule cells (Schoppa 2006). Neurons in the frontal cortex also show correlated responses which have been tagged as behaviorally relevant (Abeles et al. 1993; Vaadia et al 1995). Similar evidence is available from the motor system, where neuronal synchronization has been discovered during both preparation and execution of movements (Murthy and Fetz 1992; Riehle et

al. 1997). Synchronization between sensory and motor assemblies has been reported and has been shown to be important for sensorimotor integration (Roelfsema et al. 1997).

Plasticity in cortical spike synchrony

Spike synchrony is a dynamic property of the cerebral cortex. It is extremely malleable and changes with the ongoing information processing in the brain, especially after altered sensory experience and adaptive behavior. Although there are very few studies indicating the plastic short term and long term changes in functional coupling of neurons, they are widespread and observed in several sensory cortical areas. Changes in the topographic mapping of sensory areas are often accompanied by significant changes in neuronal coincidence properties. Simple map changes produced by intracortical microstimulation are followed by an increase in correlation between distant neurons in the forelimb area of monkey somatosensory cortex or in the auditory cortex of anesthetized cats (Dinse et al. 1993; Maldonado and Gerstein 1996; Valentine and Eggermont 2003). More dramatic changes in neuronal synchrony can be observed following electrical kindling (Valentine et al. 2004). Similar map changes with other mechanism were also accompanied by changes in correlated discharge. In auditory cortex, pairing 9 KHz tones with stimulation of dopamine releasing neurons of the ventral tegmental area, increases the cortical representational area for 9 KHz and the selectivity of the neural responses to that particular tone and were accompanied by an emergence of long-range synchronous discharges between A1 and in the secondary auditory cortical areas (Bao et al 2001). Weaker than normal correlations between

neighboring A1 neurons in addition to disruption of the other RF characteristics of A1 were also observed when rat pups were exposed to pulsed white noise from 9-28 PND. However, this negative effect was not observed when adult rats (older than 30 PND) were exposed to similar noise stimulus. These results indicate that patterned neural activity in A1 appears to play a crucial role in shaping correlated neural discharge and thus neuronal processing/decoding circuits, in the primary auditory cortex during a critical period (Zhang et al. 2002). Similarly, it has been shown that in rat barrel cortex certain conditioning procedures leads to gross topographical map changes, followed by changes in neuronal synchrony (Erchova and Diamond 2004). In this study, two pairs of whiskers were stimulated for a 50 min conditioning procedure during spontaneous “bursts” of the neurons (burst-conditioning procedure) or during the inter-burst periods and infragranular neurons were recorded from the representative barrels before and after the conditioning procedures. Only burst conditioning led to a significant expansion of the cortical area that got activated by the paired whisker stimulation just after 10-15 min. Significant levels of stimulus-induced synchrony was observed between the two cortical barrels during both burst-conditioning and inter-burst conditioning. However, a significant increase in the stimulus-induced correlated activity was reported 30 min after the start of the burst conditioning only but not during the inter-burst conditioning. After the termination of the procedure, the stimulus-induced correlation between the barrels receiving inter-burst conditioning came back to its pre-conditioning spontaneous levels but the synchrony for the burst-conditioned barrels remained elevated for at least 25 min (Fig. 1-7). JPSTH analysis confirmed that the increase in correlation between the barrels was also evident after the stimulus and was not only a feature of spontaneous

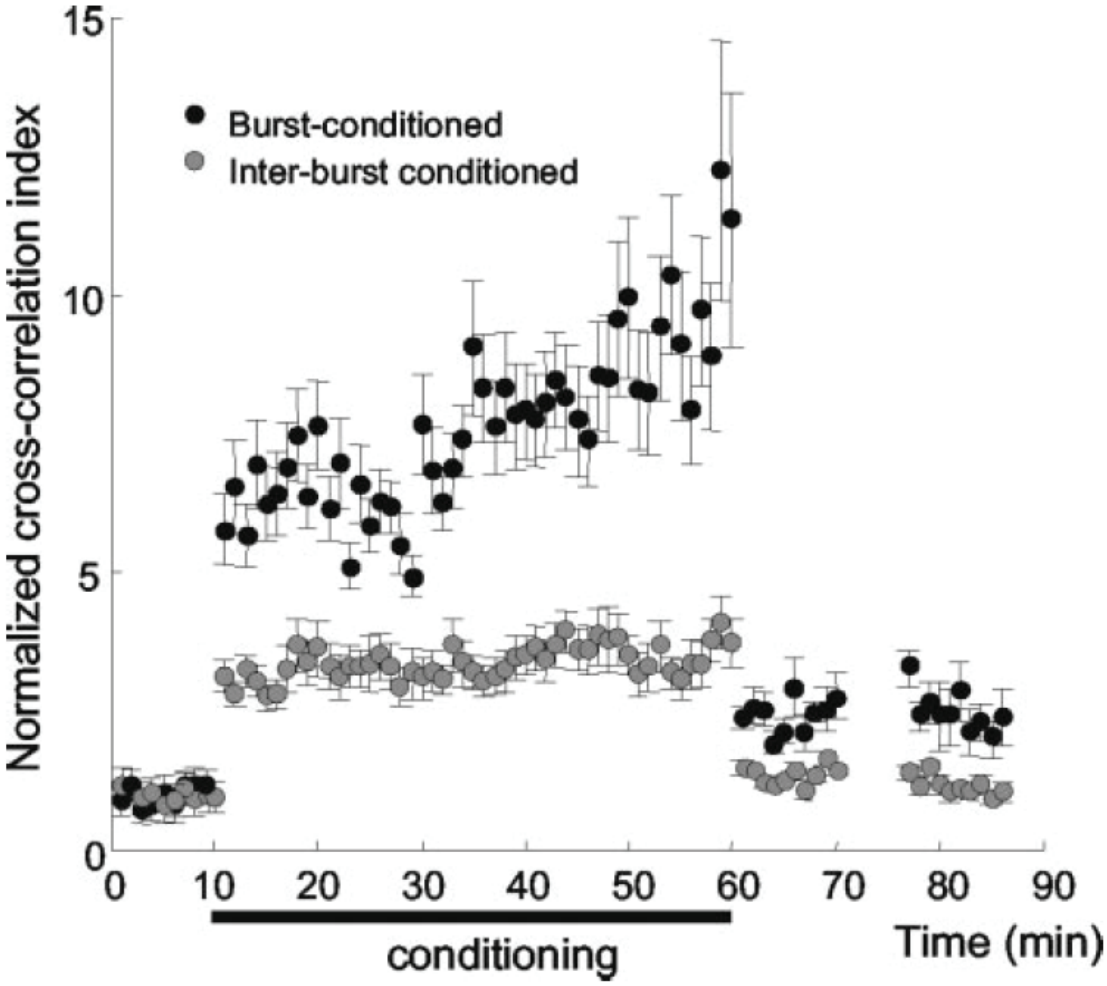

Figure 1-7. Plasticity in synchronous activity in barrel cortex
Correlations between 2 infragranular neurons in separate barrel columns were measured during burst-conditioning and inter-burst conditioning (see text). Significant stimulus-induced synchrony was observed between the two cortical barrels during both burst-conditioning and inter-burst conditioning. However, a significant increase in correlation index was observed only between pairs receiving burst-conditioning (black dots) only but not for pairs receiving the inter-burst conditioning (gray dots). After the conditioning procedure, the stimulus-induced correlation between the barrels receiving inter-burst conditioning came back to its pre-conditioning spontaneous levels but the synchrony for the burst-conditioned barrels remained elevated (Erchova and Diamond 2004).

correlations. These differences in correlated activity were only observed in the infragranular layers but not in L-IV. The results together show that the conditioning procedure led to a disparity in intercolumnar connectivity, favoring the column receiving paired inputs during cortical bursts relative to the columns receiving the same quantity of paired inputs during inter-burst intervals. The authors suggest that repetitive co-stimulation of two whiskers, timed to coincide with transient increases in cortical plasticity caused the burst- conditioned cortical barrel columns to become more strongly connected, presumably through Hebbian mechanisms (Hebb 1949; Erchova and Diamond 2004). Thus correlated neural activity may act as a driving force for functional changes in the cortex (Eggermont 2007).

Stimulus adaptation has been shown to have profound influence on cortical spike synchrony. As mentioned earlier, barrel cortical neurons, unlike thalamic neurons, have significant changes in their synchronous responses following adaptation to a particular direction of stimulus (Khatri et al. 2009). Similarly in V1, adaptation of neurons can be observed after a prolonged exposure to a non-preferred orientation leading to an attractive (neuronal tuning curve moves towards the adapting orientation) or a repulsive (neuronal tuning curve moves away from the adapting orientation) shift. Correlated activity evoked by the initial preferred orientation stimuli significantly and reversibly increases after adaptation although no changes in synchrony were observed for the adapting orientation stimuli. Thus synchronization can be dynamically modulated by adaptation-induced plasticity of tuning properties (Ghisovan et al. 2008). Similarly, sensory experience has also been shown to be critical for stimulus-induced “active” synchrony between spatially separate neurons. It was shown that cats with induced

convergent squint had differences in there amplitude of correlated discharge between neurons driven by the amblyopic eye and the normal eye (Roelfsema et al. 1994). Pairing electrical stimulation of the basal forebrain with an acoustic sequence (high frequency tone-low frequency tone- noise burst) also led to an increase in the population synchronous discharge of A1 neurons which was not seen after pairing simple tone, tone trains or broadband stimuli. This shows that stimulus-induced synchronized responses in A1 can also be substantially altered in an experience-dependent fashion (Kilgard and Merzenich 2002).

It is clear from the discussion above that neural spike synchrony is indeed a common phenomenon in the somatosensory areas as it is in other areas of the cortex. The generality of the incidence of spike synchrony makes it even more likely to be a candidate in being responsible for encoding information either independently or in conjunction with other neural response characteristics. With this as the background, we wanted to further characterize neuronal synchrony in barrel cortex. In this thesis we show, how this cortical property is developmentally regulated in barrel cortex including plausible mechanisms for the same and show how it can be modified and dissociated from the rate code during multisensory interactions in primary sensory cortex.

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CHAPTER II

EARLY BILATERAL SENSORY DEPRIVATION BLOCKS THE DEVELOPMENT OF COINCIDENT DISCHARGE IN RAT BARREL CORTEX

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Introduction

The neural code of a sensory experience is the result of ensemble neural activity that contains all of the information needed to generate a perception. The code can be based on instantaneous firing rates, integrated firing rates, mean interspike interval duration of a group of specialized neurons, or a cluster of neurons having correlated responses (Eggermont, 2001; Eggermont, 2006; Eggermont 2007). Thus, the correlated spiking activity within a neural assembly can improve the accuracy of the neural code (Abbott and Dayan, 1999; Miller, 1996; Rolls et al., 2003).

Sensory information is used continuously by animals when they explore their environment, and correlated neuronal discharge in the sensory pathway is one important mechanism for adapting to new stimuli in the environment. Detecting correlation allows an animal to make inferences about the environment. For example, Engel et al. (1991) showed that neurons in visual cortex exhibit correlated spiking activity when they have similar orientation sensitivity, and such cells are found over large extents of VI cortex. Examples of significant correlated neural discharge exist in the auditory system, such as those produced by binaural interactions (Brosch and

Schreiner, 1999),and in the second somatic sensory area (S-II) as a correlate of attention (Roy, et al., 2007)

Sensory deprivation in rats beginning near birth has been shown to degrade the function of the cerebral cortex, and the loss persists even after normal activity levels are restored later in life. Normal kittens raised in complete darkness for 4-6 months appear behaviorally blind when they start using vision as adults, and they improve only marginally over time (Wiesel and Hubel, 1965). One carefully defined deficit produced by visual sensory deprivation in cats is a loss of normal acuity when tested by lines and gratings (Mitchell, 1988). In the rat whisker system sensory deprivation degrades their ability to discriminate between stimuli of different spatial roughness, and this loss of acuity also persists for prolonged periods (Carvell and Simons, 1996). The deficits in acuity coupled with the requirement for correlated discharge in neural coding lead to the hypothesis that the maturation of network assemblies requires activity-based correlated discharge to develop normal function in cortical neuronal assemblies. If so, sensory deprivation produced by trimming rat whiskers would be predicted to interfere with the development of coincident neuronal firing in whisker-recipient barrel cortex. Although the cortical barrel looks structurally normal following bilateral whisker trimming from birth to maturity, the perturbations in neural coding and the consequent behavioral deficits might be accounted for by the failure of barrel neurons to develop appropriate correlated firing. We tested this hypothesis and found that sensory deprivation strongly interferes with the development of correlated discharge between neuronal pairs in barrel cortex. Thus, in this chapter, instead of focusing on the various characteristic details of correlation in barrel cortex, we have tried to identify and characterize the differences in

the degree of correlations between normal and sensory deprived rats in barrels and septa.

Methods

All of the experiments carried out for this report were approved by the Vanderbilt University Animal Care Committee (IACUC), and were in accordance with the guidelines of the NIH and the Society for Neuroscience in an AAALAC approved animal facility.

Sensory deprivation: Long-Evans rats (250-350 g, 2-3 mo) from 4 litters (8 animals total) were used for this study. Of these, 4 had all their whiskers trimmed on both sides of the face (bilaterally deprived group or BD) and 4 animals were handled but not trimmed (control or CON group). Whiskers were trimmed to the level of the fur for at least 60 days beginning at birth: the first 30 days (when the whisker growth rate was faster) the whiskers were trimmed every day and after P-30 the whiskers were trimmed every other day. During pre-weaning whisker trimming, the whisker trimmed BD pups were caged with their CON littermates and nursed by their dam. After weaning same sex animals were housed in groups of 3-5 animals depending on their size. Five days prior to recording sessions the whiskers were allowed to re-grow to a length sufficient for stimulation without moving the fur.

Surgery and Recording: The rats were anesthetized with urethane (1.5 g/kg, 30% aqueous solution, i.p.), and then mounted in a head holder that allowed free access to the whiskers (Narashige, Japan). Urethane is a general anesthetic that has been shown to have little effect on glutamate or GABAergic currents, but blocks Ba^{+}-

sensitive K^+ channels (Sceniak and Maciver, 2006). A craniotomy was made from 4 to 7 mm lateral to the midline and from 0 to 5 mm posterior to Bregma to expose the left barrel cortex. Body temperature was maintained at 37°C with a feedback actuated heating pad (Harvard Apparatus, Holliston, MA). Supplementary injections (10% of the initial dose) were given as needed to maintain the anesthesia at stage III-3 (Friedberg et al. 1999). After making a small opening in the dura mater microelectrodes were advanced in columnar penetrations perpendicular to the cortical surface. Contact with the pia was identified visually through an operation microscope. We used 3 quartz glass insulated, platinum/iridium microelectrodes having 2-6 MΩ resistance (Thomas Recording, Giessen, Germany). The electrodes were separated by 305 μm and advanced into the brain independently using an Eckhorn microdrive system (Thomas Recordings, Giessen, Germany). The analog waveforms from the Thomas system were transferred to a Plexon MAP system (Plexon Inc., Texas) where they were digitized at 40 kHz. Multiunit activity was viewed online using Sort Client software (Plexon Inc., Texas), and stored for offline analysis. The electrodes were first advanced to layer IV where receptive fields were mapped manually, and the 3 whiskers that evoked the largest amplitude responses were identified for each electrode based on multiunit poststimulus time histograms (PSTH's) constructed online in PeriEvent Client (Plexon Inc., Texas). Multiunit activity was recorded from layer IV (450-800μm, Li et al., 2005). The recording depth was measured on-line by Eckhorn microdrive readings and later correlated with histological reconstructions of the electrode tracks and microlesions. Electrodes were advanced or retracted in 100 μm vertical increments to reduce the probability of recording twice from the same unit. Moreover, recording was discontinued

when spindling activity in the cortex was observed and resumed only after spindling had stopped.

Whisker Stimulation: After the re-growth period, the whiskers in both CON and BD animals were trimmed to 5 mm beyond the fur to keep stimulus distance equivalent. A piezoelectric stimulator was used to deliver 100 stimuli in a caudal direction at 0.5 Hz (600 μm amplitude, 4 ms duration, 2 ms rise time with custom rounded peak). The piezoelectric wafer was actuated by a "custom" waveform programmed in a digital stimulator (DS8000 WPI, Florida) that in turn was controlled by a Spike 2 (CED, Cambridge, UK) script program.

Histology: At the end of each recording session the electrodes were moved to 700 μm below the pial surface and electrolytic lesions were made using a DC current of 1 μA for 2 minutes. This current level produces a clearly visible lesion in cytocrome oxidase stained tangential slices through layer IV, and can be used to localize the electrodes in barrel or septum columns without destroying the electrodes. At the end of each experiment the animals were given a lethal overdose of urethane and perfused transcardially with PBS followed by phosphate buffered 4% paraformaldehyde. Brains were postfixed overnight and saturated in 30% sucrose. The cortex was flattened, sectioned tangentially and stained for CO activity to assign penetrations and lesion sites to barrels or septa.

Data analysis: Offline cell sorting of single units was carried out using Offline Sorter (Plexon Inc.). Typically 2-3 units per electrode could be selected for further analysis. Only cells well separated as 3-D clusters and with qualitatively distinctly different waveform shapes (Fig. 2-1A and B) were included for further analysis. The

magnitude of response was defined as the total number of spikes in the 100 ms following the stimulus and was calculated for all stimulated whiskers for each cell. There was no threshold set for the level of response to be included. We then determined the Principal Whisker (PW) as being the whisker eliciting the highest magnitude of response for barrel cells. For septal cells responses to all whiskers stimulated were included. PSTHs were constructed for each cell in response to stimulation of the principal whisker using a custom NEX script (provided by Dr. Alexander Kirillov, NEX Technologies, MA) and custom software (Mathworks, MA).

Spike Time Synchrony: There are multiple time scales in which temporal correlation can be examined. To assess precise spike synchrony, small time bins (1-5 ms) often have been used; however, such small bins require relatively huge spike counts to be valid. Coarse temporal correlation is considered at time scales > 20 ms. Bin sizes between 1-15 ms are commonly reported (e.g., in S1: Aertsen et al., 1989; Hsiao et al., 1993; Roy et al., 2007). In the present study, spike correlation was examined over 2000 ms windows divided into 200 bins so that 10 ms bins would still include the response transient peak. Ten ms bins allowed us to analyze coincident discharge over time especially for the period of relatively low firing during sustained 'spontaneous' activity. The correlation

coefficients were extracted by subtracting the mean values of number of spikes for each cell:

$\delta n_i^{(k)}(\mu) \equiv n_i^{(k)}(\mu) - \langle n_i(\mu)\rangle$ and $\delta n_j^{(k)}(v) \equiv n_j^{(k)}(v) - \langle n_j(v)\rangle$ respectively,

Where, $n_i^{(k)}(\mu)$ and $n_j^{(k)}(v)$ are the numbers of spikes for each time bin across each trial k of each cell (μ and v), and where $\langle n_i(\mu)\rangle$ and $\langle n_j(v)\rangle$ are the means values

of spikes at each bin (I and j) for each cell (μ and v). So that the unconnected part of the correlation is defined as: $\langle \delta n_{i(\mu)} \delta n_{j(v)} \rangle \equiv \langle n_i(\mu) n_j(v) \rangle - \langle n_i(\mu) \rangle \langle n_j(v) \rangle$.

When normalized this value becomes a coefficient of correlation, which was renamed a joint post-stimulus time histogram (JPSTH) by Gerstein and colleagues, having values ranging from -1 to +1 (Gerstein et al., 1989).

The JPSTH matrix represents earlier time in the lower left corner and later time in the upper right; the bins along this main diagonal measure the coincidences with 0 time lag (Fig. 2-1C). The JPSTH can be summarized in two additional plots. First, the time-averaged cross-correlogram is computed by summing the JPSTH bins parallel to the main diagonal; the cross-correlogram measures the average positive or negative correlation across the entire interval of analysis. Second, the coincidence histogram represents the coincident or near-coincident firing of the cells time-locked to values in each PSTH. Although the bin-by-bin dynamics of the correlation are not analyzed here, examination of the JPSTH revealed that the correlated spike times occur during the sustained portion of the stimulus-driven activity, more than at the transient responses to stimulus onset and removal. The spike timing coincidence will be examined elsewhere. To test the reliability of the normalized JPSTH, we repeated the JPSTH calculation on a set of spike trains in which no correlation should be present but the spike train variability is preserved. This *shuffled* JPSTH was generated by shuffling the order of the trials for one of each pair of neurons. It is important to emphasize that the trial-by-trial variability present in the normalized JPSTH is preserved within the *shuffled* JPSTH even if no correlation should be observed. Only pairs showing no correlation for the *shuffled* JPSTH were considered for further analysis. Second, a *simulated* JPSTH was

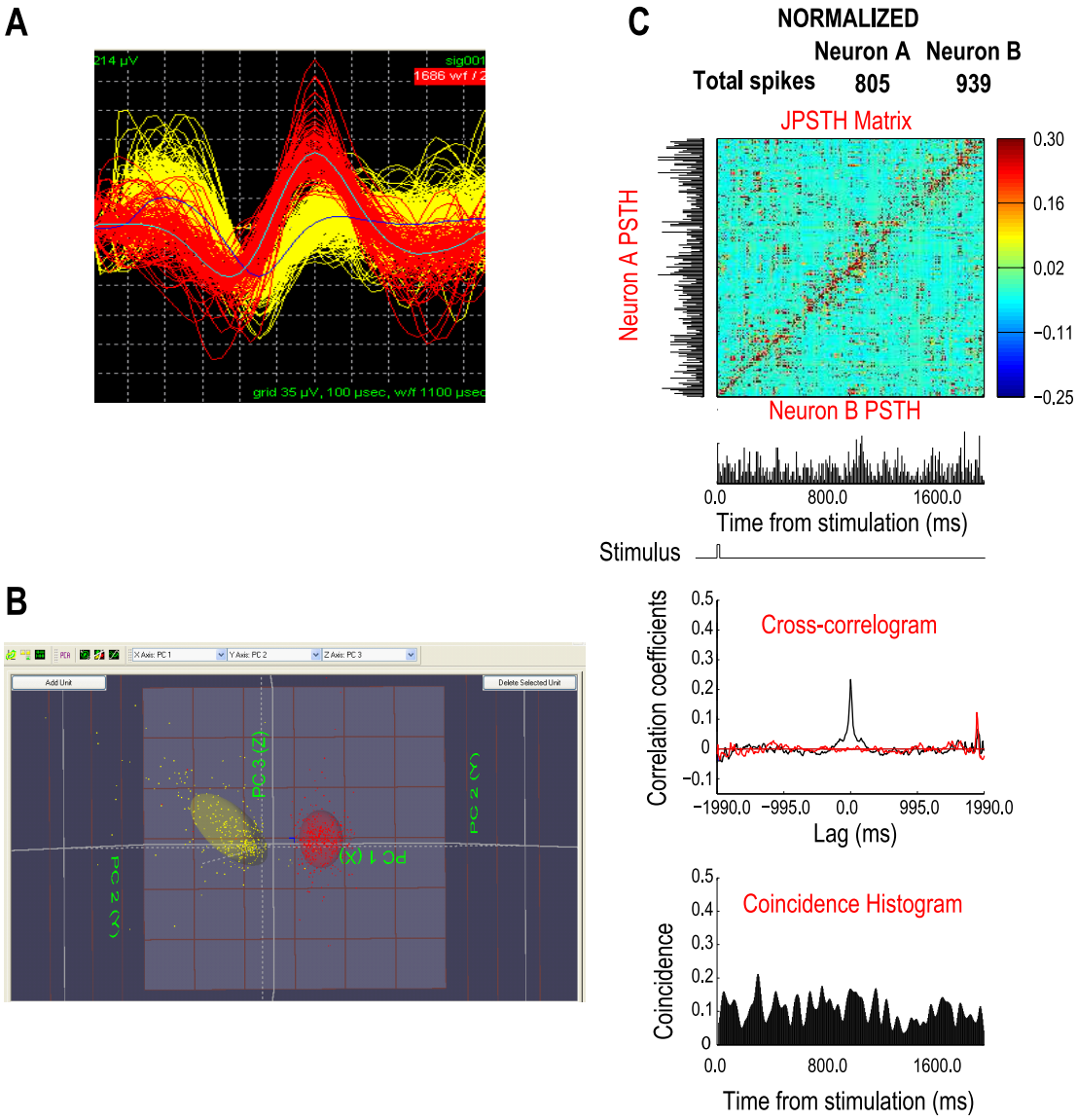

Figure 2-1. Details of spike sorting and JPSTH elements

A. Waveforms assigned to 2 neurons that are color coded in red and yellow. They were recorded from a single electrode and later superimposed on each other after offline sorting. The waveforms have a qualitatively different shape from which we infer that they are likely to be generated by 2 different cells. B. PCA clusters of the 2 neurons whose waveforms are displayed in A using the Plexon NeuroExplorer off-line sorter Principal Component (PC) 3D feature space algorithm. The three axes displayed are PC1, PC2 and PC3 respectively. The2 clusters are well separated in 3D space, which adds another criterion to assign waveforms to one or the other of the 2 neurons recorded by a single electrode. C. Normalized JPSTHs of the 2 neurons (whose waveforms and clusters are displayed in A and B) showing the 3D JPSTH matrix (X, Y, and correlation level), the PSTHs of the 2 neurons, the stimulus trace showing a brief stimulus (4 ms) at 0 after which the 2 neurons were recorded simultaneously, the cross-correlogram (average correlation across time at different time lags), and the coincidencehistogram (change in degree of correlation over time). Strong correlation can be observed along the diagonal of the JPSTH matrix indicated by warmer colors, by the narrow peak of correlation at 0 lag in the cross-correlogram, and by the coincidence histogram. The normalized cross-correlogram trace in black (with a recognizable peak) has been superimposed with the shuffled (See Methods section in paper for details) trace in red (no recognizable peak).

performed on a series of trials with spike trains derived from a non-homogeneous Poisson process with the rate derived from the actual average spike density function. Specifically, for each 1 ms interval we drew a random sample from the uniform distribution [0,1]. If, and only if, the sample was less than the normalized average spike density function value at that interval, would a simulated spike be counted. The simulated JPSTH was calculated from 50 simulated trials generated from the average spike density function for each pair of neurons. The two previous procedures allowed us to discard inappropriate normalizations.

Statistical analysis: The non-parametric Wilcoxson rank sum test was used to determine significant differences ($p<0.05$) between the cumulative distributions of peak-correlation coefficients. Paired one-tailed Students t –test was performed to find the significance of the normalized correlation coefficients over simulated and shuffled coefficients and to analyze the difference between the peak-correlation coefficients during the stimulus driven and the spontaneously active response epoch

Results

Calculating correlated discharge in barrel cortex neuronal pairs

JPSTHs were constructed as described to show the occurrence of neuronal synchrony over time after a stimulus and to determine the average peak-correlation values between pairs of neurons in control (CON, n=4) and bilaterally whisker trimmed (BD, n=4) rats. The broad 2 sec time scale was chosen for the JPSTH analysis to show coincidence of spikes both in stimulus-driven short latency epochs and in long latency

(1000 to 2000 ms post-stimulus) spontaneously active period as displayed in the coincidence histograms. For each animal group the peak-correlation observed between the neuronal pairs in the normalized JPSTH was identified as significant ($p < 0.001$) by comparing it with those obtained in the simulated and the shuffled JPSTH using the paired t-test. The correlation peaks observed in the cross-correlograms of neurons in barrel cortex were narrow and almost always had a time lag close to 0. Moreover, the cross-correlograms did not show oscillations, but there were noticeable oscillations in the coincidence histograms for the neuronal pairs.

Neuronal synchrony within a single barrel

Spike synchrony was calculated in 78 CON and 70 BD pairs of barrel neurons. The JPSTH analysis showed that most of the neuronal pairs in CON barrels were highly correlated in their discharge. This spike synchrony was observed for the entire 2 sec post-stimulus period of recording after principal whisker (PW) stimulation. Fig. 2-2A illustrates the typical correlated activity observed between two barrel neurons (color coded waveforms for each cell in insert) in a CON barrel. The high degree of correlated discharge is represented by the hotter colors in the diagonal of the JPSTH matrix and by the narrow average cross-correlation peak with a maximum value of 0.1993 centered near a lag of 0 ms. As shown in the PSTHs in Fig. 2-2A, the firing rate of these two neurons changes dramatically over time after the stimulation. Rapid changes in firing rate could affect the quantification of spike synchrony. Thus, it is important to note that high correlation was detected even when the firing rate of the two neurons were low and had returned to background levels (i.e., from roughly 200 to 2000 ms after the stimulus).

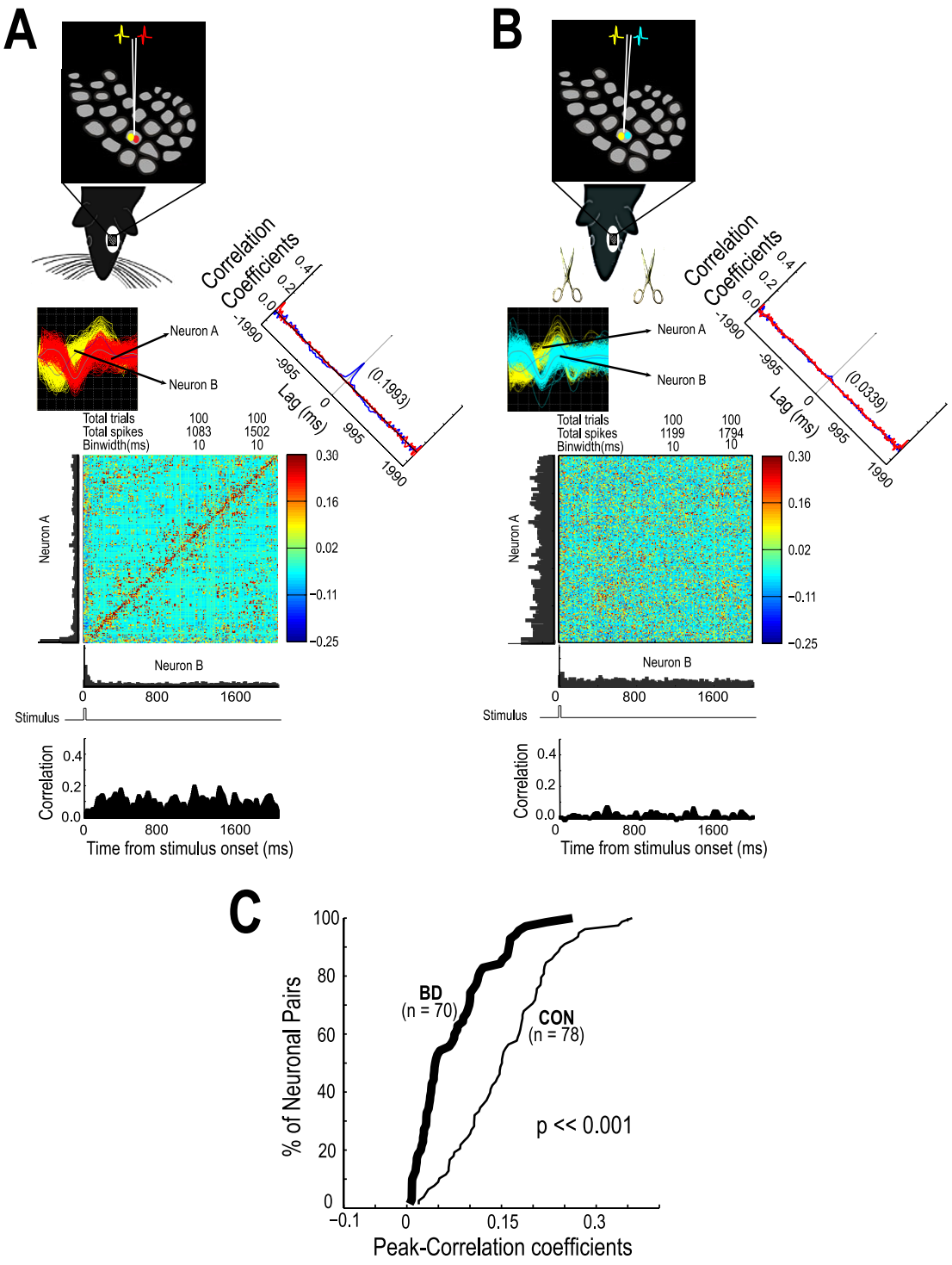

Figure 2-2. Correlated discharge of two neurons in the same barrel
A. JPSTH of a single pair of CON barrel neurons (Neuron A and B waveforms in inset) showing the PSTH's for each cell after 100 trials. The color-coded JPSTH matrix of correlation values, and the cross-correlogram (angled display) with a peak correlation coefficient of 0.1993 at 0 time lag show the degree of correlated discharge between this pair of neurons. The stimulus trace represents a 4 ms whisker stimulus beginning at time 0 following which the 2 neurons were simultaneously recorded. The degree of correlation over time along the diagonal (coincidence histogram) is shown at the bottom of A. In the cross-correlogram the normalized trace (in blue) has been superimposed with the shuffled trace (in red). B. Similar data from a pair of barrel neurons in a BD rat. Note that these cells had nearly the same total number of spikes as the control pair, but these cells show no clear peak in the cross-correlogram and a peak-correlation coefficient of 0.0339. C. Cumul -ative distribution of peak correlation in all barrel cell pairs in CON (n=78 pairs) and BD (n=70 pairs) conditions. The CON pairs show significantly higher correlation than the BD cells (Wilcoxson nonparametric test; p<0.001).

When pairs of recorded neurons were located in a cortical barrel of a BD animal, correlated discharge could not be demonstrated. Fig. 2-2B illustrates this difference with an example of correlated discharge between a single pair of neurons in a 'deprived' barrel. The cross-correlogram (average peak correlation of 0.0339) and the coincidence histogram show the low level of correlated discharge during the entire 2 S post-stimulation period. An increase or reduction in the total number of spikes could also potentially affect the quantification of spike synchrony (Brody, 1999; Ventura et al., 2005). BD animals generally have been reported to show a low response magnitude (Popescu and Ebner 2010). However, the example in Fig. 2-2B, shows 2 exceptional 'deprived' neurons, that produced a nearly identical number of spikes (to the same type and duration of stimulus as that of CON) compared to the CON example, but still failed to develop any synchrony. Thus, the diminution in the total number of spikes can't be the sole determinant of reduced correlated discharge levels in deprived barrels.

Fig. 2-2C shows a cumulative distribution of peak-correlation for the entire population of the CON and BD neuronal pairs in barrels. The CON distribution clearly has a higher range of peak-correlation coefficients than the 'deprived' distribution within the barrel, and the values were significantly different ($p << 0.001$, Wilcoxson non-parametric test of cumulative distribution). Overall, there was a 53% decrease of the mean peak correlation coefficients in BD neuronal pairs when compared to the CON group.

Neuronal synchrony between barrels and septa

Pairs of CON neurons (n = 125) and BD neurons (n = 122) were analyzed using two electrodes (~300 μm apart), one in a barrel and another in an adjacent region of the septum. The correlated discharge between pairs of barrel-septal neurons was high in CON animals. Fig. 2-3A shows a representative CON barrel-septal neuronal pair. The correlation peak was narrow and centered around a lag of 0 ms. Again, even if the firing rate strongly changes over the course of a trial, the coincidence histogram shows high coincident spiking activity throughout the entire 2 sec post-stimulation period. On the other hand, the spike synchrony in BD barrel-septal pairs was again extremely low, as illustrated in Fig. 2-3B, despite spike magnitudes (for this particular example) that were comparable with those of the CON example (Fig. 2-3A). The persistent lack of spike synchrony was revealed by the low average peak-correlation value (0.0785), and the coincidence histogram (Fig. 2-3B bottom). Overall, the population cumulative distribution for the peak-correlation of the BD group was significantly different (Fig. 2-3C; $p<<0.001$, Wilcoxson non-parametric test of cumulative distribution) when compared to the CON group. Moreover in comparison to the CON, the population mean peak-correlation of the BD was reduced by 44%. Thus, BD had a strong negative impact on synchrony between cells in barrels and in the surrounding septa.

Neuronal synchrony within the septa

Correlated discharge between neurons in the septa was much less affected by BD than cells in other barrel cortex locations. Unlike the barrel and barrel-septum neuronal pairs, we observed that, not all the septal neuron pairs showed reduced

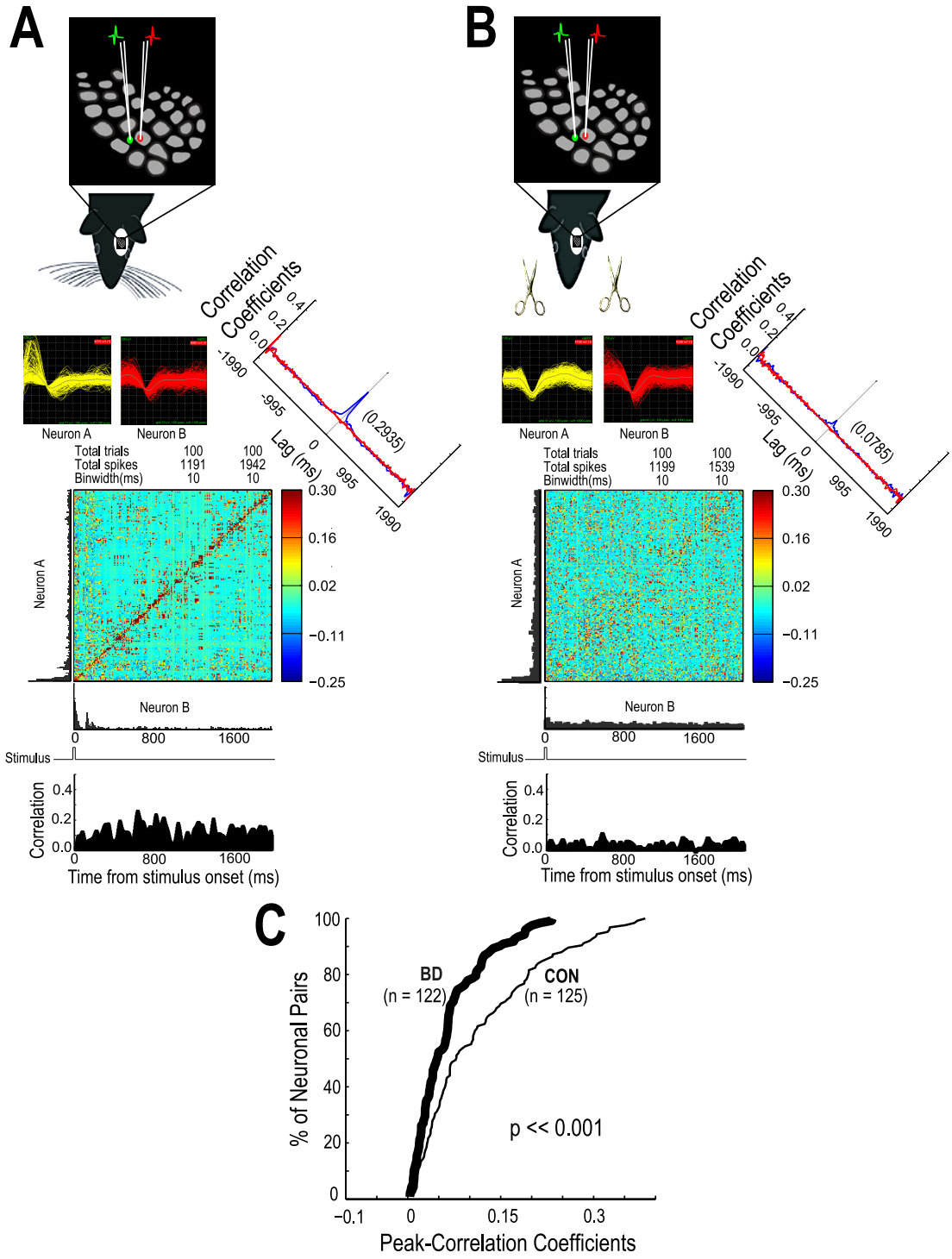

Figure 2-3. Correlated discharge of two neurons, one in a barrel and the other in a nearby septal location

A and B. Here the neurons are ~300 µm apart recorded with their firing rates simultaneously from 2 adjacent electrodes following a 4 ms whisker stimulus represented by the stimulus trace. The waveforms are highly unlikely to be from the same cell even when the waveforms are similar in shape (waveforms displayed in separate inserts because they were recorded by 2 electrodes). Display similar to Fig. 2-1. Here there is a detectable peak in the BD cross-correlogram (peak-correlation coefficient of 0.0785), but it is much reduced compared to CON pairs (peak-correlation coefficient of 0.2935). C. The difference is clearly seen when the population cumulative distribution of peak correlation coefficients of the BD (n=122) and CON (n=125) neuron pairs are compared with the Wilcoxson nonparametric test (p<0.001).

synchrony in the BD animals. This result is illustrated in Fig. 2-4A and B with a typical example of a CON pair of septal neurons showing a peak-correlation coefficient of 0.1330 and a comparable BD septal pair with a similar peak-correlation coefficient of 0.1374. Fig. 2-4C shows the cumulative distribution of the population peak-correlations for the CON (n=125) and BD (n=122) rats. The two distributions were statistically not different ($p > 0.05$; Wilcoxson non-parametric test of cumulative distribution), but the plots reveal that there are an important percentage of neuronal pairs in the BD septa that are characterized by lower peak-correlation coefficients than CON (Between arrows Fig. 2-4C). The population peak-correlation of the BD septa was on average reduced by 29% below CON.

To analyze the apparent multimodal cumulative distributions of the CON population peak-correlation coefficients (Fig. 2-4C), and the reduction of its mean value we examined the frequency and probability distributions of the peak-correlations of the septal CON and BD populations (Fig. 2-5A, B). As shown in Fig. 2-5A, the frequency and probability distributions indicate that the CON septa have a range of peak-correlation coefficients varying from 0-0.4, with only a sub-population of cells showing relatively high (defined arbitrarily as >0.15) correlation (To the right of the red dashed line in Fig. 2-5A). Interestingly, in the BD distribution (Fig. 2-5B), the frequency of neuronal pairs with high correlation (>0.15 indicated by the red-broken line in Fig. 2-5B) was reduced by 49% ($p<0.01$; Wilcoxson non-parametric ranksum test), but many neuronal pairs with relatively lower, but significant ($p<0.01$), correlation continued to be frequent in the BD septa suggesting that they were less affected by sensory deprivation.

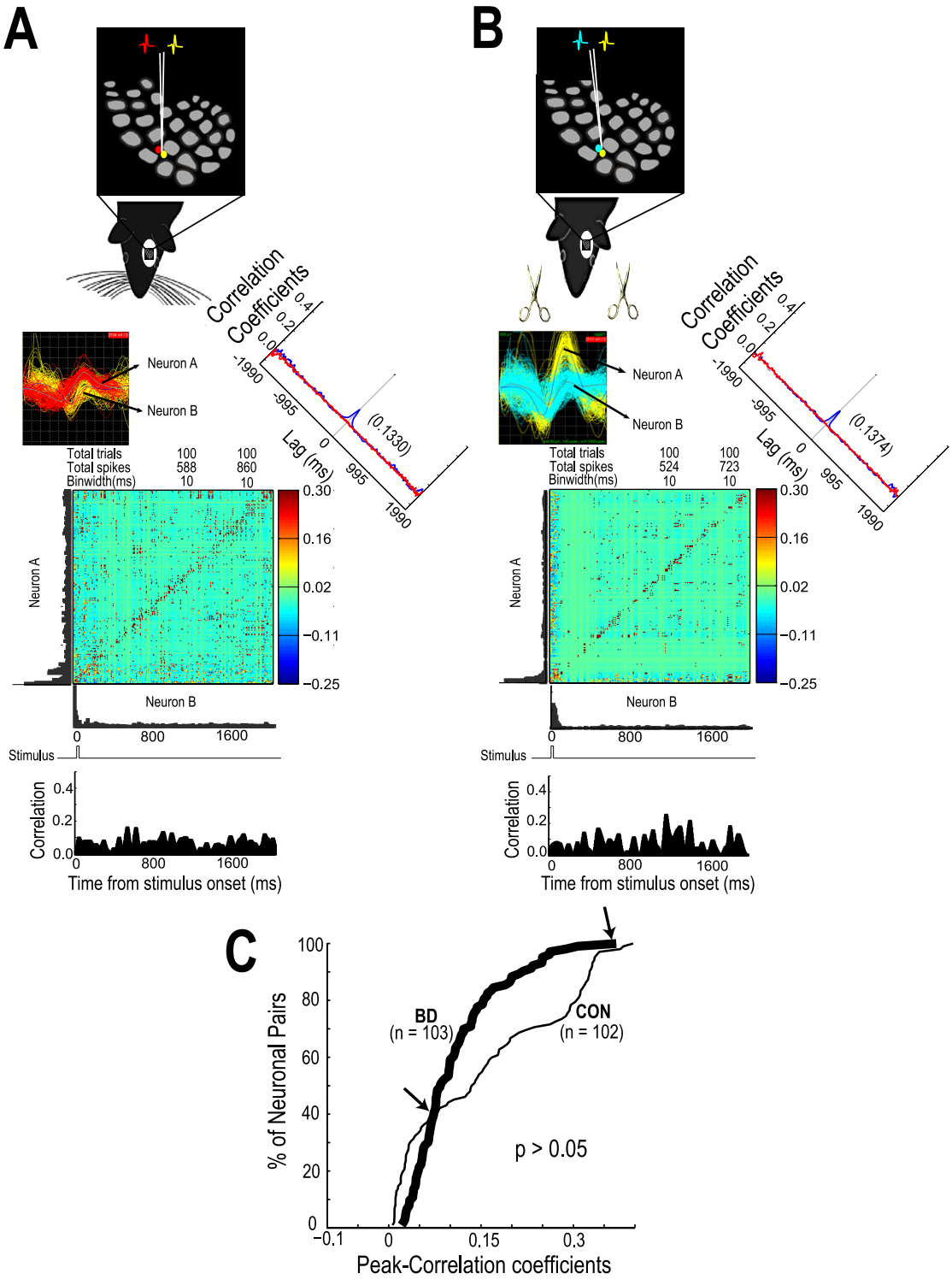

Figure 2-4. Correlated discharge of two neurons in the septal area between barrels
A and B. Display similar to Fig. 2-1. In the septum the degree of correlated discharge is less than in barrels and similar levels are maintained after BD in these example cell pairs. C. Cumulative distribution of peak correlation coefficients in all septal cell pairs in CON (n=102 pairs) and BD (n=103 pairs) conditions. Althoughthe Wilcoxson nonparametric test shows no statistical difference between the 2 distributions, there is a noticeable percentage of BD neuron pairs (between arrows) characterized by lower peak-correlation co--efficients than CON. These were further analyzed in Fig. 2-5.

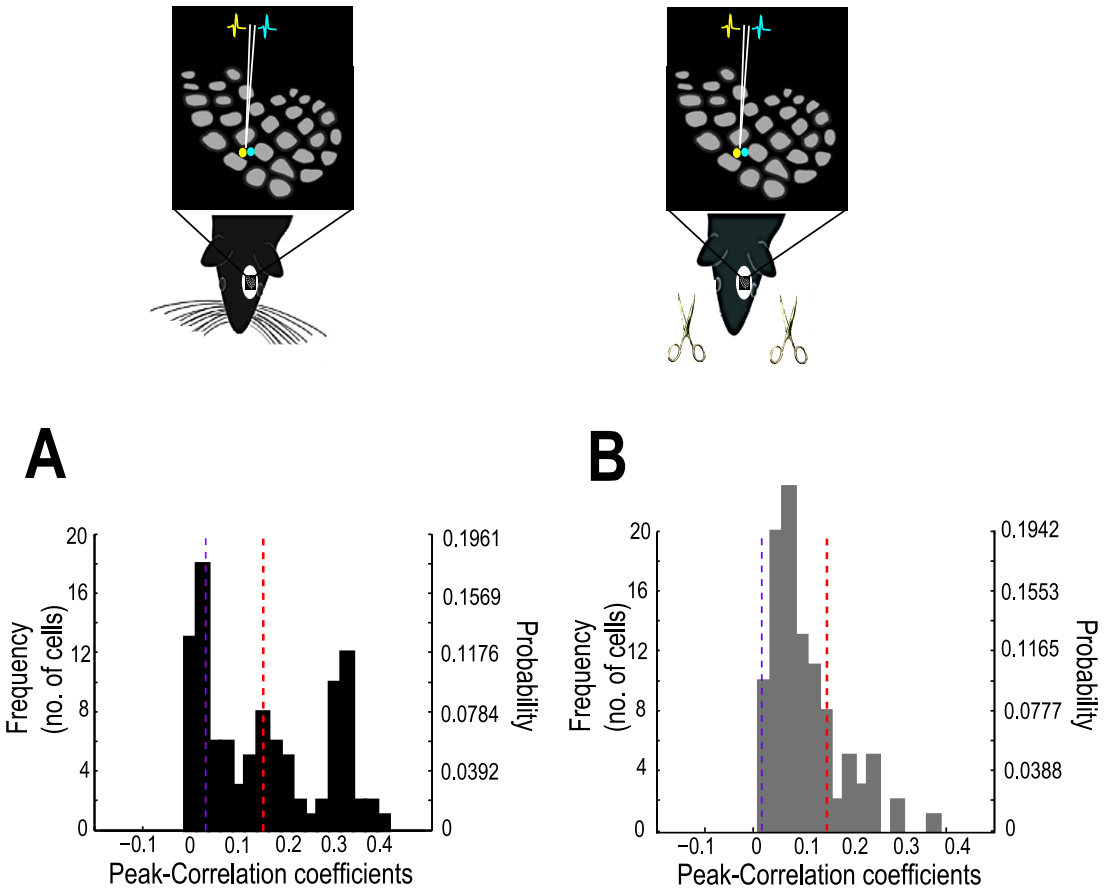

Figure 2-5. Frequency and probability distribution of peak correlations in pairs of septal neurons

The blue broken line represents the upper value of confidence interval for a mean average of 0 ($p<0.01$). Bin size=0.025. A. Frequency (number of cells) and probability distribution of CON peak-correlation co--efficients in septum-septum neuronal pairs showing the range of peak-correlations. The red dashed line has been arbitrarily placed at 0.15 to show a subpopulation of cells having relatively high peak correlation values (to the right of the dashed red lines). B. Frequency and probability distribution of BD peak-correlation coefficients in septum-septum neuronal pairs. The broken red line is placed at the same point in the X-axis of the distribution as the one in the CON case, to show the change in the distribution of cells. The low frequency of cell pairs to the right of the dashed red line reflects loss of the highly correlated neuronal pairs in the septum after BD. However, as seen to the left of the broken red line, the septal neuronal pairs with low but significant peak-correlation coefficient values became more frequent after sensory deprivation.

In other words, the effect of bilateral deprivation in reducing synchrony appears to be limited to a particular sub-population of septal cells.

Neuronal synchrony during stimulus driven and spontaneous firing periods

The pairs of PSTHs considered for the analyses showed a brief evoked response (~0-50 ms epoch) following the short duration (4 ms) whisker stimulus, and a much longer (~50-2000 ms) post-stimulus epoch of background discharge. Since neuronal spike coincidence may provide an important platform for stimulus encoding, it is possible that the onset of a stimulus modifies the existing neuronal synchrony, rendering it different from the synchrony that occurs during the rest of the inter-stimulus interval. To test this idea we calculated the average coincidence of each neuronal pair during the stimulus driven and the spontaneously active period. In the coincidence histogram, the 'first' 50 ms (0-50 ms post-stimulus) represented the time period containing almost all of the whisker stimulation driven activity (Petersen and Diamond, 2000) and the 'last' 50 ms (1950-2000 ms post-stimulus or the 50 ms before the next stimulus) was assumed to be long enough after the stimulus to represent a spontaneous activity period for the neurons. The average coincidence during the first 50 ms post stimulus was calculated and compared with the average coincidence of the last 50 ms post stimulus using a Student's t-test for neuronal pairs in a group of barrel, septa and barrel-septal pairs in both CON and BD rats (Fig. 2-6). In the CON animals the average coincidence of the last 50 ms was significantly higher than that of the first 50 ms for neuronal pairs in the barrel ($p < 0.001$), septa ($p < 0.01$) and also for barrel-septal pairs ($p < 0.001$) (Fig. 2-6A, B, C white bars). However, this difference was not

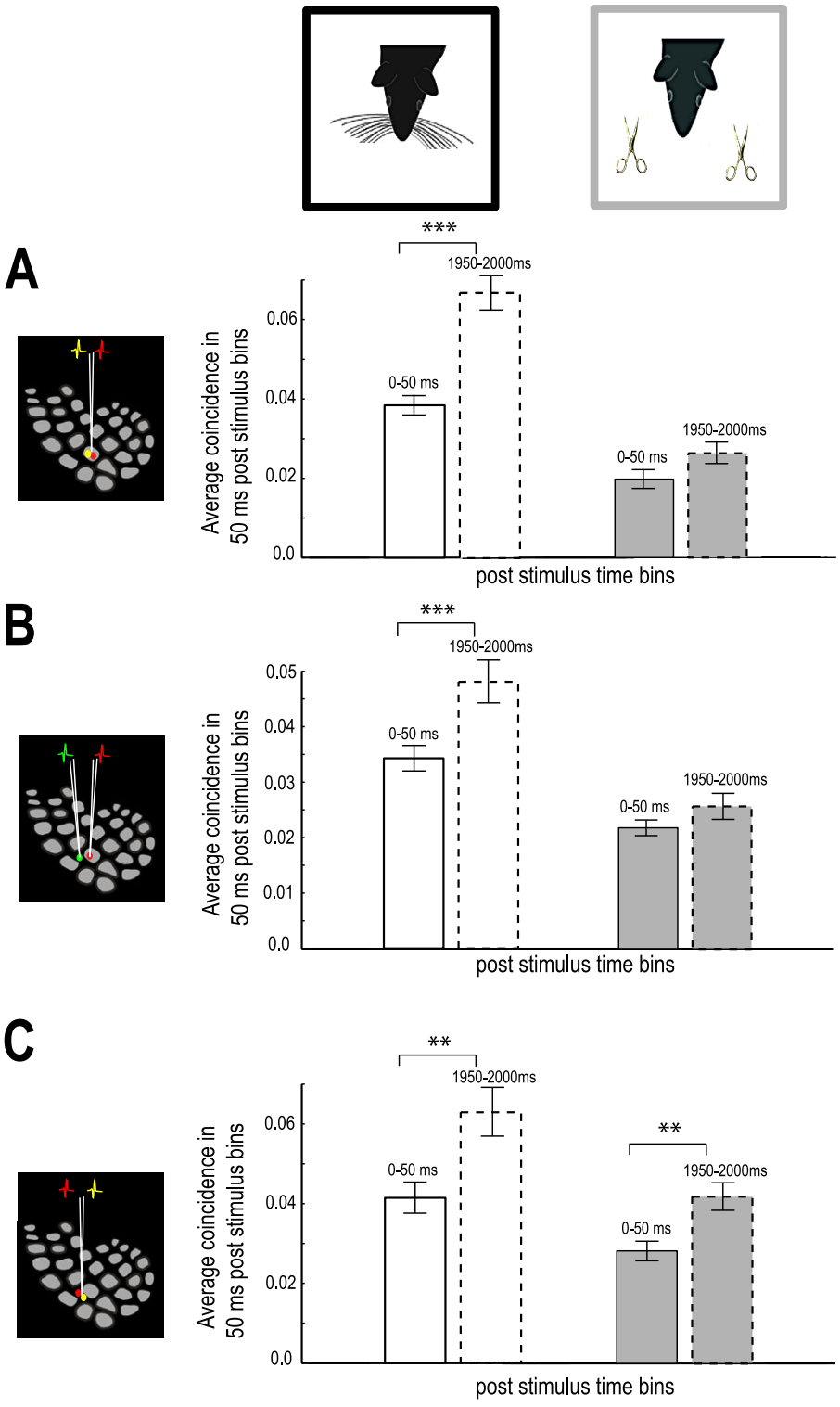

Figure 2-6. Comparison of the average coincidence in neuronal pairs in the 'first' 50 ms after the stimulus (solid bars) and the 'last' 50 ms (1950-2000 ms post-stimulus, dashed bars) after best whisker stimulation in the three locations

Error bars represent standard error of mean or S.E.M. The triple asterisks (***) and double asterisks (**) represent a significance level of <0.001 and <0.01, respectively. A. In barrel cells of CON animals (white bars) the 'last' 50 ms (background activity epoch) shows significantly higher correlation than the 'first' 50 ms (evoked response epoch) ($p<0.001$; one-tailed t-test). This difference is eliminated after bilateral sensory deprivation (gray bars; $p>0.05$; one-tailed t-test). B. Similar data when one cell is in a barrel and the other is in the septum. C. When both cells are in a septal zone the 'first 50, last 50' difference is maintained after sensory deprivation, showing a resistance of septal cells to this effect of bilateral sensory deprivation.

significant after BD for within-barrel and barrel-septal neuronal pairs ($p > 0.05$) (Fig. 2-6A, B, grey bars). Interestingly, the difference between the average coincidences of the spontaneously active and the stimulus driven firing period in the within-septa neuronal pairs were unaffected ($p < 0.01$) by bilateral sensory deprivation (Fig. 2-6C grey bars). Similar results were produced when tested with the non-parametric rank sum test. The temporal evolution of coincidence after the brief stimulus was also calculated as average coincidences in 5 successive epochs of 50 ms post-stimulus time bins spaced regularly at 200 ms intervals during the 2000 ms post-stimulus period (Fig. 2-7). However, further statistical analysis is required in order to completely characterize the pattern of the evolution of coincidence post-stimulus.

Discussion

Sensory deprivation has been previously shown to have adverse effects on single cell neurophysiology in sensory cortex (Lee et al., 2007; Popescu and Ebner, 2010; Rema et al., 2003; Shepherd et al., 2003; Simons and Land, 1987; Shoykhet et al., 2005; Wiesel and Hubel, 1965). The present study adds a new dimension to the effects of sensory deprivation by showing that neuronal ensemble functions are strongly degraded by sensory deprivation. The results show that sensory deprivation severely degrades coincident discharge in pairs of barrel-barrel and barrel-septum neurons in barrel cortex. The results confirm the presence of “synchrony at rest”, that is, synchronous discharge of neurons during periods of ‘spontaneous’ activity in this neural system (Swadlow et al., 1998; Zhang and Alloway, 2006). The correlation peaks were narrow in the crosscorrelograms and almost always had near 0 lag. These results

emphasize that barrel cells fire together coherently, and we conclude that sensory pathway activity during the first postnatal month is important for developing synchrony among barrel neurons,

Correlated firing of neurons can occur in at least six ways: 1) a common input arriving near simultaneously to the pair of cells; 2) horizontal cortical connections, 3) responses in unconnected neurons to a common stimulus, 4) statistical coincidence artifact due to firing rate (Reed et al., 2008), 5) Oscillatory variations in neuronal excitability (Eckhorn, 1994; Gray et al., 1990), and/or 6) Synchronous drive from the thalamic afferents (Swadlow and Lukatela, 1996).

In agreement with Swadlow et al. (1998), the correlated discharge observed in the present study was not dependent on the peripheral, transient, stimulus as the synchrony persisted throughout the 2 sec post-stimulus period even after the response to the brief stimulus (4 ms duration) was undetectable (e.g. 1000-2000 ms post-stimulus). Thus a near simultaneous arrival of a common input to the pair of cells is very unlikely to be a suitable explanation for the prolonged spontaneous correlated discharge we observed here. The stimulus driven correlated discharge can be seen clearly in the raw JPSTH using 1 or 10 ms bin widths, but almost all of the stimulus-driven correlated discharge disappears after the shift predictor control in cortex as it has been shown to do in the thalamus (Temereanca et al., 2008). Fig. 2-7 shows snapshots of coincident spike discharge in 50 ms epochs every 200 ms for the 2000 ms duration of the inter-stimulus interval. The first epoch, containing correlations during the evoked response, always shows lower correlation than the remaining epochs, but the higher correlation in

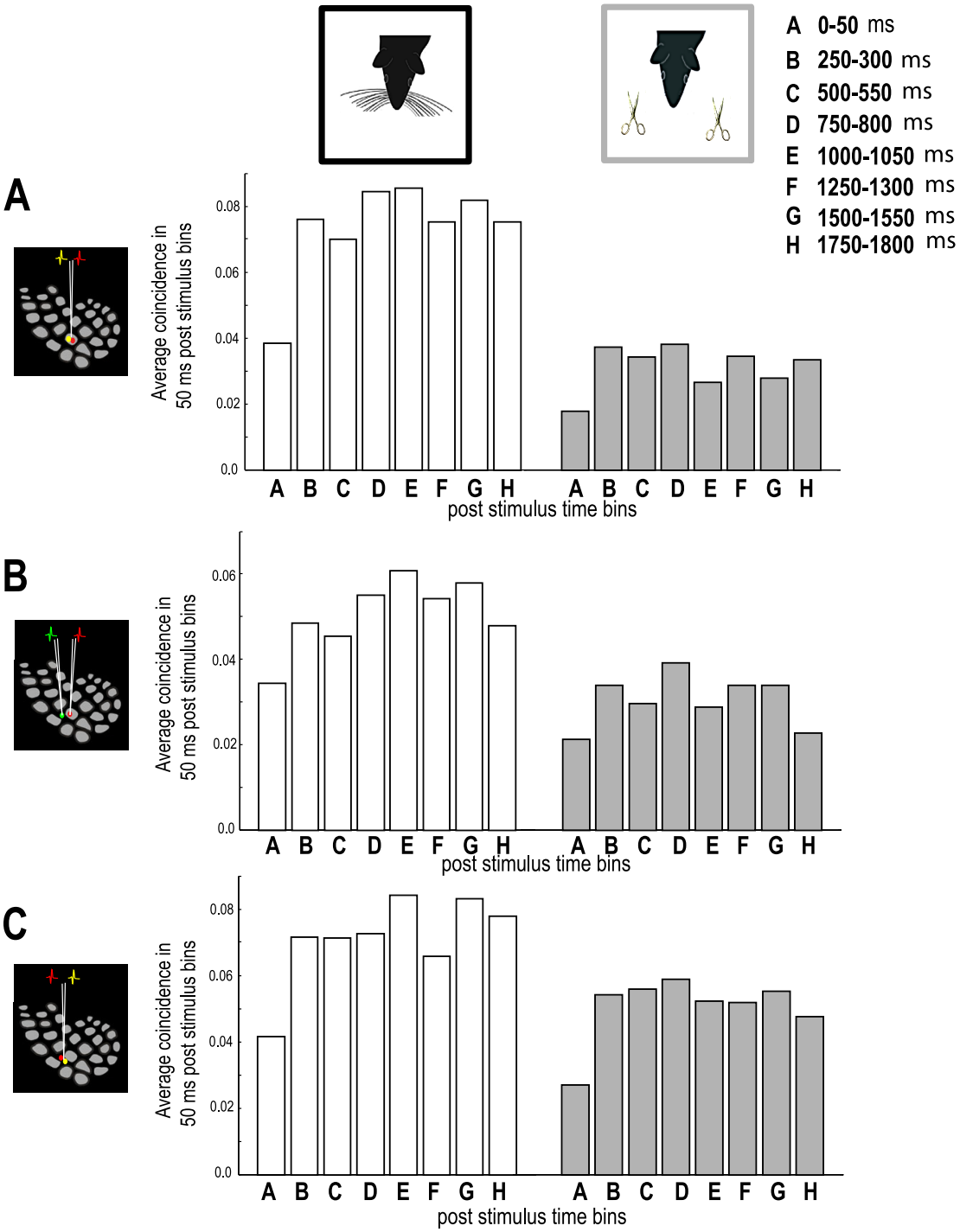

Figure 2-7. Evolution of spike coincidence in a population of neuron pairs across post stimulus times

Average coincidence was measured in 50 ms post stimulus bins and represented in white (CON) and gray (BD) bins. Eight such bins (A-H; 200 ms apart) have been chosen to represent the temporal evolution of coincidence of all the neuron pairs. A. Coincidence of the population of neuron pairs in CON and BD barrels over post stimulus time. The average coincidence appears to be higher after 200 ms post stimulus CON (Whitebars) which mainly comprises background activity since the stimulus duration (4 ms) is very short. In BD (Gray bars) pairs there is an overall decrement in the average coincidence (as discussed in the chapter) and probably less differences between evoked and background coincidental activity. B. Similar results when one neuron is in a barrel and one in the septa. C. Similar results when both the neurons are recorded from the septa.

the post-response epochs don't show any particular trend up or down during the period sampled.

The methods applied in our study greatly reduce the probability of correlation being produced by a common stimulus (reason 3) or firing rate (reason 4). The PSTHs of the neuronal pairs and the cross-correlograms also were free of oscillatory activity, thus, reducing oscillatory variations in neuronal excitability (Eckhorn, 1994; Gray et al., 1990) as a probable candidate for correlated discharges observed here. However, oscillatory activity was observed in the coincidence histogram indicating a possible waxing and waning of the level of spontaneous correlated discharge by the barrel cortical cells. However, more experiments and extensive analysis are necessary before concluding that the oscillatory variations in neuronal coincidence are affected by early sensory deprivation.

The degree of change in synchronous discharge due to early sensory deprivation is complex and selective: when synchrony is almost obliterated between cell pairs in a barrel or barrel and septum, partial synchrony can be maintained by some neuron pairs in the septum. In control (CON) rats, the population of septal cell pairs shows a multimodal peak-correlation distribution which may reflect distinct subsets of cell pairs coupled by low, intermediate and high levels of correlated discharge. In bilaterally whisker trimmed animals, intermediate and highly synchronized neurons are severely affected, whereas, low levels of correlated discharge develop among some septal cells. In addition to this overall dramatic loss of synchrony, the results show that the synchrony of the neurons in all parts of the barrel cortex is particularly reduced during the response to the stimulus (0-50 ms post stimulus). Interestingly, this relatively lower

synchrony is not apparent in the ‘deprived’ barrel and barrel-septal cell pairs whereas the ‘deprived’ septal cell pairs show a reduction of coincident firing during the stimulus driven period similar to controls. Ongoing studies will determine whether this reduction in synchrony during the response to stimulation occurs in awake, attending animals.

One explanation for these results is that the abnormally low lemniscal activity coming into cortical barrels during the first month of life leads to a failure to develop correlated discharge among barrel neurons. A logical corollary is that perturbation of synchrony among barrel neurons in turn desynchronizes barrel to septum transmission, whereas, connectional complexities and diversities in the different inputs and outputs of the septum (Kim and Ebner, 1999) permits it to preserve certain aspects of synchrony.

Behaviorally, a period of sensory deprivation similar to that used in the present study caused a loss of acuity in texture discrimination that persists even after normal activity levels are restored for months before and during behavioral training (Carvell and Simons, 1996). The deprived animals could distinguish between smooth and rough, suggesting that they were not cognitively impaired, but they could not distinguish between even grossly different rough surfaces that normally reared animals could distinguish at high performance levels. After normal rearing, synchronous spiking activity has been observed between almost all inhibitory barrel interneurons that receive potent monosynaptic input from the ventral posterior medial thalamic nucleus, but similar correlated discharge was not found among layer 5 corticofugal neuron pairs that do not receive thalamic inputs (Swadlow et al., 1998). On the other hand, spiking synchrony between barrel columns can be found if multiple whiskers are stimulated simultaneously or sequentially (Zhang and Alloway, 2004; Zhang and Alloway, 2006).

Such results have been interpreted as evidence for thalamocortical inputs being the underlying organizer of spike synchrony in primary sensory areas (Swadlow and Lukatela, 1996). Also, it has been suggested that precisely correlated activity among thalamic neurons may strengthen the excitatory effects of thalamic input on cortical neurons through temporal summation (Alonso et al., 1996; Temereanca, et al., 2008), which may be necessary because of the low efficacy of individual thalamocortical synapses (Bruno and Sakmann, 2006). The state of thalamic synchrony is predicted to change ms by ms as the whiskers are moved to make high velocity contact with objects in the environment (Temeranca, et al., 2008). Functionally, the summation of excitatory postsynaptic potentials may be especially effective in enhancing activity in the synaptic targets when peripheral stimulation is weak or non-optimal (Kyriazi and Simons, 1993).

Septal cells with normally low correlation probably include neurons which have connections with structures outside the barrel field, and these areas may be less affected by deprivation. On the other hand, the septal cells that receive direct connections from nearest-neighbor barrels might develop high or intermediate synchrony with other types of septal cells during the course of maturation. It is possible that following deprivation when the ultra-low lemniscal activity is insufficient to synchronize barrel cell activity, the septal cells connected to these deprived barrel neurons similarly fail to develop the expected synchrony. This feature of septal function is consistent with the idea that while the barrel neurons are strongly sensory, the septal neurons participate in more sensorimotor integrative functions (reviewed by Alloway, 2008). However, a counterpoint to such a simple argument is that the septum is a relatively narrow structure, and the highly correlated septal cells might involve

accidentally recorded neurons from the edge of the barrels. Clear histological cases showing localization of the electrodes in the septum and a significant percentage of neuronal pairs in the high correlation subset makes it an unlikely alternative interpretation, although we cannot completely rule it out.

The fact that correlated discharge in barrel cortex is reduced during the response period after a stimulus and that following deprivation this difference does not develop in barrel-barrel and barrel-septum neuronal pairs (Fig. 2-6) hints at the importance of this reduction in correlated discharge for processing information in the barrels that is needed to guide behavior. This finding suggests that reduced correlated firing during the stimulus evoked epoch also develops through the influence of the active thalamocortical connections during normal rearing. In the population of deprived septal cells, this feature of correlated discharge continued to be present, possibly due to other connections to the septum from outside the barrel field. The requirement for background spike synchrony in cortex coupled with the coding-based deficits in behavioral acuity lead to the hypothesis that the maturation of barrel network assemblies requires correlated discharge during development to generate normal processing in cortical networks. This model of normal and deprived cortical development is illustrated in Fig. 2-8.

Fig. 2-8A illustrates a number of features of circuit maturation in barrel cortex that may be activity-sensitive during normal post-natal development. Activity imposes changes in cortical circuitry leading to the well-established excitatory-inhibitory-excitatory response sequence well-documented in barrel cortex (Agmon and Connors, 1991; Simons and Carvell, 1989). Within this response there is a strong theoretical

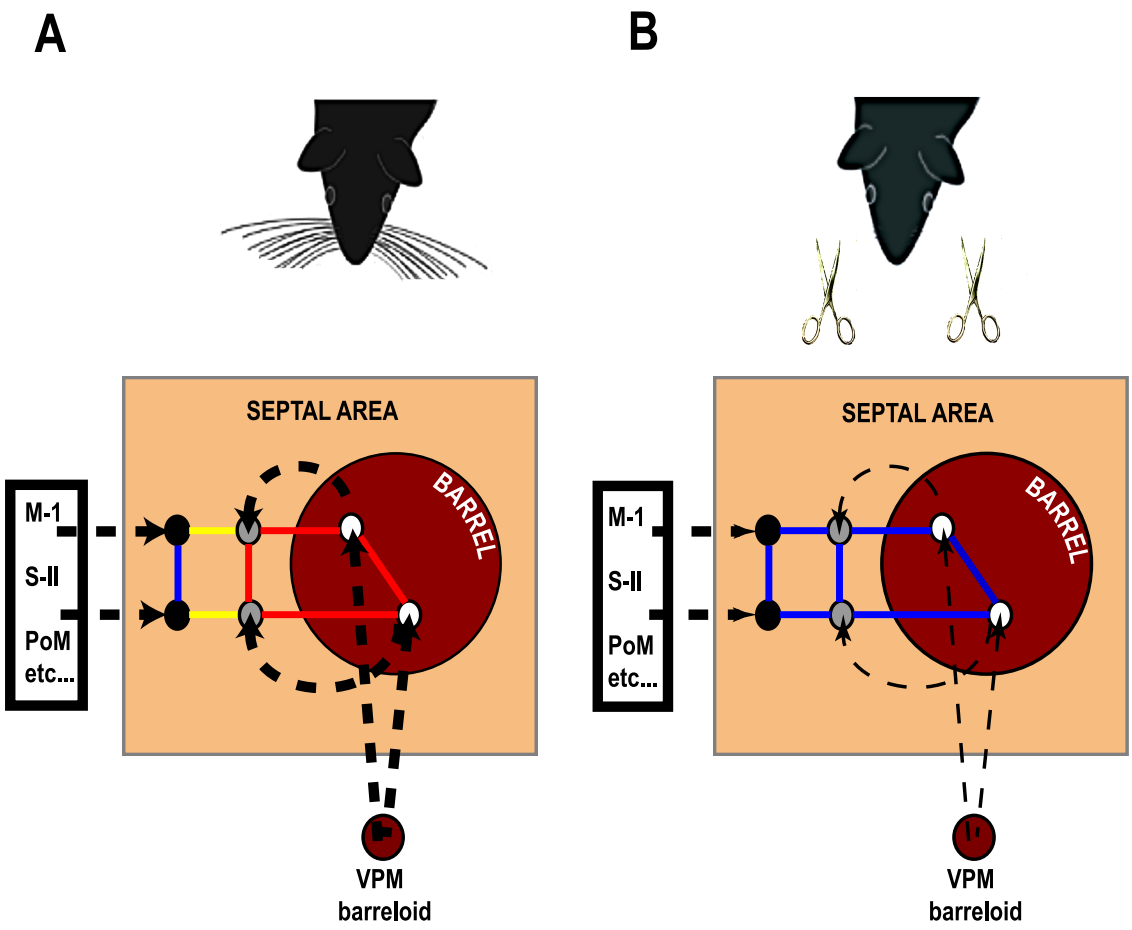

Figure 2-8. Model of the effect of sensory deprivation on the integrative properties of neurons in barrel cortex

Dashed lines with arrowheads represent connections and solid colored lines represent correlated activity. Thickness of the dashed line indicates the level of neuronal activity. Red, yellow and blue colored solid lines represent high, intermediate and low correlated activity. A. During normal rearing the lemniscal pathway through the relay nucleus of the thalamus (VPM) is very active and leads to strong correlated discharge (red lines) among barrel neurons (white circles) that receive VPM thalamic input. Barrel neurons, in turn, project to the septum around the barrel developing strong correlated circuits (red lines) with a subset of septal neurons (gray circles) to which they are directly connected. Other neurons (black circles) in the septum are assumed to receive diverse inputs, many from outside the barrel cortex (box on the left) and these septal neurons develop only modest (blue line) correlated activity among themselves, and intermediate correlated activity (yellow lines) with the barrel-dominated septal neurons. B. Sensory deprivation drastically reduces activity in the lemniscal pathway, and low activity blocks the development of correlated discharge between neurons in a barrel, and between barrel and a subset of septal neurons (blue lines). The non-barrel dominated septal cells maintain their low level of correlated discharge even when the lemniscal inputs are low, presumably due to diverse inputs from outside the barrel cortex.

case for almost all (~91%) of the information about whisker location being conveyed by the first spike after the stimulus (Panzeri et al., 2001; Petersen et al., 2001). Following a brief burst of firing after a whisker contacts an object there is widespread inhibitory activity in the barrel, orchestrated by the synchronous discharge of nearly all inhibitory neurons in a barrel: cell activity within a barrel is thus effectively quenched after the initial high discharge rate (Swadlow et al., 1998). This level of correlated discharge, even at rest, is unusual and could maintain a high level of synaptic strength ("readiness") to respond as an ensemble to thalamic input volleys. Excitatory activity then rebounds at longer latency until the discharge in the barrel circuit returns to a low background level that is no longer directly tied to the stimulus, but continues to be correlated among neurons in each barrel (present results). Bilateral sensory deprivation has a negative impact on each of these activity-based events. First, the response of both excitatory and inhibitory neurons to a thalamic volley in deprived barrel cortex is significantly weaker when individual cells respond to single test stimuli (fewer spikes per stimulus), compared to that elicited in normally reared animals. Further, deprivation disrupts the timing of the first spike post-stimulus and the high probability of short (7-10 ms) latency discharge following repeated stimulations (Popescu and Ebner 2010). Finally, the barrel neurons fail to develop the characteristic "synchrony at rest" found in numerous studies of normal barrel cortex (Petersen et al., 2001; Swadlow et al., 1998; Zhang and Alloway, 2006; present results). The net result is a sensory deprived cortex that looks anatomically quite normal, but that cannot process sensory information with normal precision. Importantly, this synchronous spontaneous discharge is also seen in awake preparations, and it is not abolished by anesthesia (Swadlow, et al., 1998).

Awake behaving preparations will be necessary to determine whether synchronized activity is increased by attention to task as it is in the secondary somatic sensory area of primates (Roy, et al., 2007).

Despite the observation that spike synchrony is severely reduced following early sensory deprivation, it remains to be directly tested whether or not spike synchrony is a sufficient or even a necessary mechanism for development of normal sensory processing. It is possible that changes in synchrony will be correlated with the rapid fluctuations in plasticity that occur in barrel cortex (Erchova and Diamond, 2004). If so, low levels of correlation also could be a cause of reduced plasticity in barrel cortex following early sensory deprivation. However, evidence from awake behaving animals would be necessary to conclusively analyze the behavioral consequences of the loss in neuronal synchrony caused by sensory deprivation.

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CHAPTER III

UNILATERAL WHISKER TRIMMING LEADS TO UNIQUE PATTERNS OF NEURONAL COINCIDENT DISCHARGE IN RAT BARREL CORTEX NEURONS

Introduction

In the previous chapter, it was shown that sensory experience can affect the development of cortical spike synchrony in rat barrel cortex. Non-traumatic reduction in peripheral activity via bilateral whisker trimming led to a general reduction of spike synchrony in L-IV barrel cortex. However, whether spike synchrony is dependent upon the methods adopted for producing a sensory deprived animal model has not yet been studied. Sensory deprivation has been shown by means of bilateral and unilateral whisker trimming to have dramatically different effects on magnitude of responses in barrel cortex (Popescu and Ebner 2010). While unilateral deprivation (UD) leads to an increase in spine density (Vees et al. 1998) as well as the appearance of inhibitory synapses on dendritic spines (Micheva and Beaulieu 1995), bilateral deprivation (BD) led to a significant decrease in spine numbers and head diameter (Briner et al. 2010). Moreover, following BD rearing there is a general overall decrease in magnitude and increase in onset latency, while UD leads to increased responsiveness in the septum and negligible effects on onset-latency (Popescu and Ebner 2010). Thus, we wanted to characterize whether UD rearing would have a characteristically different effect on network properties similar to or different from spike synchrony that exists after BD rearing. In this chapter, we show using JPSTH analysis that the effect on cortical spike

synchrony is significantly different following UD rearing than it is following BD rearing in rats.

Methods

All data acquisition methods utilized here are identical to those described in detail in chapter II, with the main difference being unilaterally deprived rearing conditions (n=5) in which only the whiskers on one side of the face were trimmed from PND 0-60.

Results

Recording from barrel cortex and calculating spike synchrony among neuronal pairs using JPSTH analysis in control (CON) and unilaterally whisker trimmed (UD) rats.

Extracellular single unit recording was carried out in the barrel cortex of CON (n=4) and UD (n=4) rats that had all of their mystacial whiskers trimmed on one side of their face from PND 0-60. Recordings from single cortical neurons were made using three microelectrodes (~300microns apart) in response to 100 repetitions of a short duration (4ms) whisker deflection (600 µm) at 0.5 Hz. The recorded neurons from each electrode were sorted offline and segregated into 6 groups (Fig. 3-1A), depending on their location in L-II/III or IV of a histologically determined barrel or septal column. JPSTHs were constructed as described previously (Ghoshal et al. 2009; Chapter II) to show the incidence of neuronal spike synchrony between pairs of neurons in a single

barrel or septal column and also between one barrel and one septal column in both CON and UD rats.

For each animal group, the peak correlation observed between the neuronal pairs in the normalized JPSTH was identified as significant ($p < 0.001$) by comparing it with those obtained in the simulated and the shuffled JPSTH using the paired *t* test. In general, the correlation peaks observed in the cross-correlograms were quite sharp and generally had a time lag close to 0. Moreover, the cross-correlograms did not show oscillations, but oscillations in the coincidence histograms were observed.

The significant average peak correlation values computed by the JPSTH were then compared statistically between CON and UD rats for each location. The average coincidence was also calculated from the coincidence histogram in an early epoch post the whisker stimulus (0-50 ms), representing the stimulus-evoked response period, and a late post-stimulus epoch (1950-2000 ms) representing a putative spontaneously active period. The average coincidences were then compared across the early and late epochs within and between each group.

Fig. 3-1B and C displays two typical examples of CON and UD JPSTHs constructed from pairs of neurons located in the supragranular layer of a septal column (a.k.a. "above-septum" location, Fig. 3-1B) and granular layers of a barrel column (a.k.a. "within-barrel" location (Fig. 3-1C). The left panel of Fig. 3-1B shows that two above-septal neurons (waveforms shown in the inset) from a CON rat have significant and sharply correlated discharge at near 0 lag with a peak-correlation coefficient (PCC) of 0.1325. However, in an UD rat a pair of neurons (right panel of Fig. 3-1B) in a similar location within barrel cortex, with similar response magnitudes to that of the CON

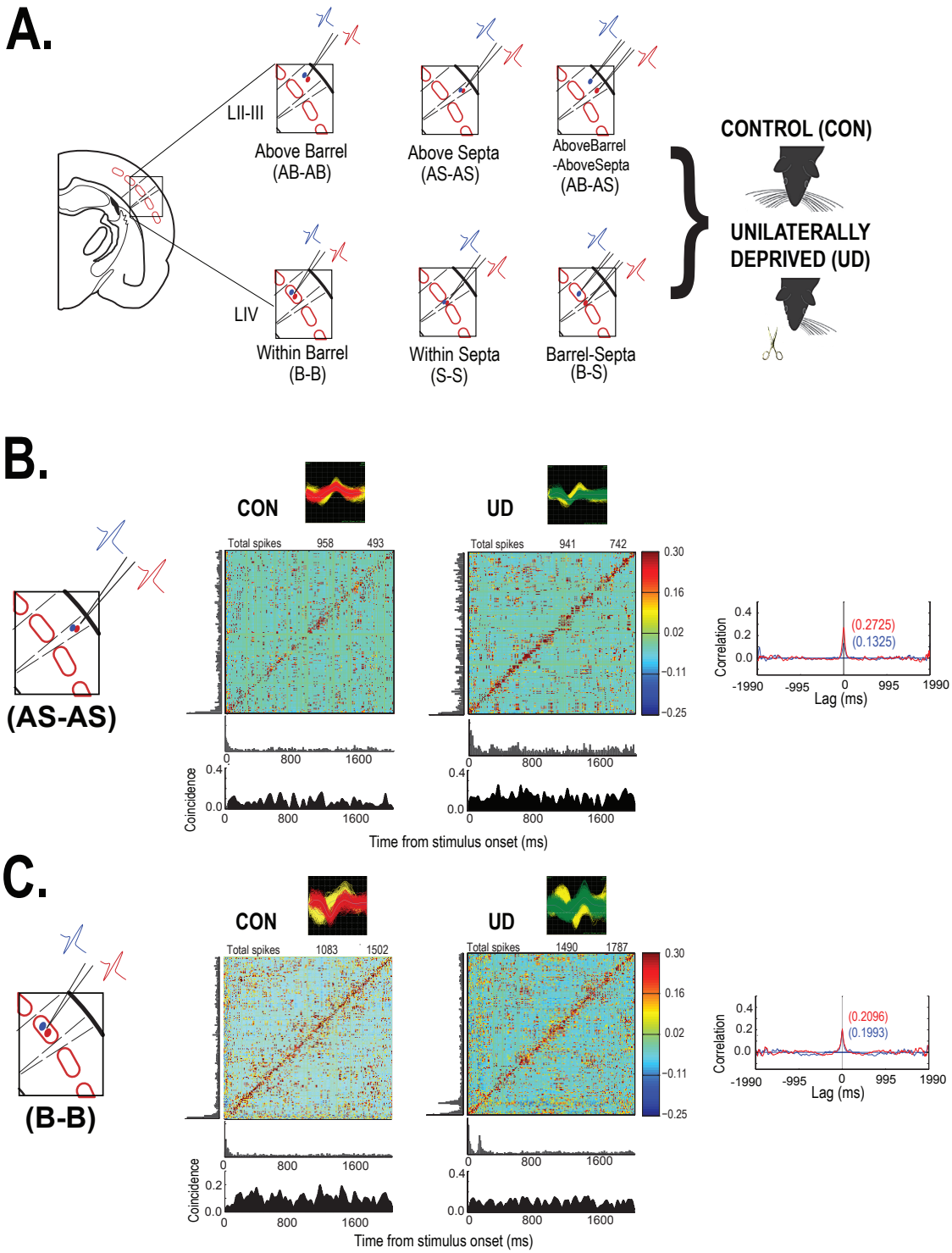

Figure 3-1: Experimental design and typical examples

A. Neuronal pairs were recorded from CON and UD rat barrel cortex and cross-correlations were computed using a JPSTH. The neuronal pairs were grouped into 6 different conditions depending on their location. Neurons recorded from layer II-III (LII-III) of the same barrel and septal column were considered as above-barrel (AB-AB) and above-septum (AS-AS) conditions. In the above-barrel-above-septum condition (AB-AS), one neuron was recorded from LII-III of a barrel column and the other from the LII-III of an adjacent septal column. Similarly, when a pair of neurons were recorded from a single layer IV (LIV) barrel or a septal column, they were considered as the within-barrel (B-B) and within-septum (S-S) conditions respectively, while in the B-S condition one neuron was located in a LIV barrel and the other in the adjacent LIV septum. B and C. A typical JPSTH constructed for an AS-AS pair (B) and a B-B pair (C). In the cross-correlogram the red trace represents the UD, while the blue trace represents the UD. The peak correlation coefficients for CON and UD are mentioned in the cross-correlograms. In this example, there is a noticeable increase in spike synchrony above-septa, but not within-barrels after UD

neurons are much more highly correlated as observed in the JPSTH matrix (prominent correlation in the diagonal), the coincidence histogram (higher coincidence across time) and the cross-correlogram (higher peak with a peak correlation coefficient (PCC) over twice as high (0.2725). On the other hand, the lower left panel (Fig. 3-1C) shows the JPSTH of a neuron pair in L-IV barrels in a CON rat indicating that they are highly correlated with a PCC of 0.1993. Unlike the previous example, there are negligible differences in the degree correlated discharge of a pair of comparable barrel neurons (right panel of Fig. 3-1C) in an UD rat (PCC of 0.2096) from that of the CON neurons. In general, there was an overall increase in spike synchrony observed in the UD barrel cortex in all locations except in the L-IV barrels. Thus, the maximum increase following UD rearing compared to the CON rats was observed in supragranular layers of the UD septal column.

Changes in neuronal spike synchrony in the barrel column after unilateral deprivation:

JPSTHs were constructed for 111 CON and 79 UD neuronal pairs that were located in a single barrel column. Of these 33 CON and 29 UD neuronal pairs were located in the supragranular layers above a barrel (AB-AB pairs) and 78 CON and 50 UD pairs were located in L-IV barrels (B-B pairs). Fig. 3-2 displays cumulative distributions of the CON and UD PCCs for AB-AB (Fig. 3-2A) and B-B pairs (Fig. 3-2B). With unilateral deprivation there is a marked and significant increase in the distribution of PCCs of AB-AB pairs when compared with CON pairs in the same layers ($p<0.01$, Wilcoxon nonparametric test), indicating that neurons in the supragranular layers of UD

barrel cortex are more correlated than comparable neurons in normal barrel cortex. Overall, there was 87% increase of the mean PCC in UD AB-AB pairs when compared with the CON group. On the other hand, the PCCs of CON and UD B-B pairs were not significantly different ($p>0.05$, Wilcoxon nonparametric test) indicating unilateral whisker deprivation does not affect the magnitude of correlated discharge of adult L-IV rat barrel neuronal pairs.

Changes in neuronal spike synchrony in septal columns after UD.

Spike synchrony was also calculated for neuronal pairs located in the same septal column of CON (n=222) and UD rats (n=222). The neuronal pool in the septal column was further subdivided on the basis of their laminar location and JPSTHs were calculated for 120 CON and 120 UD neuronal pairs located in L-II/III above a septum (AS-AS pairs) and for 102 CON and 102 UD L-IV septal pairs (S-S). Fig. 3-2C and D show the cumulative distributions of the CON and UD PCCs for AS-AS (Fig. 3-2C) and S-S pairs (Fig. 3-2D). After unilateral deprivation there is a striking increase in correlated discharge in both L-II/III ($p<0.001$, Wilcoxon nonparametric test) and L-IV of a septal column ($p<0.01$, Wilcoxon nonparametric test) as indicated by the range of PCCs in the cumulative distributions of the UD AS-AS and S-S pairs. The mean PCC increases by ~200% in UD L-II/III septum and 48% in L-IV septum when compared with the CON group.

As described in Chapter II, the CON L-IV septal PCC distribution has more than one observable peaks. Figure 3-3D shows the frequency/probability distribution of CON L-IV PCCs indicating a minimum of two distinct levels of correlated discharge where

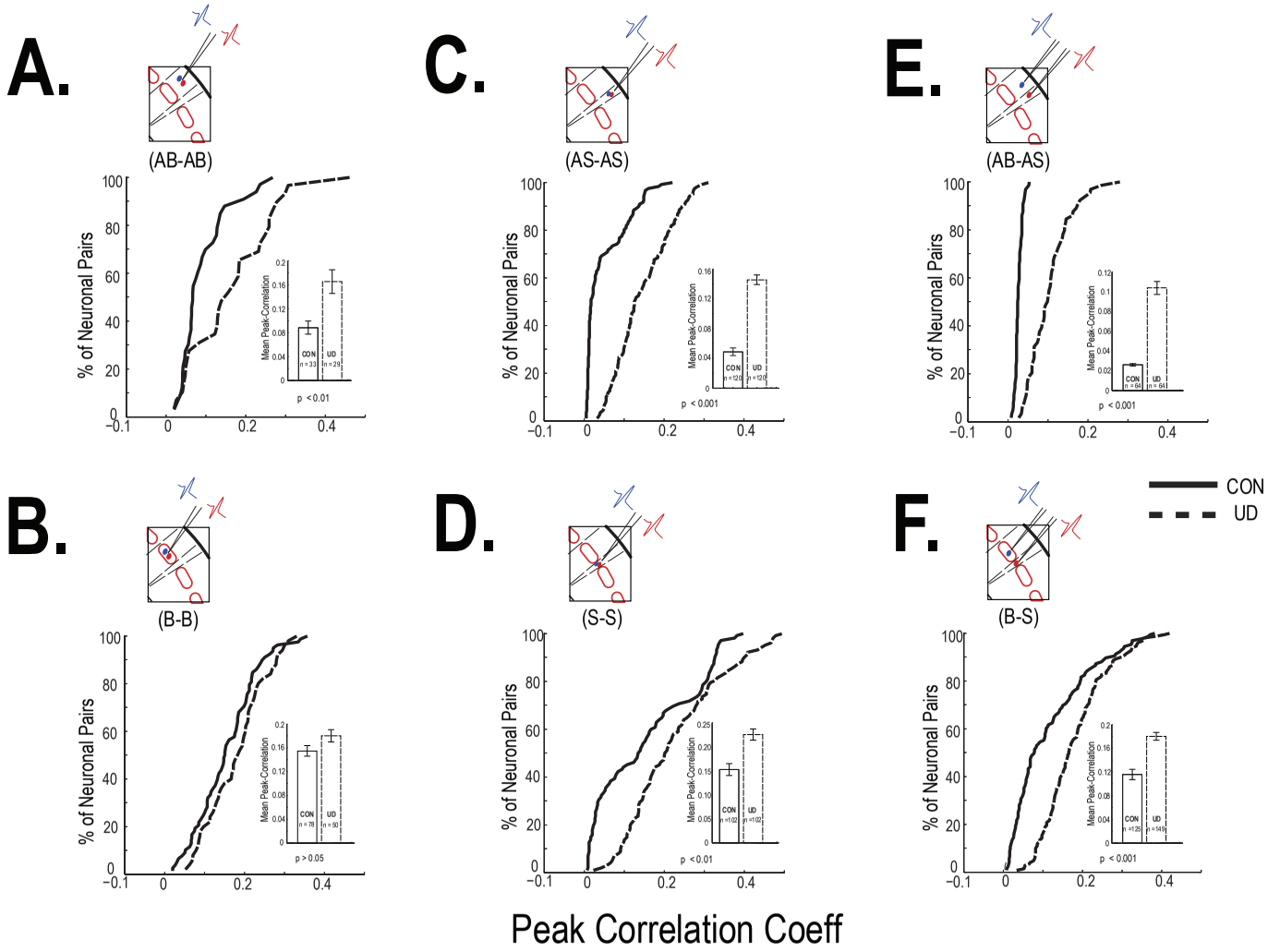

Figure 3-2: Population cumulative distributions and means of peak correlation coefficients for CON and UD rats
Cumulative distributions of peak correlation coefficients are displayed for the CON (solid line) and UD (dashed line) rats when calculated for neurons pairs located in the supragranular and granular layers of a single barrel column (A and B) (AB-AB: CON n=33, UD n=29; B-B: CON n=78, UD n=50) or a single septal column (C and D) (AS-AS: CON and UD n=120; S-S: CON and UD n=102). The distribution for correlation coefficents between barrels and septal columns are displayed in E and F (AB-AS: CON and UD n=64; B-S: CON n=125, UD n=149). The mean bar-graph is shown in the inset for each distribution. Overall, there is a significant increase in correlation strength with UD for all neuron pairs except for the B-B pairs. Error bars in the bar-graph represent the standard error of means or S.E.M.

only a subpopulation of CON septal cell pairs show a relatively high correlation (with a PCC of >0.15). However, frequency distribution of CON AS-AS pair correlations (Figure 3-3C) does not show the existence of two separate levels of synchrony. After UD, there is a significant increase in the number of highly correlated L-IV septal neuronal pairs and the entire S-S pair PCC frequency distribution (Fig. 3-3D) is shifted to the right. There are no apparent multiple peaks. UD also leads to an increase in the number of relatively highly correlated (>0.15) AS-AS pairs compared to the CON group.

Changes in synchronized discharge between barrel and septal columns after UD.

JPSTHs were also constructed between neuronal pairs recorded from two electrodes one of which was located in a barrel column and the other in the adjacent septal column in CON and UD rats. Fig. 3-2E displays the PCC cumulative distribution of CON and UD barrel-septal pairs, specifically when both the neurons were located in the supraganular layers (AB-AS pairs; CON and UD: n=64). The CON AB-AS correlations are extremely low which is highly and significantly increased after UD ($p<0.001$, Wilcoxon nonparametric test). There is ~300% increase in the mean PCC of AB-AS pairs after UD when compared to the CON. UD also led to an increase in spike synchrony in the L-IV barrel-septal pairs (B-S pairs; CON: n=125; UD: n=149) as shown by the cumulative distributions in Fig. 3-2F. The increase in the UD group is highly significant ($p << 0.001$, Wilcoxon nonparametric test of cumulative distributions) with an overall increase of ~56% in the mean PCC as compared to the CON group. Thus, there is an overall increase in correlated discharge between a barrel column and its adjacent septal column after UD.

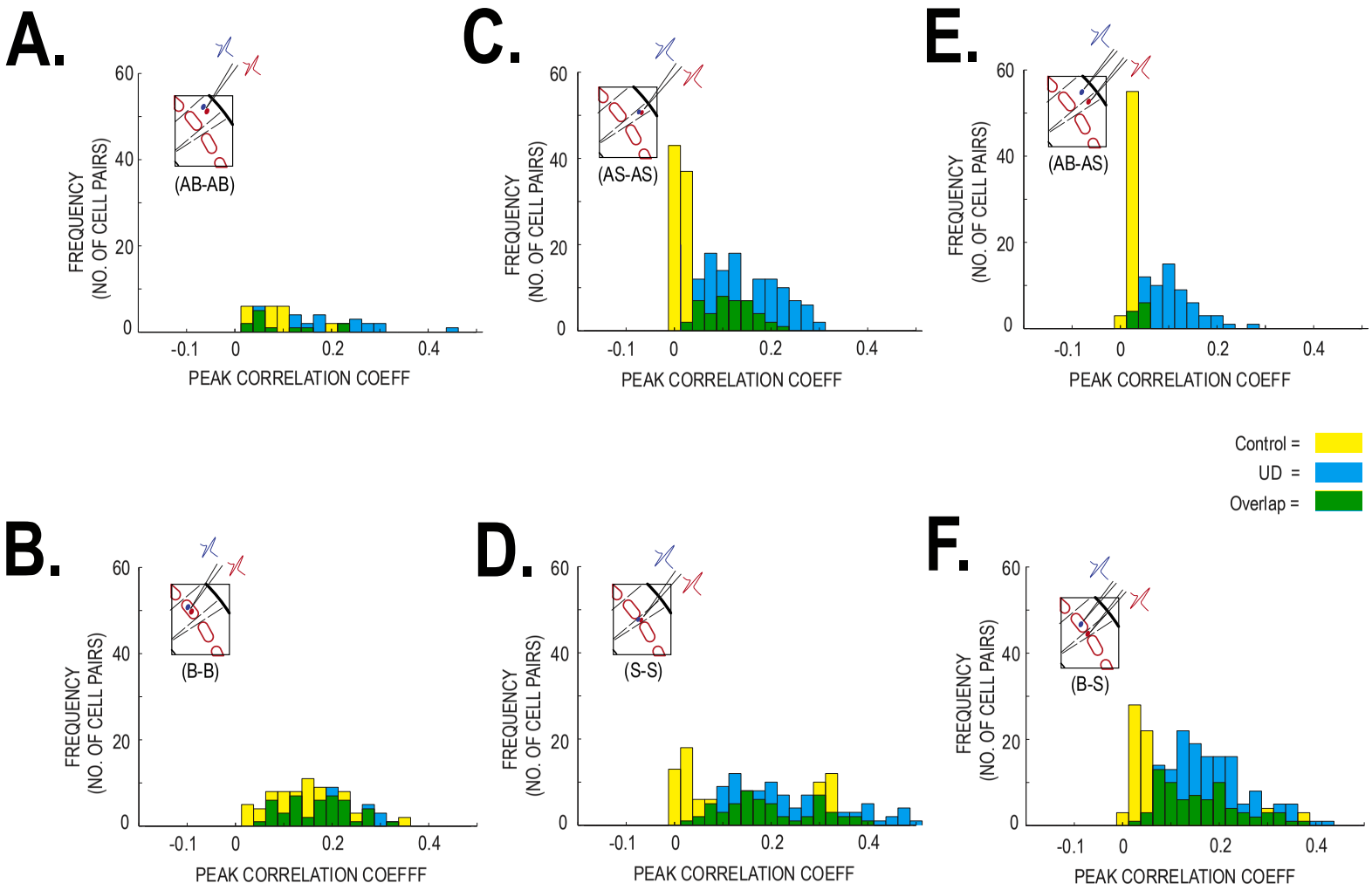

Figure 3-3. Frequency distributions of peak correlation coefficients in the different regions of barrel cortex of CON and UD rats
Frequency distributions for the peak correlation coefficients for the different neuronal pairs are displayed for CON (yellow bars) and UD (blue bars) rats. The green bars represent the overlap of the distributions. The distribution for the B-B pairs (B) are almost completely overlapped, however, for the rest of the pairs the UD distribution is shifted to the right indicating an overall increase in the number of neuron pairs in that region, with a higher correlation strength. Moreover, for the S-S pairs (D), the multimodal distribution in CON is transformed into a more uniform distribution for the UD rats.

Differences in neuronal spike synchrony during stimulus evoked response period and spontaneously active periods.

Fig. 3-4 displays the average coincidence in a 50 ms stimulus evoked response period (0-50 ms post-stimulus epoch) and in a more spontaneously active 50 ms period from 1950 to 2000 ms post-stimulus in the different locations of the barrel cortex. These average coincidences were compared within and between the CON and UD rats. As shown before, the CON rats showed a reduction in response period coincidence in L-IV B-B, S-S and B-S pairs when compared with those of the spontaneous periods ($p<0.001$ for all). However, in the supragranular layer of CON rats only the AB-AB pairs showed this reduction ($p<0.001$). The AS-AS and AB-AS neuronal pairs failed to show any such significant reduction ($p>0.0125$). In UD rats, the response period coincidence was also significantly lower than that of the spontaneous period for the L-IV B-B, S-S and B-S pairs ($p<0.001$). In L-II/III, both AB-AB and AB-AS pairs showed significant reduction of coincidence with the response when compared to the spontaneous epochs ($p<0.001$ for both). The increase in neuronal coincidence with UD compared to CON rats was observed in the stimulus evoked response period for the septal pairs in L-IV ($p<0.001$) and the above barrel ($p<0.01$), above septum ($p<0.001$) and AB-AS ($p<0.001$) in L-II/III. Finally, with UD, the average coincidence in spontaneous epochs was increased in all areas of barrel cortex except layer IV barrels when compared with CON rats (S-S, B-S, AB-AS, AS-AS: $p<0.001$; AB-AB: $p<0.01$; B-B: $p>0.05$).

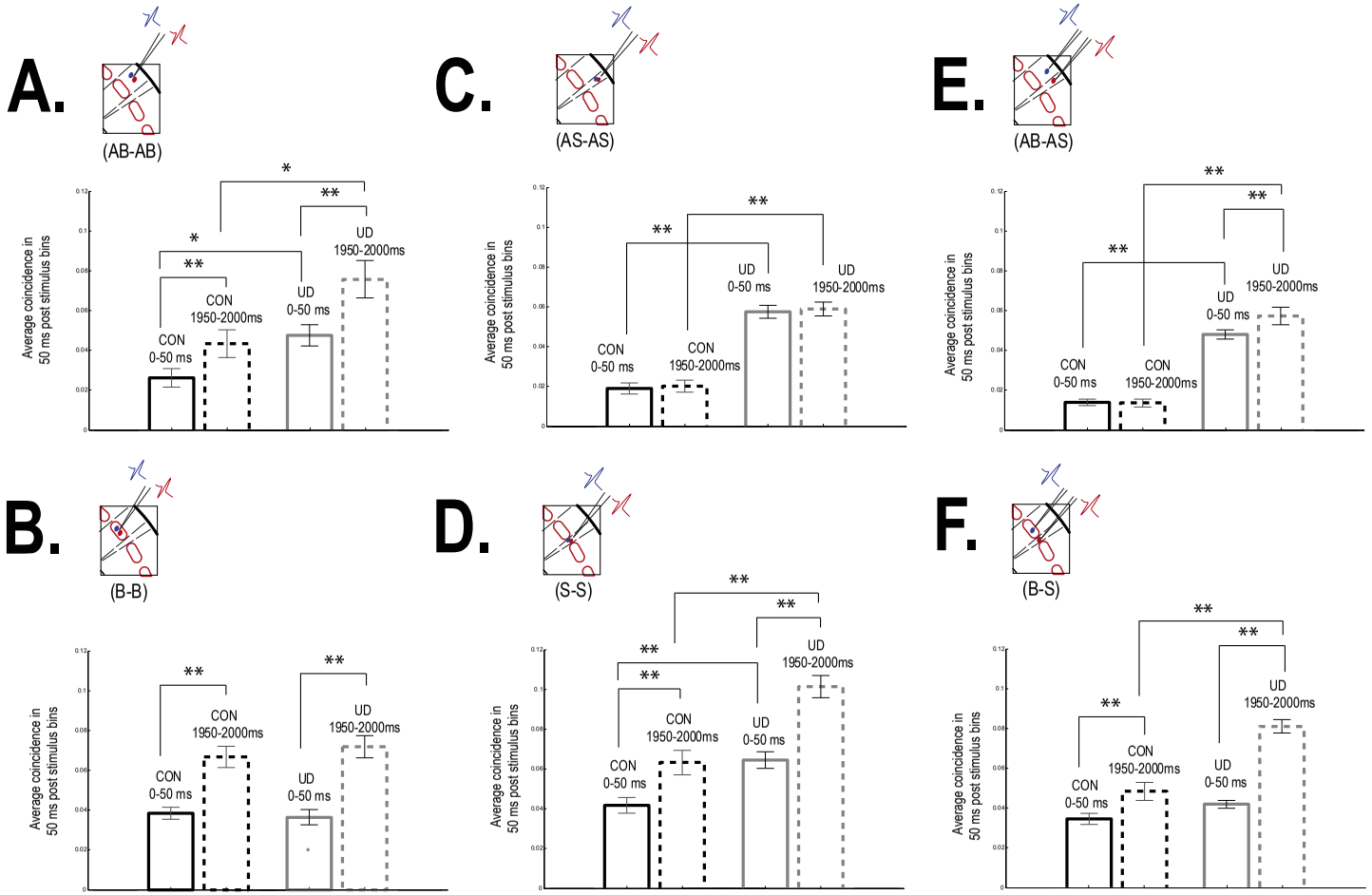

Figure 3-4. Comparison of spike synchrony between response periods and relatively spontaneous periods for CON and UD rats
Comparison of the average coincidence of spikes in neuronal pairs in the 'first' 50 ms (solid bars; response period) and the 'last' 50 ms (1950-2000 ms post-stimulus, dashed bars; relative spontaneous period) after best whisker stimulation in CON (black bars) and UD (gray bars) rats. There is a reduction in correlation strength in the response period when compared with that of relative spontaneous periods within CON and UD rats in all areas of barrel cortex except above-septum (C). Furthermore, only in CON rats such a reduction is also absent for the AB-AS pairs (E). When compared between CON and UD rats, there is an increase in response period coincidence for UD rats for all neural pairs except the B-B (B) and B-S (F) pairs. On the other hand, an increase in coincidence in the relative spontaneous periods is observed in UD for all areas of barrel cortex except in LIV barrels (B). Error bars represent standard error of mean or S.E.M. The double asterisks (**) and single asterisk (*) represent a significance level of <0.001 and <0.01, respectively.

Discussion

Previously, our lab has shown how single cell magnitude and onset-latencies are affected following UD. Here, we focus on the effects of UD on ensemble neuronal property called spike-spike synchrony. The results described above provide clear evidence that UD rearing spanning the developmental critical period for cortical plasticity alters spike-spike synchrony in several different locations within barrel cortex. Interestingly, the changes observed with UD were strikingly different from those observed following BD as reported earlier in Chapter II. In summary, with UD there was a significant increase in the amount of coincident discharge in all regions of the barrel cortex except in the layer IV barrels, when compared with CON rats. This increase in synchronized spike discharge between pairs of neurons was evident both in the whisker stimulus evoked response periods as well as during spontaneously active periods. The maximal change was observed in the supragranular layers and predominantly in the septal column with an increase of approximately 200% of PCC compared to that of CON in L-II/III septum and 300% increase in L-II/III barrel-septum pairs.

Previously it has been postulated that the high synchrony found in normal L-IV barrels and some L-IV septal cells can be a property of dominant lemniscal or paralemniscal activity from the ventral posteromedial nucleus (VPM) or the medial division of the posterior nucleus (POm) of the thalamus, respectively, during the developmental critical period (Ghoshal et al. 2009; Chapter II). In addition, the sub-population of L-IV septal cells that are highly correlated could also be the subset of neurons that have been reported recently to receive their dominant input from the ‘head’ sub-region of VPM (Furuta et al. 2009). In short, the dominant thalamocortical activity

during the developmental period might be responsible for the highly correlated activity observed in L-IV barrel cortex but not in L-II/III. Fig. 3-6 represents the revised hypothetical model based on the basic assumption mentioned above and the observations from CON and UD rats to explain the variability of neuronal spike synchrony across the different areas and layers in barrel cortex.

Following UD, the activity from the lemniscal or paralemniscal pathway is greatly reduced to the deprived half of the hemisphere during the critical period. Instead, activity from the intact whiskers on the other side of the face might allow the non-deprived hemisphere to emerge as the dominant driving force providing active inputs to the deprived hemisphere. The ipsi and contralateral barrel cortex are interconnected through the corpus callosum. Callosal inputs from the ipsi barrel cortex are known to terminate in a topographic manner primarily in the supra and infragranular layers, and in L-IV these connections innervate only the septal region carefully avoiding terminations in L-IV barrels (Akers and Killackey 1978; Ivy and Killackey 1981; Olavarria et al, 1984; Hayama and Ogawa, 1997). These callosal inputs which are thought to be modulators of bilateral responses in CON animals might become driving inputs and/or develop higher efficacy in UD rats. Such a phenomenon would up-grade the callosal inputs during the developmental critical period in UD rats, and thus would be a logical candidate to account for the sharp increase in spike synchrony within L-II/III and septal regions of barrel cortex (Fig. 3-6B). In the forelimb representation of S1, callosal inputs contact the inhibitory cells preferentially (Pluto et al. 2005), and the inhibitory cells are capable of producing synchronized states in cortex (Cardin et al. 2009; Vierling-Claassen et al. 2010), and may help to explain a possible mechanism of up-regulation

of coincident discharge in the deprived hemisphere. Interestingly, the increase in coincident discharge among neurons was observed in areas of barrel cortex that matched the connection pattern of the callosal inputs. Thus, L-IV barrels that are primarily devoid of any callosal innervations also did not show any significant increase in spike synchrony. These findings add further support for the hypothesis that callosal inputs take over when thalamocortical sensory activity is deficient during the critical period and that the development of cortical synchrony is activity dependent.

The effect of neuronal coincidence after UD was strikingly different from the ones that were observed following BD rearing. In fact, the changes observed in L-IV after UD were nearly the polar opposite to that of BD, which is characterized by severe reduction of synchronized discharge L-IV of barrel cortex (Ghoshal et al. 2009; Chapter II). The UD L-IV barrels were spared of any changes unlike the BD L-IV barrels. Moreover, the PCCs of septal neurons which had a heterogenous distribution comprising of distinct peaks of low and high correlated discharge in CON animals had more uniform distribution that resembled the distribution of L-IV barrels of CON comprising primarily of highly correlated neurons in UD rats. This was in stark contrast to BD where the L-IV septal PCC distribution was reduced to predominantly to low correlated pairs. Also, following BD there were no changes in neuronal synchrony in the supragranular layers of barrel cortex (Fig. 3-5). However, with UD there was a considerable increase in the magnitude of coincident discharge both in above-barrel and above-septal locations. Very low and negligible neuronal synchrony was observed in above-barrel-above-septal (AB-AS) pairs of CON and BD animals. However, with UD there was a predominance of AB-AS synchrony indicating a possible emergence of connections between barrel and

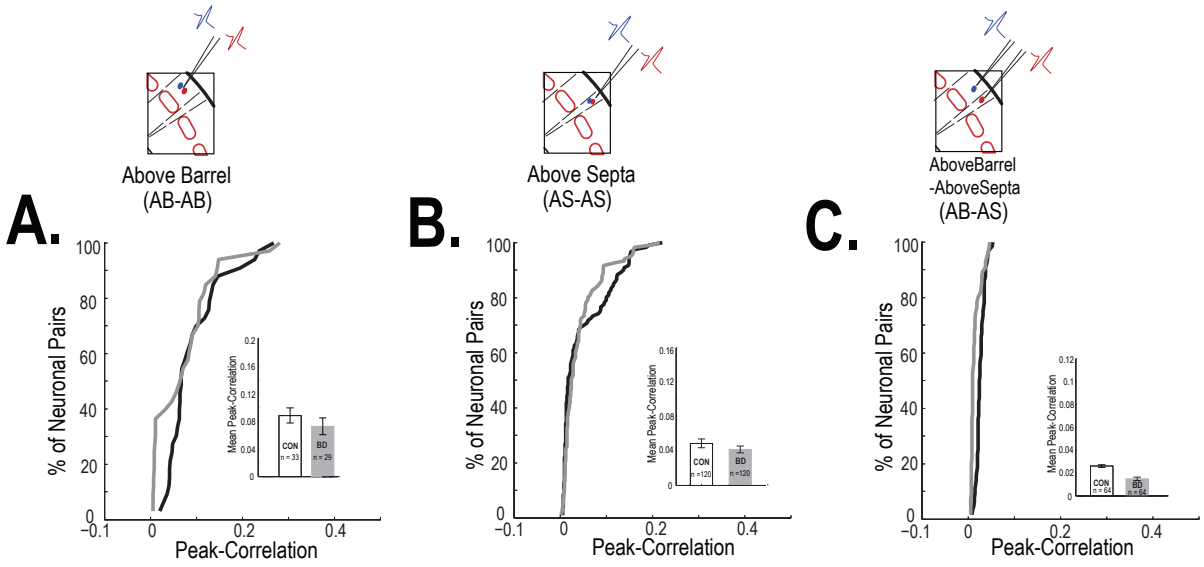

Figure 3-5. Cumulative distributions and means of peak correlation coefficients supragranular neuronal pairs of CON and bilaterally whisker trimmed (BD) rats

Cumulative distributions of Above-barrel (A, CON n=33; BD n=29), above-septum (B, CON and BD n=64) and above-barrel-above-septum (C, CON and BD n=120) pairs of CON (black line) and BD rats (gray line) are displayed in this figure. With BD there is no change in correlation strength ($p>0.05$ for all comparisons) for the neuronal pairs located in layers II and III. Mean bar graphs are displayed in the inset for each distribution. Error bars represent standard error of mean or S.E.M.

adjacent septal columns in the supragranular layers in UD rats which are not readily observed in CON rats (Alloway 2008). Such changes with BD only in L-IV can be explained by reduced thalamocortical activity and the absence of compensatory activity in L-IV from the 'normal' hemisphere (a revised model for BD is depicted in Fig. 3-6C). All of these clear differences in the single cell neurophysiology (Popescu and Ebner 2010) as well as ensemble neuronal properties described above between UD and BD indicate how much the primary sensory area is differently affected by different peripheral manipulations, and why neurophysiological evidence from unilateral or partial whisker deprivation on one side of the face should not be compared with the behavioral deficits that are observed with loss of all whisker sensations in BD.

The hypothetical model (Fig. 3-6) described above explain almost all the changes observed in UD animals. However, one might expect a reduction in L-IV barrels in UD since there no known compensatory anatomical connections in such structures. Reduction of L-IV barrel activity was reported from studies in slice preparations following short term (P-9-14) UD by Shepherd et al. (2003). In contrast, *in vivo* study from Popescu and Ebner (2010) failed to confirm a reduction in L-IV barrels following UD. One possible explanation can be compensatory mechanisms occurring at the subcortical level in *in vivo* preparations prevent the reduction of magnitude or cortical synchrony in L-IV barrels. Active connections from the adjoining up-regulated septum could also prevent such reductions. Finally, the possibility of exuberant callosal connections spilling over into the L-IV barrels in UD rats cannot be ruled out as an explanation for such an absence of reduction of magnitude and synchrony after UD *in vivo* preparations with much longer periods of deprivation.

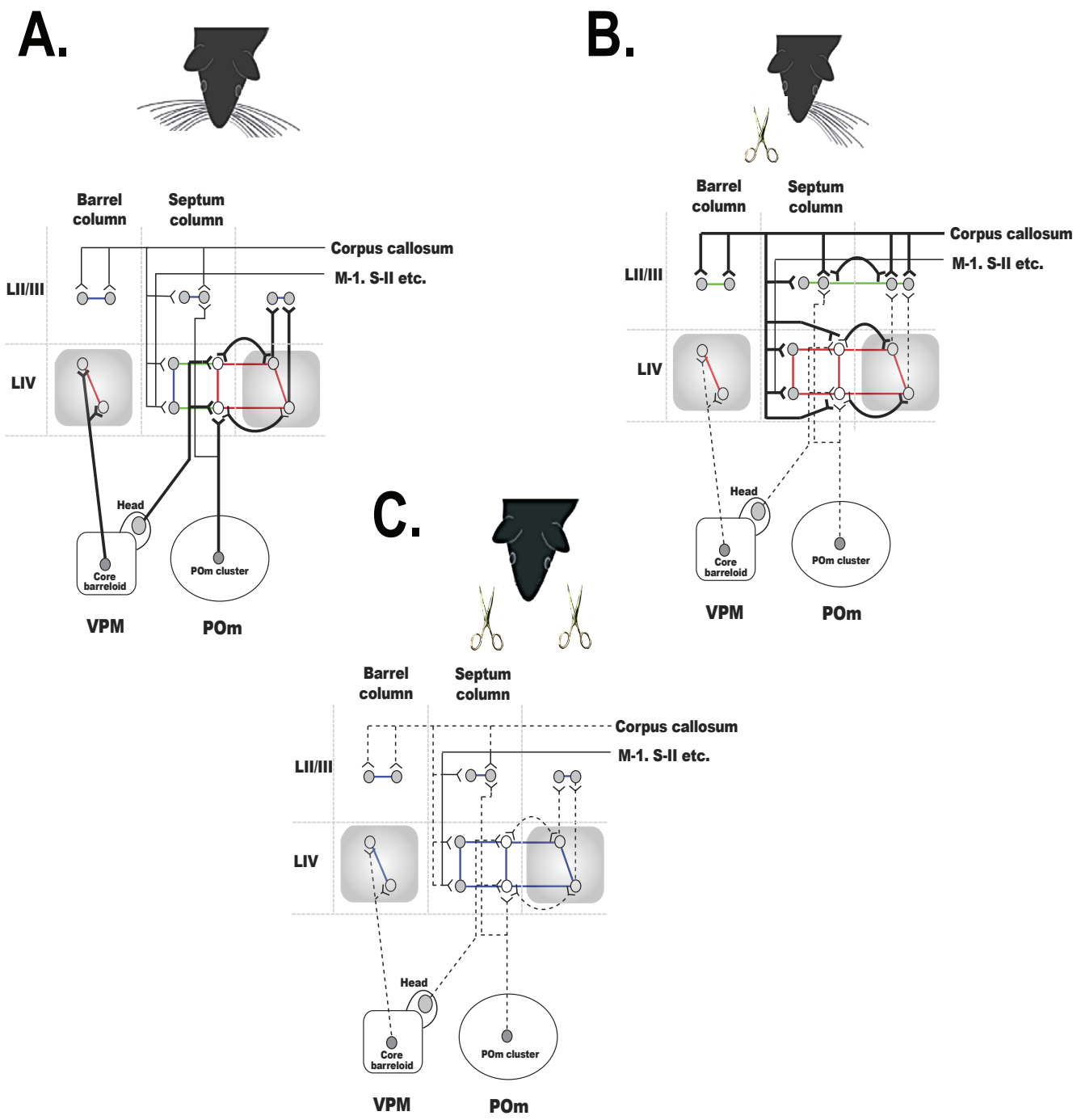

Figure 3-6. Hypothetical model explaining the probable mechanisms for the differences observed in neuronal spike synchrony following sensory deprivation.

According to this model spike synchrony is activity dependent. Thus, high synchrony observed specifically in LIV barrels of CON rats (A) can be due to the dominant activity of thalamocortical inputs arising from VPM core. In the LIV septum, cells receiving inputs from the VPM head cells and/or POm cells could represent the subpopulation of septal neurons with high correlations. In the UD rats (B), loss of contralateral whiskers may lead to a reduction of thalamocortical activity and instead, callosal inputs from the active hemisphere terminating in LIV septum and LII-III of the deprived hemisphere could become the dominant driving input. More activity in the callosal inputs, thus can be responsible for an increase in correlation strength for neurons residing in such locations. With BD (C) reduced activity in the thalamocortical inputs and also in the opposite hemisphere may lead to a failure of the development of high cortical spike synchrony anywhere in barrel cortex. (See text for detailed explanations).

Black lines indicate known anatomical connections. Bolded lines indicate dominant activity and dashed lines indicate reduced activity. The colored lines indicate correlation strength between neuronal pairs with the warmer colors signifying higher correlation strength and vice versa.

Other possible mechanisms for the up-regulation of neuronal coincidence in septal and supragranular layers in UD can be competition between subcortical structures namely VPM and POm and/or preferential projections to barrel cortex from other cortical areas like MI and SII. However, such an up-regulation is not seen in BD rats which had an equal probability to that of UD rats for such mechanisms to kick in after deprivation. Thus, these mechanisms are less likely to be responsible for the changes in UD. Finally, an imbalance between excitation and inhibition might also indirectly lead to such a septal up-regulation in UD rats. An increase in the number of inhibitory synapses (Micheva and Beaulieu 1995; Vees et al. 1998) coupled with less robust firing of putative inhibitory neurons (Lee et al. 2007) and weakened inhibitory receptive fields (Shoykhet et al. 2005) in L-IV barrels after UD might generate an imbalance of excitatory and inhibitory levels in the deprived hemisphere. But, such excitation-inhibition imbalance in UD was not evident in the studies conducted by Popescu and Ebner (2010), also making it a less likely mechanism for the changes observed in this study.

As described before the stimulus evoked response period coincidence was significantly lower in CON rats than the coincidence measured in the relatively spontaneous periods for all of L-IV (Ghoshal et al. 2009; Chapter II). Such a relationship between the response period coincidence and spontaneous period coincidence is not true for the CON supragranular layers except above-barrel pairs. It has been postulated that such a reduction in L-IV barrels with whisker related responses could be important for information processing in barrels. And since such reductions were absent in BD L-IV barrel cortex, it was further hypothesized that reduced correlated firing during the

response period develops through the influence of the active thalamocortical connections during normal rearing (Ghoshal et al. 2009). Interestingly, with UD such reduction in spike coincidence during the response was maintained in L-IV as well as in the AB-AB neuronal pairs. Moreover, the L-II/III barrel septal (AB-AS) pairs in UD also showed the response period-related reduction in correlated discharge. This could be the possible substrate that could explain why behavioral disabilities with UD rats are rarely reported (although few behavioral studies use this paradigm).

Our results also indicate that following UD there is an up-regulation in septal column activity which might reflect a shift from barrel-to-above barrel transmission in CON rats to septa-to-above septa transmission of information in UD rats (also see Shepherd et al. 2003). This shift in information transfer can be a consequence of homeostatic plasticity that might occur due to the imbalance of peripheral activity from the ipsilateral intact whiskers vs the contralateral deprived whiskers. Numerous papers show that neurons are equipped with homeostatic mechanisms which allow them to restore their firing rates to control levels following activity-dependent changes (Turrigiano et al. 1998, Burrone et al. 2002, Turrigiano 2007). This is the first case we have encountered that shows that such homeostatic mechanism can also lead to adjustments of the temporal properties of ensemble neurons. Whether such neurophysiological homeostatic mechanism is the basis of or important for the compensatory behavioral attributes of UD rats, remains to be tested but our results indicate for such a possibility.

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CHAPTER IV

CROSS-SENSORY MODULATION OF PRIMARY SENSORY CORTEX IS DEVELOPMENTALLY REGULATED BY EARLY SENSORY EXPERIENCE

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Introduction

The convergence of information from more than one sensory modality greatly improves the sensitivity of an animal's perceptually guided behavior (Stein and Meredith 1990; Burnett et al. 2004). Psychophysical experiments have shown that multisensory interactions also modulate perception in humans. For example, in illusions, such as the "Parchment-Skin Illusion", somesthetic perceptions for rough and smooth surfaces are dramatically modulated by concurrent sounds (Jousmaki and Hari 1998; Guest et al. 2002). Similarly, there is evidence for somesthetic-visual and audio-visual interactions. However, in this report we concentrate on somesthetic-auditory interactions.

Primary sensory cortex has been considered devoid of multi-sensory interactions. In the last two decades, however, a strong case has emerged for the notion that multi-sensory modulation of sensory responses is a common feature of both primary (V1, S1, A1) and secondary (V2, S2, auditory belt) sensory areas. Such

phenomena are often referred to as ‘cross-sensory’ or ‘crossmodal’ interactions (see Foxe and Schroeder 2005; Ghazanfar and Schroeder 2006 and Kayser and Logothetis 2007 for reviews). Physiological evidence supports the concept of a linkage among all combinations of visual, auditory and somatic sensory inputs to cortex. Somatic and visual stimuli can strongly activate single and multiunits in A1 and the posterior auditory belt area when the stimuli are relevant to an over-trained task (Brosch et al. 2005). Lakatos et al. (2007) found ERP responses in the supragranular layers of Macaque A1 cortex in response to somatic sensory stimuli suggesting a modulatory as opposed to a driving type of cross-sensory influence in the primary auditory area, even when overtraining was not a variable. Moreover, bimodal stimuli composed of paired somatic sensory and auditory stimuli led to a supralinear multisensory interaction both for field potentials and multi-unit activity in all layers of A1 (Lakatos et al. 2007). Only a few studies have investigated multisensory properties in S1 cortex. In monkeys performing a haptic discrimination task, Zhou and Fuster (2000, 2004) located neurons in Brodmann’s areas 3a, 3b, 1 and 2 that could be activated by visual or auditory cues when linked to the haptic task. Dynamic cross-sensory plasticity is also well documented where plasticity refers to the ability of active sensory modalities to recruit or strongly modulate neurons in a deprived area of cortex (Review see, Bavelier and Neville 2002). Sensory deprivation of a particular modality during development diminishes the response magnitude of the deprived neurons (Wiesel and Hubel 1965; Simons and Land 1987; Stern et al. 2001; Popescu and Ebner 2010) and disrupts neuronal synchrony (Ghoshal et al. 2009) in primary cortical areas representing that modality. The net effect is that ‘deprived’ cortical neurons become less responsive to

natural stimuli. However, deprived cortex, in turn, has a remarkable capacity to respond to cross-sensory stimulation or reorganize in response to the suppression of the natural sensory inputs. (Reviews see Collignon et al. 2009; Kral 2007).

In the present study we investigated whether neuronal responses in rat S1 barrel cortex could be modulated or driven by an auditory click stimulus after early somatic sensory deprivation. It has been shown before that rearing rats with auditory clicks in the background can reorganize auditory cortex (Zhang et al. 2002), and thus, a further goal was to determine whether increased auditory stimulation during whisker deprivation would augment the cross-sensory activation of barrel cortex. Our results show that whisker responses in rat S1 barrel cortex are modulated by auditory inputs after normal rearing, and that this influence is significantly increased following altered early sensory experience.

Methods

All of the experiments in this report were approved by the Vanderbilt University Animal Care Committee (IACUC), carried out in an AAALAC approved animal facility, and were in accordance with the guidelines of the NIH and the Society for Neuroscience.

Animal Groups: The experimental design consisted of 4 rearing groups using seventeen male (ten) and female (seven) Long-Evans rats (250-350 g, 2-3 mo at the time of recording). The newborn pups were raised either: 1) with a continuous 1 Hz auditory click stimulus in addition to normal laboratory noises (click reared: CR+) or 2) with normal laboratory noise only (CR-). Half of the animals in each of these groups

were reared with bilateral whisker trimming (WBD) and half without any sensory deprivation (sham trimming [CON]) to generate 4 rearing conditions: $N_{CON/CR^-} = 5$; $N_{CON/CR^+} = 4$; $N_{WBD/CR^-} = 4$; $N_{WBD/CR^+} = 4$ as diagrammed in Fig. 4-1A.

Bilateral whisker deprivation: Two groups of rats were bilaterally sensory deprived (WBD) by trimming all whiskers from both sides of the face to the level of the fur, for a period of 60 days beginning on the day of birth (PND 0-60). Two groups of animals were handled but not trimmed during the same period to serve as control (CON) groups. Whiskers were trimmed every day for the first 30 days (when the rate of the whisker growth was most rapid) and every other day thereafter. During pre-weaning whisker trimming, the whisker-trimmed pups were caged with their control littermates and nursed by their dam. After weaning, same sex animals were housed in groups of 3-5 animals per cage depending on their size. Five days prior to recording sessions trimming was discontinued and the whiskers were allowed to re-grow to a length sufficient to stimulate each whisker without moving the small facial hairs (~5 mm). This 5-day period of whisker re-growth was the only recovery period in these studies.

Auditory click rearing: One group of WBD and one group of CON rats were reared from P0 to P60 with a repeated click stimulus delivered at 1 Hz continuously through a speaker kept above the rat cages (click duration: 10ms; Amplitude: 75 dbspl at 20 mm from the speaker). The click stimulus was generated by a square wave pulse generator (Grass Technologies, Rhode Island) and consisted of an envelope of frequencies (range: 1 Hz to 50 KHz).

Surgery and Recording: Over a period of days from 1 week to 1 month after the end of the rearing period each rat was anesthetized with urethane (1.5 g/kg, 30%

aqueous solution, i.p.), and mounted in a head holder that allowed free access to the whiskers and did not require the use of ear bars (Narishige, Japan). A craniotomy was made from 4 to 8 mm lateral to the midline and from 0 to 5 mm posterior to Bregma to expose the left barrel field cortex (BFC). Body temperature was maintained at 37°C with a feedback actuated heating pad (Harvard Apparatus, Holliston, MA). Supplementary injections (10% of the initial dose) were given as needed to maintain the anesthesia at stage III-3 (Friedberg et al. 1999). After making a small opening in the dura mater, microelectrodes were advanced in columnar penetrations perpendicular to the cortical surface. Contact with the pia was identified visually through an operation microscope. Commercial quartz glass insulated, platinum/iridium microelectrodes were used having 2-6 MΩ resistance (Thomas Recording, Giessen, Germany). The electrodes were advanced into the brain using an Eckhorn microdrive system (Thomas Recordings, Giessen, Germany). Analog waveform signals were amplified by Thomas preamps and collected by a Plexon MAP system (Plexon Inc., Texas) in which the waveforms were digitized at 40 kHz. Multiunit activity was viewed online using Sort Client software (Plexon Inc., Texas), and stored for offline analysis. The electrode was first advanced to layer IV of the BFC where receptive fields were mapped manually. The whisker was identified online that evoked the largest amplitude response in a multi-unit post-stimulus time histogram (PSTH), which was designated as the “Principal Whisker” (PW) (PeriEvent Client, Plexon Inc., Texas). Multiunit activity was collected from layers II and III (depth 100-450μm) and layer IV (450-800μm) of the BFC (Li et al. 2005).

The multiunit spike stream collected on-line was sorted offline to isolate single neuron waveforms, and the magnitude and spike timing of responses to each of the three stimulus conditions were compared.

Electrodes were advanced or retracted in 100 μm intervals to minimize the probability of recording twice from the same units. Lesions were made by passing DC current (1 μA for 2 min) at depths of 600 μm in each penetration and their location was later verified anatomically using cytochrome oxidase stained tangential sections through the BFC. However, the lesions were too variable in size to precisely locate the recording location to either a barrel or a septum.

Whisker and Auditory Stimulation during recording: Prior to recording, the whiskers were trimmed at 5 mm beyond the fur in all groups to keep the whisker length uniform. A piezoelectric bimetal wafer was used to deliver 100 stimuli to the whiskers in a caudal direction (1 Hz, 600 μm amplitude, 4 ms duration, 2 ms rise time). The piezoelectric wafer was actuated by a "custom" waveform programmed in a digital stimulator (DS8000 WPI, Florida) that in turn was controlled by a Spike 2 script program (CED, Cambridge, UK). The auditory stimulus was generated by a second channel on the DS8000, which was also controlled by a Spike 2 script to activate a speaker (Kenwood, 20-20,000 Hz response). The speaker was positioned 20 mm away from the right ear of the rat. Auditory stimuli were delivered at 1 Hz (75 db spl, 10 ms duration square wave either alone or 10 ms prior to a whisker stimulus). Five different sets of stimuli were presented in each recording location always in the same sequence (Fig. 4-1B):

a. Contralateral PW stimulation alone (W1) prior to the CS stimuli (100 trials).

b. Contralateral Auditory Stimulation alone prior to the CS stimuli (A1) (100 trials).
c. Contralateral auditory stimulus preceding whisker stimulation by 10 ms (Cross-sensory stimulus, CS) (100 trials).
d. Contralateral auditory stimulation after CS (A2) (100 trials).
e. Contralateral Principal Whisker stimulation 5 min after CS (W2) (100 trials).

We locked the inter-stimulus-interval (ISI) between the auditory click and the whisker stimulus at 10 ms and didn't test other possible ISIs due to technical limitations. That is, some of the effects, especially in the WBD/CR+ rats, were longer lasting making it difficult to interpret whether the effect observed for a particular ISI was characteristic for that temporal interval or due to the residual effect of the previous ISI tested.

Since under our conditions, neither A1 nor A2 evoked a significant spiking response in LII/III or L-IV barrel neurons in any neurons in any of the 4 rearing groups these stimulus conditions were not analyzed further for magnitude differences.

Data analysis: Principal component analysis and template matching were used in Offline Sorter (Plexon Inc.) for sorting spike waveforms to separate single units from the mulitunit stream. Typically two to three units per electrode could be isolated for further analysis from each electrode recording position. Post stimulus time histograms (PSTH's) were constructed from waveform time stamps for each cell in response to the different stimulus conditions using a custom NEX script (provided by Dr. Alexander Kirillov, NEX Technologies, MA). Raster plots generated by the NEX software were used to display individual cell trial-by-trial spike frequencies and latencies. PSTHs and raster plots for W1, CS and W2 conditions were constructed with the onset of the whisker stimulus as the reference event under each condition, whereas, those for A1

and A2 were constructed with the onset of the square wave input to the speaker as the reference event. Significant responses to each stimulus condition were determined using a 99% confidence interval calculated by the NEX software. The magnitude of response for each neuron was calculated as spike counts in the first and second 15 ms post-stimulus epoch (0-15 and 15-30 ms post-whisker stimulus). The magnitude for spontaneous firing of neurons was calculated as the total spike count in a time window of the last 500 ms before each successive stimulus during the block of 100 trials. The response modulation index (RMI) for each rearing group was calculated as the ratio of Magnitude(CS)/Magnitude(CS+W1) and represented a quantitative measure for the amount of cross-sensory influence. A high RMI value thus indicates a relative facilitation of responses in the CS stimulus, whereas, a low RMI value represents suppression. These values were compared statistically between rearing groups and/or across stimulus conditions within a rearing group. Population PSTHs were constructed by averaging the responses of all neurons from the same group and producing graphical display (after smoothing the graphs with 3 Gaussian filters) using NEX software and a custom MATLAB script.

Significant increases in response magnitude of individual neurons in either the 0-15 or the 15-30 ms epoch were identified when the average of 100 trials in the CS condition exceeded the sum of the mean plus 2 times the standard error (SE) of 100 W1 trials. A decrease in response magnitude for individual neurons was identified for either of the epochs when the trial average in the CS condition was lower than the value of the mean plus 2*SE for 100 W1 trials. This analysis was performed to evaluate the

percentage of individual neurons in each rearing group that showed either the facilitative or suppressive cross-sensory modulations described in the results.

Statistical Analyses: We carried out analyses testing: (1) the overall effects of postnatal rearing, and stimulus variables on spike counts; and, (2) a series of designed comparisons addressing more focused questions. Spike counts summed across two post-stimulus time periods (0-15 ms and 15-30 ms) served as the dependent measures. To test effects, we used a generalized linear mixed effects model (GLMM), which is an extension of the generalized linear model (GLM). GLMs specifying that the data have either a Poisson or negative binomial distribution are commonly used to analyze counts (e.g. Hardin and Hilbe, 2007). In turn, GLMM's are an extension of GLMs that are appropriate when independence of observations is violated due to repeated measures or other factors that reflect clustering of observations (e.g., Fitzmaurice et al. 2004). In the present study there were several sources of clustering that needed to be accounted for: the correlated responses of a given neuron recorded across the three stimulation conditions; the shared correlation among all the neurons of a given rat; and the correlated responses of neurons recorded from the same cortical layer within a given animal (a more local factor that might serve to heighten the correlation among neurons at the same depth), Such structures can be modeled by the specification of random effects that allow for estimation of the shared variance among all observations within a given source of clustering. The random effects specification for stimulation condition within rat included an additional scaling parameter that corrected for potential over-dispersion (i.e., under-estimation of standard errors) in the data. By these means we were able to insure that hypothesis tests were more valid for the effects of primary

interest (i.e. the effects of rearing group and stimulus condition) (e.g., Fitzmaurice et al., 2004). All random effects were assumed to be normally distributed. Because the numbers of counts per epoch were generally high, and because in the limit the Poisson distribution approaches a normal distribution, an alternative approach would have been to use a linear or generalized linear mixed effects model that specified a normal distribution of residuals. In fact, the GLMM approach that we used and these alternative approaches yielded results that were highly similar and conclusions that were identical.

Our initial models tested for the main effects and interactions among Rearing Condition (CON/CR-, CON/CR+, WBD/CR-, WBD/CR+), Stimulus Condition (W1, CS, W2), and Cortical Layer (II/III or IV). Main effects refer to the overall effects of a given factor averaging across all relevant neurons. Thus, for example, the main effect of Rearing Condition tested the global null hypothesis that the means of the four rearing conditions computed by averaging across all neurons and stimulus conditions were equal to one another. Interactions tested whether the effects of a given factor were conditional upon the specific levels of other factors. To generate a suitable number of observations, we aggregated cortical layer into two groups: layers II/III (100-450 μm in depth) and layer IV (450-800 μm in depth) to create the Cortical Layer factor.

Analyses were conducted using SAS PROC GLIMMIX software, Version 9.1.3 of the SAS system for Windows. Copyright © 2002-2003 SAS Institute Inc. SAS and all other SAS Institute Inc. products or service names are registered trademarks of SAS Institute Inc., Cary, NC, USA (Littell et al. 2006). Consistent with the count nature of the dependent variables, both the 0-15 ms and 15-30 ms models specified a Poisson distribution for the dependent variables. In addition to the global tests of interest, we

tested several a priori contrasts of prime interest. Specifically, we compared the counts of the W1 condition to those of the CS and W2 condition, within each of the four rearing groups. To control familywise Type 1 error rates, we set the critical alpha level per group at .05/4 = .0125 and then used a stepdown Bonferroni procedure to evaluate the two contrasts within each group. These sets of contrasts were performed separately for the 0-15 ms and 15-30 ms time windows. Using a similar Bonferroni procedure we compared the W1 and CS conditions within each of the two cortical layer groupings for each rearing group. More exploratory analyses are also described in the Results, such as whether excitatory vs. inhibitory status of neurons moderated the effects. The rationale behind the statistical approach is explained in detail in the supplementary methods.

Results

Somatosensory and auditory interactions in normal, whisker deprived and/or click reared rat barrel cortex:

In the present experiments we tested the hypothesis that auditory stimuli can influence the responses of the barrel cortex neurons under normal conditions and under conditions of whisker deprivation coupled with auditory enhanced rearing. Three out of 5 stimulus conditions (W1, CS, W2; Fig. 4-1B) elicited significant responses from the barrel cortex neurons under all 4 rearing conditions (Fig. 4-1). The responses from W1, CS and W2 were divided into early and later post-stimulus time epochs of 0-15 ms and 15-30 ms because deprivation and click rearing produced consistent response

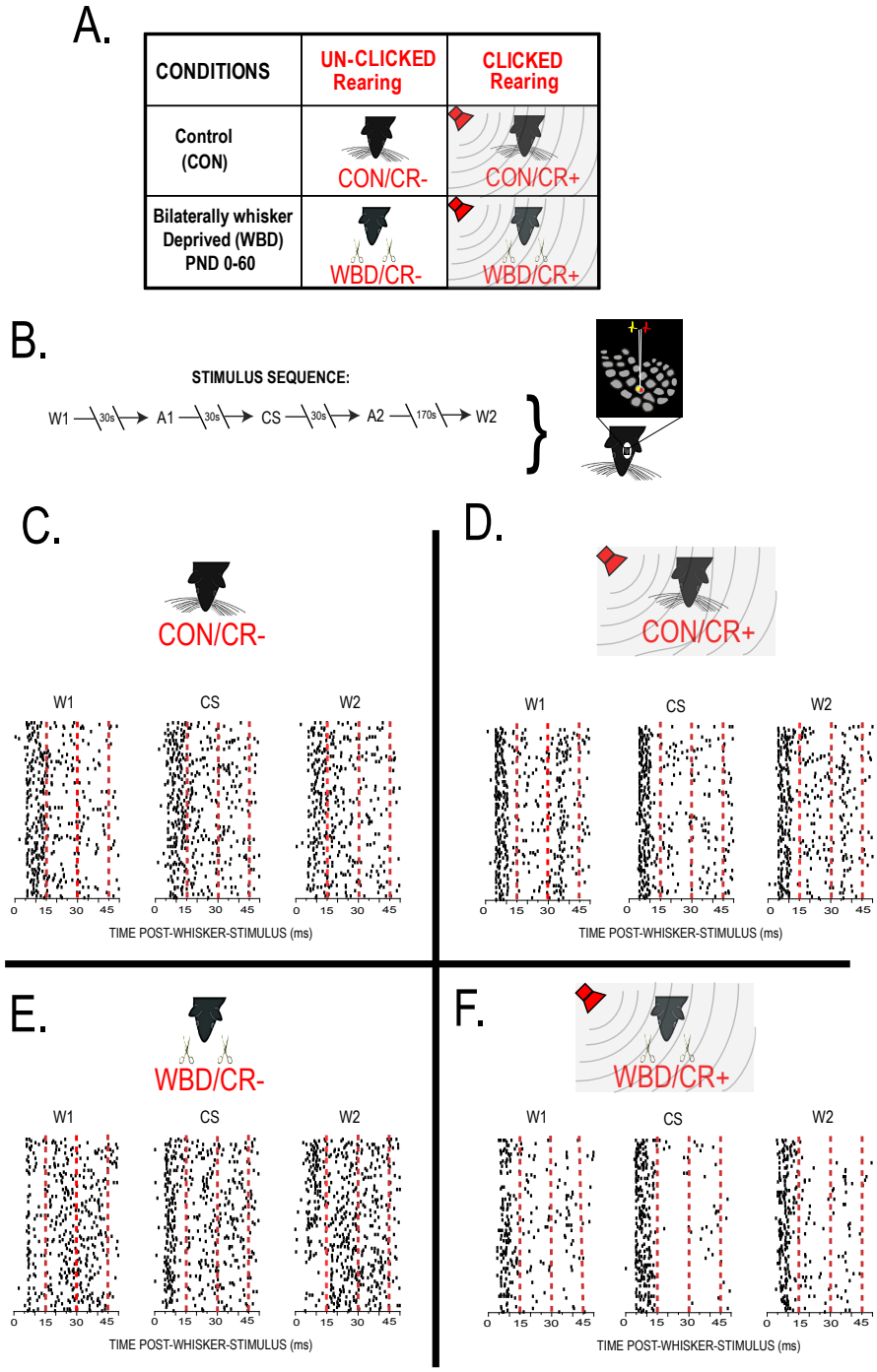

Figure 4-1: Experimental Design and single cell examples demonstrating cross-sensory interactions in barrel cortex

A. Experimental design showing the four rearing conditions from birth to PND 60. Rats with intact whiskers (CON) or bilaterally whisker trimmed (WBD) were raised either with normal laboratory noise (No Click Rearing [CR-]) or reared with 1 Hz auditory clicks (Click Rearing [CR+]). B. Sequence of stimulation used to test single cell responses in barrel cortex of all animals. Neuronal responses were analyzed to whisker-only stimuli before (W1) and after (W2) a cross-sensory stimulus (CS). W2 was always carried out 5 minutes after the last CS stimulus. Click alone (A1 and A2) always produced a null response. C-F. Raster plots of typical single neurons from each of the rearing groups showing responses to the W1, CS, and W2 stimulus over a 50 ms post-whisker -stimulus time period. Zero ms on the X-axis is the onset of the whisker stimulus in each stimulus condition. Vertical, red, dashed lines in the plots demark the first three 15 ms post-whisker-stimulus epochs. The increase in the number of spikes in the 0-15 ms epoch of the CS stimulus condition was significant in all the rearing groups when compared with W1. The responses in the 15-30 ms time window were more variable across the rearing groups, with the WBD/CR+ (F) showing a dramatic suppression of response in the CS stimulus condition. The W2 stimulus, delivered 5 minutes after the CS condition appear to be not different from the W1 responses.

modifications in these two time windows. Changes in response magnitude were analyzed in each epoch to compare the three stimulus conditions. In addition, most of the neurons recorded in each rearing group were stimulated with auditory click stimulation alone (A1, A2; Fig. 4-1B) both before and after the CS stimulus. However, the click stimulation alone failed to produce detectable changes in firing rate from barrel cortex neurons in any of the rearing groups (data not shown).

Raster plots (Fig. 4-1C-F) show the responses of representative single neurons from each of the 4 rearing groups in response to W1 (whisker before CS), CS (auditory-whisker, cross-sensory stimulus) and W2 (whisker after CS) stimuli. The plots show that there is a modulation of a neuron's response under the CS stimulus conditions when compared to W1. The responses in the W2 condition are similar to the W1 response pattern. The response modulations in a single cortical neuron (Fig. 4-1C-F) closely resemble the population effects discussed below (Fig. 4-2). For example, in Fig. 4-1F the CS stimulus produced an increase in response magnitude in a single neuron in WBD/CR+ rats in the 0-15 ms epoch, followed by a striking suppression of response in the 15-30 ms time window. This effect shows up clearly in the population of neurons recorded from WBD/CR+ rats (Fig. 4-2D).

Omnibus analyses of the magnitude of cross-sensory responses:

To analyze group effects by rearing, stimulus and cortical layer, we carried out generalized linear mixed model (GLMM) analyses on spike counts occurring during the post-stimulus windows of 0 to 15 ms and 15 to 30 ms {Rearing (CON/CR-, CON/CR+, WBD/CR-,WBD/CR+) X Stimulus Condition (W1/CS/W2) X Layer (II-III, IV)} (Table 4-1).

TABLE 4-1:

REARING GROUPS	0-15 ms epoch			15-30 ms epoch		
	W1	**CS**	**W2**	**W1**	**CS**	**W2**
CON/CR-	132.99 (22.97)	166.75 (28.66)	129.86 (22.44)	166.85 (39.28)	181.52 (42.71)	172.81 (40.68)
CON/CR+	117.40 (20.70)	148.09 (25.93)	115.92 (20.45)	123.83 (29.59)	120.73 (28.86)	123.03 (29.4)
WBD/CR-	114.41 (32.91)	154.12 (31.94)	115.61 (24.14)	158.60 (43.73)	159.91 (44.08)	165.42 (45.58)
WBD/CR+	100.36 (18.11)	121.01 (21.71)	99.68 (17.99)	76.65 (18.75)	48.98 (12.13)	67.16 (16.49)

Table 4-1: Means and standard errors of means (in parentheses) of total spike counts for 100 trials in the 0-15 ms and 15-30 ms post-whisker-stimulus epoch for each stimulus condition and rearing group. The means shown are estimated population marginal means adjusted for all fixed effects (i.e., main effects and interactions) in the model (e.g., Searle et al. 1980). The means displayed are in the scale of raw counts and are thus exponentiated versions of the estimates directly yielded by the generalized mixed model analysis. Standard errors were estimated using the delta method (Littell et al. 2006).

To account for sources of non-independence, we specified random effects for rat, cortical layer within rat, and stimulation condition within neuron. The GLMM performed on the 0-15 ms window yielded a highly significant main effect for stimulus condition ($p < .0001$), due to highly significant increase in neural responses elicited by the CS stimulus in all 4 rearing conditions. No other effects were statistically significant (all p's > .09). Subsequent multiple comparisons (Fisher LSD; Levin et al. 1994) among the three types of stimulation indicated that the response magnitude was higher in the CS condition relative to both the W1 condition, ($p < .0001$), and the W2 conditions, ($p < .0001$). The W1 and W2 conditions were not significantly different ($p > .70$) (Fig. 4-2).

The omnibus GLMM analysis conducted on the 15-30 ms epoch showed a different result. We observed significant main effects for Rearing, ($p < .05$), Stimulation, ($p < .0001$), and Layer, ($p < .05$), which were moderated by a highly significant Rearing X Stimulation interaction ($p < .0001$) (Fig. 4-2) and a significant three-way Rearing X Stimulation X Layer interaction ($p < .04$) (Fig. 4-4). Thus, unlike the short latency epoch, the effects of stimulation on the 15-30 ms time window were conditional on both rearing condition and layer. Further analyses also showed that these cross-sensory effects, especially the ones observed in the 15-30 ms epoch were somewhat dependent upon the responsiveness of the neurons, with the low responsive neurons exhibiting the cross-sensory modulations in the 15-30 ms epoch, more often than the high responsive neurons (Fig. 4-3). How each rearing group was influenced by cross-sensory stimuli is presented in detail in the next section.

Cross-sensory interactions in normal (CON/CR-) rats:

To test for cross-sensory interactions we compared the response magnitude in the 0-15 ms and 15-30 ms epochs after the W1 stimulus to that of the other two stimulus conditions (CS and W2) within each of the four rearing groups.

'Normal' rats reared with intact whiskers in a normal laboratory noise environment showed significant cross-sensory interaction in S1 barrel cortex. Population post-stimulus time histograms (PSTHs), constructed by averaging the responses of 84 neurons to W1, CS and W2 stimuli showed a significant modulation of neuronal response to the CS stimulus in normal animals (CON/CR-, Fig. 4-2A). The magnitude of spike counts following the CS stimulus was significantly greater than after the W1 stimulus during the 0-15 ms post-stimulus epoch ($p < .0001$), whereas the W1 spike counts were not significantly different from the W2 spike counts ($p > 0.05$). Over 65% of all neurons showed an increase to CS stimulation in the 0-15 ms epoch. During the 15-30 ms epoch the response magnitude to CS was also significantly greater than that to W1 in normal animals ($p < 0.005$), whereas the magnitude of response to W2 remained unaltered from the response to W1 ($p > .05$) (Fig. 4-2A). When tested individually 51% of all neurons showed the increase in magnitude to CS in the 15-30 ms epoch.

Cross-sensory interactions in CON/CR+ rats:

Control rats with intact whiskers were also reared with environmental click stimuli (CON/CR+) to show the effect of click rearing by itself. Recordings from 58 neurons of this group from L-II/III and IV were pooled together to construct the population PSTHs to

W1, CS and W2 stimuli (Fig. 4-2B). Similar to the other rearing groups, the magnitude of the neuronal response to the CS stimulus was significantly higher in the 0-15 ms epoch when compared to the response to the W1 stimulus condition ($p < 0.0001$) when the responses to W1 were not significantly different from those of W2 ($p > 0.05$). This facilitative effect with the CS stimulus was observed in 81% of neurons in the CON/CR+ group. The click rearing in normal animals failed to show any difference in response magnitude in the 15-30 ms epoch. Thus, the responses under CS and W2 stimulus conditions were not significantly different from that under W1 (W1 vs. CS $p > 0.05$; W1 vs. W2 $p > 0.05$). However, when individual neurons were analyzed 46% actually showed a decrease in magnitude in this epoch with the CS stimulus.

Cross-sensory interactions in WBD/CR- rats:

Rats whisker trimmed bilaterally, but not click reared, served as a control for sensory deprivation alone. This group also showed significant modulation of barrel cortex responses to the CS stimulus that were evident only in the initial 0-15 ms post-stimulus epoch. This result can be seen in the population PSTHs constructed from responses of 48 neurons combined from both layers II/III and IV of barrel cortex (Fig. 4-2C). The CS stimulus in WBD/CR- animals caused a significant increase in the magnitude of the neuronal responses in the 0-15 ms epoch when compared with the response to the W1 stimulus ($p < 0.0001$), without a significant W1-W2 difference in this 0-15 ms epoch ($p > 0.05$). This increase with CS stimulus was found in 81% of individual neurons in this group, showing that the sensory deprivation alone produced an enhanced response to the cross-sensory stimulus. Like the CON/CR+ rats, the

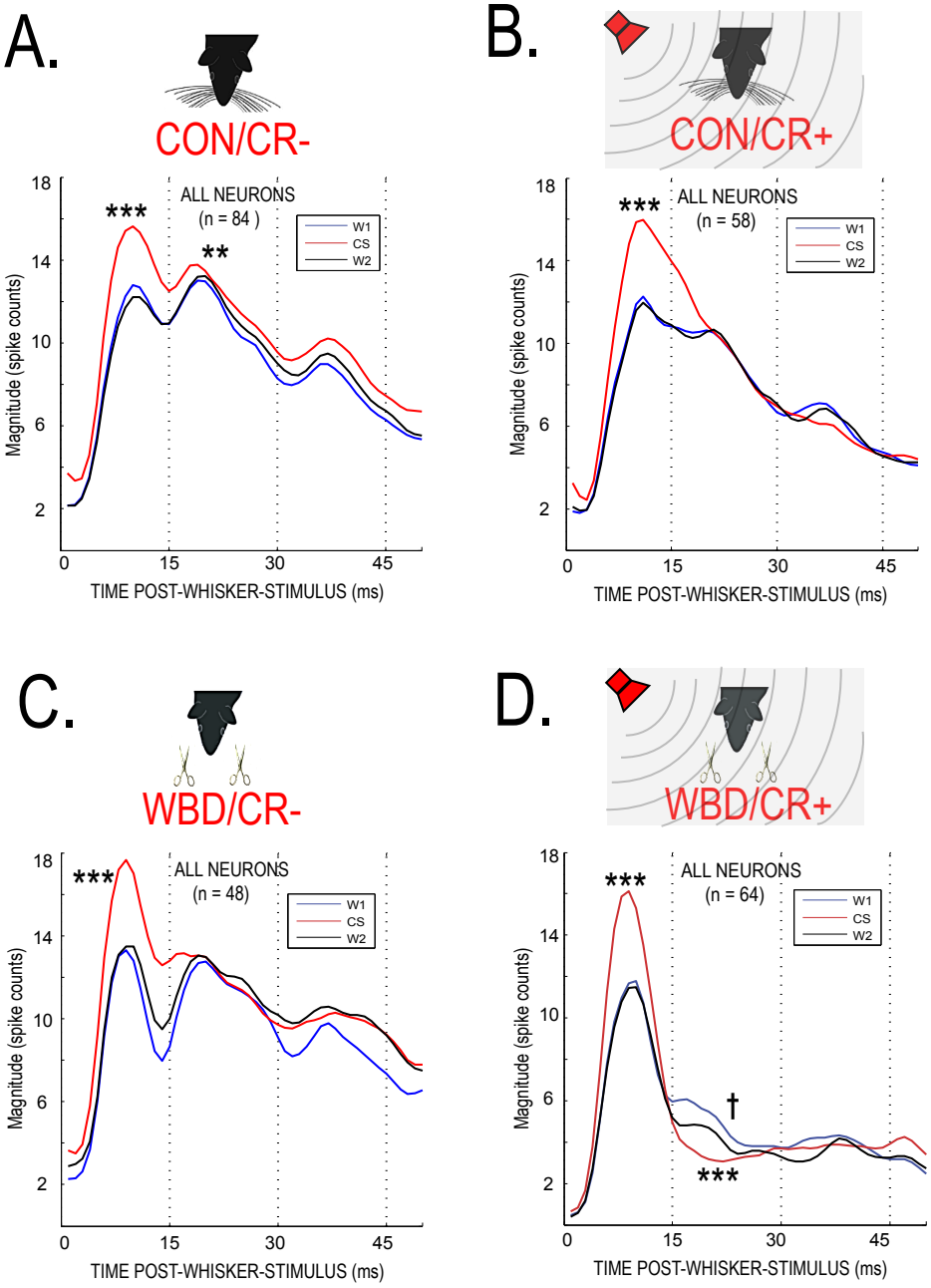

Figure 4-2: Population responses showing the cross-sensory effect on the magnitude of response in the different rearing groups

A-D: Population Post-stimulus-Time histograms (1 ms bin size) for all 3 stimuli (W1, CS and W2) under the 4 different rearing conditions. The onset of the whisker stimulus is time zero. The PSTHs were smoothed using NEX software with Gaussian filters with a filter bin width of 3 and divided into 15 ms post-whisker -stimulus time windows (dashed black vertical lines). The response magnitude to the CS stimulus was significantly increased in the 0-15 ms epoch for all 4 rearing groups, whereas in the 15-30 ms epoch there was significant CS facilitation in CON/CR- rats (A) and a significant CS suppression in the WBD/CR+ rats (D). No significant changes in response magnitude to the CS stimulus were observed in the 15-30 ms in CON/CR+ rats (B) and WBD/CR- rats (C). The response to the W2 stimulus was not significantly different from that to the W1 stimulus for any rearing groups at any time, except in the WBD/CR+ rats (D) where the responses to the W2 stimulus in the15-30 ms epoch were significantly lower. See table 1 for mean values and standard error for each epoch.

*** denotes $p < 0.001$ when CS was compared with W1, ** denotes $p < 0.01$ when CS was compared with W1, † denotes $p < 0.05$ when W2 was compared with W1.

magnitude of the longer latency 15-30ms epoch was not significantly different among the 3 stimulus conditions (W1 vs. CS: $p > 0.05$ or W1 vs. W2: $p > 0.05$).

Cross-sensory interactions in WBD/CR+ rats:

Rats with their whiskers trimmed bilaterally and reared with environmental auditory clicks from PND 0 to 60, showed the greatest modulation of response magnitude to the CS stimulus. All of the neurons recorded from the WBD/CR+ barrel cortex (n = 64) were pooled to construct population PSTHs for the different stimulus conditions (Fig. 4-2D). For the 0-15 ms epoch there was a significant increase in magnitude of the response to the CS stimulus when compared to that of the W1 stimulus ($p < .0001$), whereas, the magnitude of response to W2 remained unaltered in this epoch compared to the response to W1 ($p > 0.05$). This increase in magnitude to the CS stimulus was found in 69% of all neurons.

In contrast, in the 15-30 ms epoch, there was a highly significant decrease in the magnitude of response to CS stimulation when compared to the W1 responses ($p < 0.0001$) with 75% of neurons in the population showing this effect. Moreover, when the whisker alone was stimulated again (W2), the response remained significantly less than W1 ($p < 0.012$) in the 15-30 ms post-whisker-stimulus epoch. Thus, in the WBD/CR+ animals the CS stimuli increased the short latency whisker response and strongly suppressed the longer latency whisker response.

Dependence of cross-sensory interactions on the responsiveness of neurons:

All neurons recorded were subdivided into 'high' and 'low' responsive groups in each rearing condition. For a given rearing condition, if the total spike count of a neuron (0-30ms post-stimulus epoch) in response to whisker stimulus alone (W1) was higher than the median of the total spike counts of all neurons then it was considered a high responsive neuron and vice versa. Fig. 4-3 illustrates how the CS stimuli affected the magnitude of 0-15 and 15-30 ms epoch of the high and low responsive neurons in each rearing condition.

In the 0-15 ms epoch the facilitation of response to the CS stimulus was significant in both responsive groups for all rearing conditions, except for the high responsive neurons of WBD/CR+ rats ($p > 0.05$; Fig. 4-3D).

In the 15-30 ms epoch of CON/CR- rats, only the low responsive neurons showed a significant facilitation ($p < 0.01$; Fig. 4-3A). For the CON/CR+ rats, the responses in this epoch were not significantly different in response to the CS stimulus for both the responsive groups ($p > 0.0125$), but the low responsive neurons showed a trend toward response suppression following the CS stimulus ($p = 0.03$; stepdown Bonferroni critical $p = 0.0125$; Fig. 4-3B). In the WBD/CR- rats the magnitude in the 15-30 ms epoch of all neurons was not significantly different in the CS stimulus condition (Fig. 4-2C). However, when classified according to responsiveness, the low responsive neurons showed a significant facilitation of response ($p < 0.01$; Fig. 4-3C). For the WBD/CR+ rats, there was a significant response suppression for both the high and low responsive neurons in the 15-30 ms epoch ($p < 0.001$; Fig. 4-3D). A stepdown

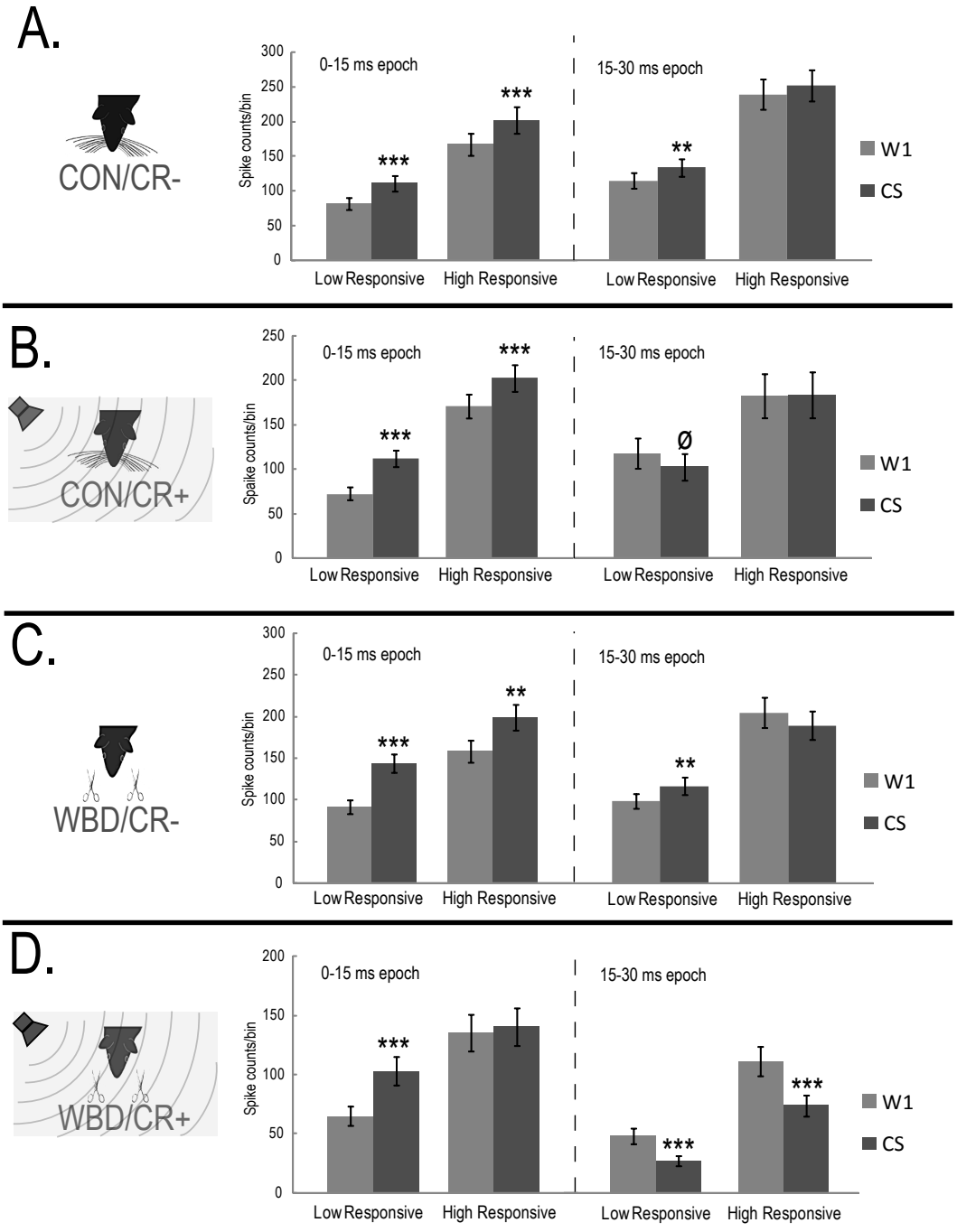

Figure 4-3: Population means of responses in each rearing group showing the dependence of cross-sensory effect on neuronal responsiveness

Neurons for each rearing group were divided into two groups depending on whether their mean response in the 30 ms post-whisker-stimulus period was below (Low responsive) or above (High responsive) the median of responses of all the neurons in that rearing group. Bar graphs for total spike counts for each such neuronal groups in either the 0-15 ms (left column) and 15-30 ms post-whisker stimulus epoch (right column) are displayed for CON/CR- (A), CON/CR+ (B), WBD/CR- (C) and WBD/CR+ (D) rats. Responses for W1 (blue) and CS (red) stimulus conditions are considered and compared statistically using a stepdown Bonferroni procedure. The response facilitation in the 0-15 ms epoch after the CS stimulus is observed in both responsive types for all rearing groups except the high responsive group of the WBD/CR+ rats. For the 15-30 ms epoch only low responsive neurons of CON/CR- and WBD/CR- rats showed significant facilitation of response (A and C), whereas with click rearing only (CON/CR+) there is a strong trend for suppression of neuronal response in low responsive cells (B). Simultaneous click rearing and whisker deprivation (WBD/CR+) leads to highly significant response suppression in both the low and high responsive neurons in the 15-30 ms post-stimulus epoch (D). *** denotes $p < 0.001$; ** denotes $p < 0.01$; Ø denotes $p = 0.03$

Bonferroni procedure was applied to adjust the p values for multiple tests (see methods).

Dependence of cross-sensory interaction on laminar position of neurons:

We further subdivided the pool of neurons according to their location in layers II/III or IV to determine whether the cross-sensory interactions observed in the 4 rearing groups were different in different cortical layers. Neurons that were recorded from depths ranging from 100-450 µm were considered to be in the supragranular layers II-III (L-II/III), whereas neurons recorded from 450-800 µm were considered to be in layer IV (L-IV) (Li et al. 2005). Average responses of the neurons from each layer were utilized to construct the population PSTHs for W1, CS and W2 stimulus in each rearing group as displayed in Fig. 4-4. In the 0-15 ms time window the increase in magnitude of response under the CS stimulus condition was consistent across all layers. That is, both L-II/III and L-IV neurons of all rearing conditions showed a significant increase in spike counts in the 0-15 ms epoch when stimulated with the CS stimulus compared to the response following W1 stimulus (CON/CR-, L-II/III, n = 38, $p < 0.0001$ and L-IV, n = 46, $p < 0.0001$), (CON/CR+, L-II/III, n = 27, $p < 0.0001$ and L-IV, n= 31, $p < 0.01$), (WBD/CR-, L-II/III, n = 18, $p < 0.001$ and L-IV, n = 30, $p < 0.0001$), (WBD/CR+, L-II/III, n = 27, $p < 0.05$ and L-IV, n = 37, $p < 0.001$).

There was a subtle relationship between the laminar positions of neurons and the cross-sensory interactions in the 15-30 ms epoch observed in certain rearing groups (Fig. 4-4). In the CON/CR- rats the increase in magnitude in the 15-30 ms under the CS stimulus condition was significant only in L-IV ($p < 0.02$) but not in L-II/III ($p > 0.05$). The

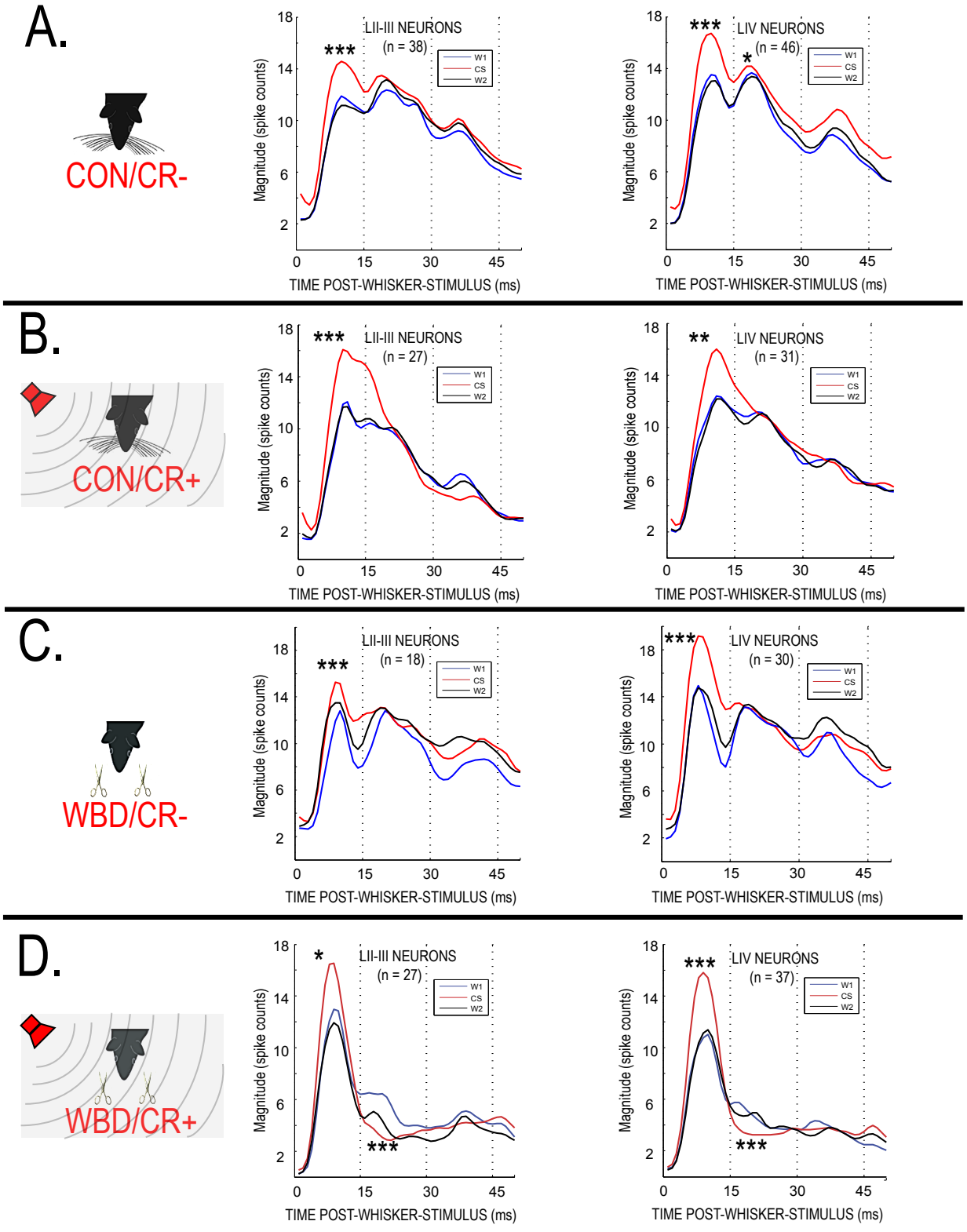

Figure 4-4. Population responses of neurons from each rearing group showing the cross-sensory effect in layers II-III and IV

Neurons from each rearing group were divided into Layer II-III (LII-III) and Layer IV (LIV) on the basis of their vertical location (see results) from the surface of the cortex. Population PSTHs were constructed after W1, CS and W2 stimuli for CON/CR- (A), CON/CR+ (B), WBD/CR- (C), and WBD/CR+ (D) rearing groups. The population PSTH's in this figure were constructed as in figure 2, but separately for the LII-III (left column) and LIV (right column) neurons. The dashed, vertical black lines indicate successive 15 ms response epochs.
*** denotes $p < 0.001$ when CS was compared with W1; ** denotes $p < 0.01$ when CS was compared with W1; * denotes $p < 0.05$ when CS was compared with W1.

lack of any evident cross-sensory interaction in this epoch, i.e., differences in the response magnitude between W1 and CS conditions for WBD/CR- and CON/CR+ was consistent in both L-II/III and L-IV ($p > 0.05$)., For WBD/CR+ rats, the decreased response magnitude to the CS stimulus was highly significant in both L-II/III ($p < 0.0001$) and L-IV ($p < 0.0001$) when compared to that of the W1 response. A stepdown Bonferroni procedure was applied to adjust the p values for multiple tests (see methods). Thus, the laminar position of neurons had only a minor effect on the cross-sensory interactions reported above.

Dependence of cross-sensory interaction on putative excitatory and inhibitory neurons:

To determine whether a particular neuron type is more susceptible to the cross-sensory interactions observed in rat barrel cortex, we divided the entire pool of neurons from each rearing group into regular spiking units (RSUs) and fast spiking units (FSUs). A neuron was designated an RSU or an FSU based on their baseline-to-baseline waveform duration (RSU >750 μs, FSU <750 μs). For each rearing group, population PSTH's were constructed averaging the responses of RSUs and FSUs to W1, CS and W2 stimulus conditions (Fig. 4-5). For the 0-15 ms epoch, the increase in magnitude of response to CS stimulus over the responses to W1 was significant for RSUs in all the rearing groups [CON/CR- (n = 53), $p < 0.0001$; CON/CR+, (n = 31), $p < 0.0001$; WBD/CR-, (n = 27), $p < 0.0001$; WBD/CR+, (n = 38), $p < 0.001$]. For FSUs, the increase was only significant for CON/CR- (n = 28; $p < 0.01$), WBD/CR- (n = 21; $p < 0.0001$) and CON/CR+ (n= 27; $p < 0.01$) rats, but not for WBD/CR+ rats (n = 26; $p > 0.05$).

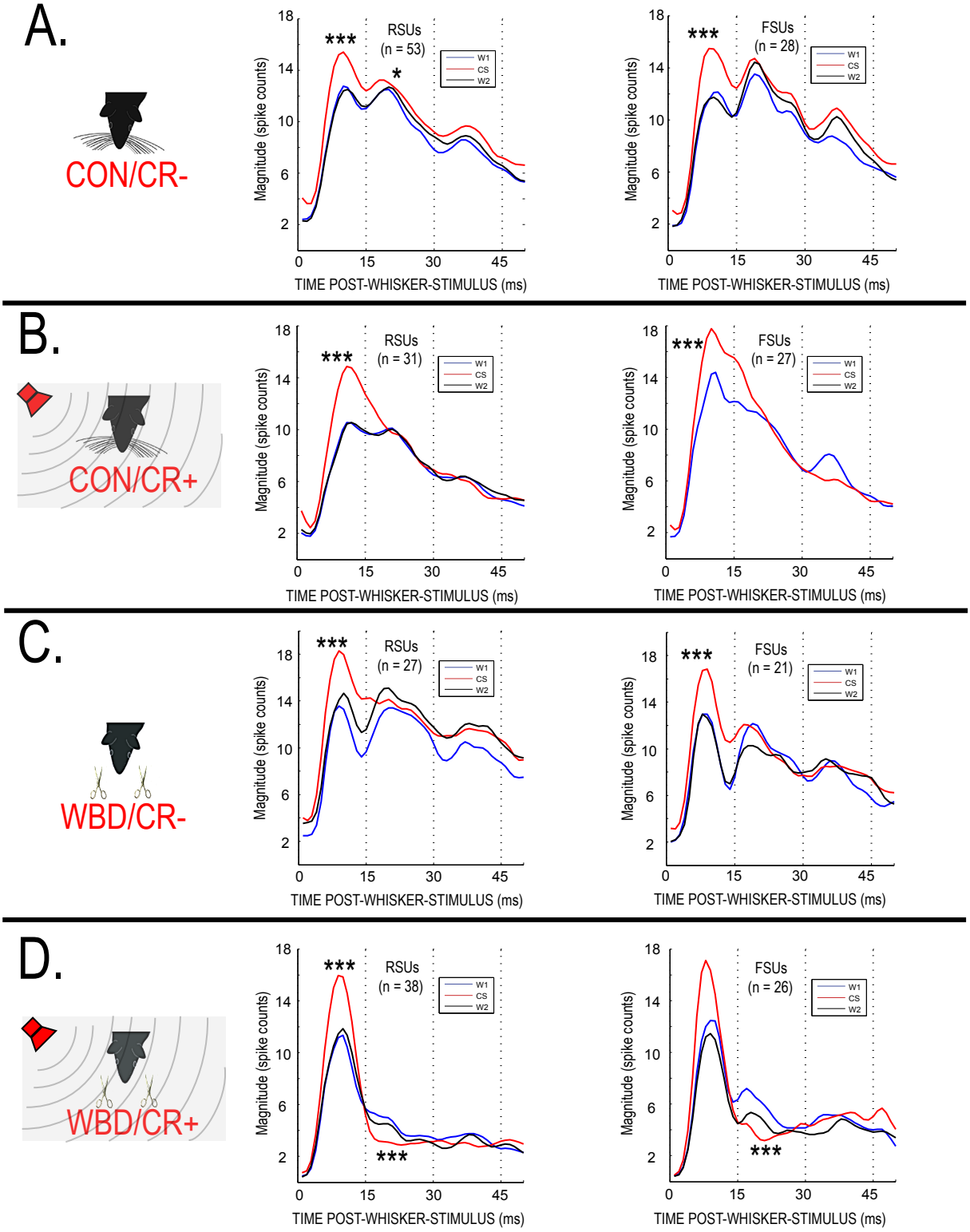

Figure 4-5. Population responses for neurons in each rearing group showing the cross-sensory effect on RSU and FSU neurons

Neurons for each rearing group were divided into presumed excitatory regular spiking units (RSUs) and presumed inhibitory fast spiking units (FSUs) on the basis of their waveform duration (see results). Population PSTHs to W1, CS and W2 stimuli for CON/CR- (A), CON/CR+ (B), WBD/CR- (C), and WBD/CR+ (D) rats displayed in this figure were constructed similarly as in figure 2, but separately for the RSUs (left column) and FSUs (right column). The black broken lines in each graph indicate the 15 ms response time windows. *** denotes $p < 0.001$ when CS was compared with W1. ** denotes $p < 0.01$ when CS was compared with W1; * denotes $p < 0.05$ when CS was compared with W1.

For the 15-30 ms epoch in the CON/CR- rats, the increase in magnitude of responses with CS stimulus over that of W1 stimulus was significant only for RSUs ($p < 0.05$) but not for FSUs ($p > 0.05$). However, in the WBD/CR+ rats the decrease in magnitude with CS stimulus compared to the W1 responses in this epoch was significant in both RSUs ($p < 0.0001$) and FSUs ($p < 0.0001$). No significant differences between W1 and CS stimulus responses were observed in this epoch for WBD/CR- RSUs ($p > 0.05$) or FSUs ($p > 0.05$) and CON/CR+ RSUs ($p > 0.05$) and FSUs ($p > 0.05$). As above, a stepdown Bonferroni procedure was applied to adjust the p values for multiple tests (see methods).

The effect of the cross-sensory stimuli on spontaneous activity:

The magnitudes of spontaneous firing rates were calculated using the total spike counts in a 500 ms time window from 500 to 1000 ms post-whisker stimulus, and was compared across stimulus conditions within each group. Both non-click reared groups (CON/CR- and WBD/CR-) showed a significant increase in spontaneous activity in this time window ($p < 0.01$) under the CS stimulus condition, but both click-reared groups (CON/CR+ and WBD/CR+) showed no change in spontaneous activity ($p > 0.05$) during the CS stimulus condition (Table 4-2).

Between group comparisons of cross-sensory interaction using the RMI:

In order to compare whether the auditory-touch interactions observed in our experimental rearing group WBD/CR+ were significantly different from the other control groups, we statistically compared the response modulation index (RMI) of the 0-15 ms

TABLE 4-2:

REARING GROUPS	500 -1000 ms spontaneous epoch	
	W1	**CS**
CON/CR-	1033.61 (365.45)	1147.32 (405.38)
CON/CR+	474.63 (172.24)	486.73 (176.54)
WBD/CR-	983.87 (404.50)	1153.52 (473.73)
WBD/CR+	171.92 (65.59)	171.9 (65.53)

Table 4-2: Means and standard errors of means (in parentheses) of total spike counts for 100 trials during the relatively spontaneous periods (500 ms to 1000 ms post-whisker-stimulus) for each stimulus condition and each rearing group. The means shown are estimated population marginal means adjusted for all fixed effects (i.e., main effects and interactions) in the model (e.g., Searle et al. 1980). The means displayed are in the scale of raw counts and are thus exponentiated versions of the estimates directly yielded by the generalized mixed model analysis. Standard errors were estimated using the delta method (Littell et al. 2006).

and 15-30 ms epoch across different groups. For each neuron assessed, the RMI was computed by the following ratio: Magnitude(CS)/Magnitude(CS+W1), separately for each epoch. We used this index for two reasons. First, it is easily interpretable because it is bounded between 0 and 1 (i.e., it assesses the proportion of the total number of counts across the CS and W1 conditions that were observed in the CS condition). Second, because the index is a proportion we were able to estimate a grouped binomial mixed model that specified that the probability distribution of the residuals is binomial (Hilbe 2009). The key feature of the analysis was the set of pairwise comparisons comparing WBD/CR+ to the other three groups on the RMI index. Dunnett's method (Dunnett 1955) was used to adjust p-values. For the 0-15 ms epoch there was no main effect for rearing group ($p > 0.05$) and the RMI for the WBD/CR+ failed to differ significantly from that of CON/CR- ($p > 0.05$), CON/CR+ ($p > 0.05$) and WBD/CR- ($p > 0.05$) (Fig. 4-6). In contrast, for the 15-30 ms epoch there was a significant main effect for rearing groups ($p < 0.01$) and the RMI for WBD/CR+ was significantly different from CON/CR- ($p < 0.01$), CON/CR+ ($p < 0.05$) and WBD/CR+ ($p < 0.05$) (Fig. 4-6B). Thus, the rearing experience significantly affected the RMI in the 15-30 ms time window, but not the early 0-15 ms epoch (Fig. 4-6).

Discussion

These results show that there is a significant cross-sensory auditory influence on the responses of S1 barrel cortex neurons in the normal adult rat that is modified by early sensory experience. There was a significant facilitation of whisker-evoked responses in the 0-15 ms time window, when an auditory click preceded a whisker

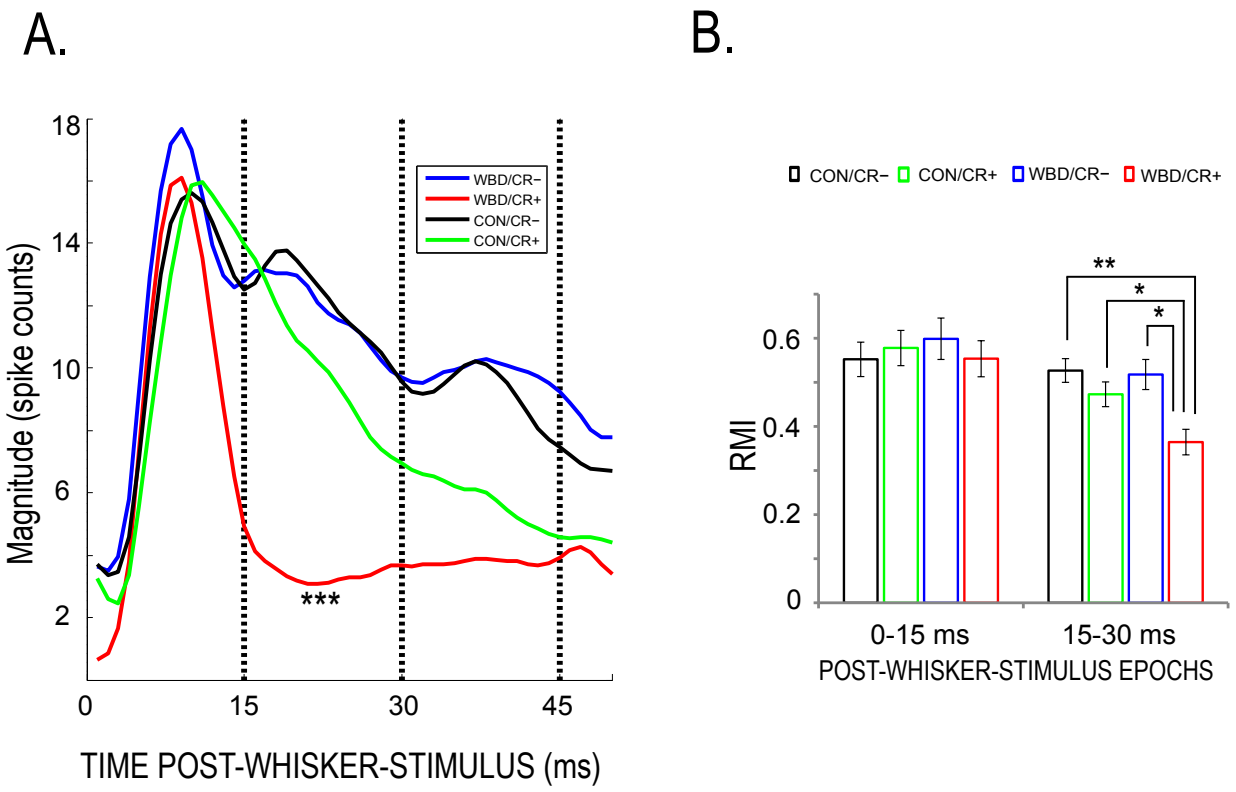

Figure 4-6. Population responses to the CS stimulus and response modulation index (RMI) in the different rearing groups showing the effect of rearing experience on cross-sensory responsiveness
A. Comparison of population PSTH's showing responses to the CS stimulus for all four rearing groups during each 15 ms post-stimulus time bins (dashed, vertical, black lines) following the whisker stimulus. *** denotes a significant main effect for the deprived and click reared group in the 15-30 ms epoch ($p < 0.001$). B. Bar graph showing the RMI of the different rearing groups calculated as the ratio [(MagnitudeCS)/Magnitude(CS+W1)] separately for the 0-15 ms and 15-30 ms post-whisker-stimulus epoch. The WBD/CR+ rats show a significant difference in the RMI when compared with the other groups, only in the 15-30 ms response epoch. *** denotes $p < 0.001$, ** denotes $p < 0.01$, * denotes $p < 0.05$. Dunnett's tests reported in the text were performed on the mean log odds (i.e., logits) rather than the raw ratios. This figure shows the raw ratios for ease of interpretation. The results were identical when comparisons were performed on the raw ratios generated by an alternative GLMM.

stimulus, even though the barrel cortex neurons failed to fire action potentials in response to the auditory click stimulus alone. The response facilitation was similar across all rearing groups. In contrast, the response modulation during the CS stimulus in the longer latency 15-30 ms time window, depended heavily upon postnatal rearing experiences. The conditioning auditory click prior to a whisker stimulus led to a modest but significant response facilitation in the normally reared CON/CR- group that was especially robust in layer IV and in low responsive neurons. A longer latency facilitation was observed only in the low responsive neurons after whisker trimming (WBD/CR-), while responses showed a strong tendency towards suppression in the low responsive neurons after click rearing (CON/CR+). However, simultaneous whisker trimming coupled with click rearing (WBD/CR+), produced a striking and significant response suppression in the 15-30 ms epoch following CS stimuli. This suppression was highly significant in both the supragranular and granular layers, for both FS and RS neurons, and for both low and high responsive neurons in the WBD/CR+ rats. Thus, auditory clicks profoundly modulate both the shorter (0-15 ms) and longer (15-30 ms) latency response epochs of barrel cortex neurons, while other combinations of rearing experiences influence primarily the longer latency cross-sensory responses. Finally, cross-sensory modulations may target subsets of neurons based on their response level, laminar location and neuron type within each rearing condition.

The fact that the auditory clicks failed to drive any barrel cortex neurons under any conditions in the present study, indicate that the cross-sensory multisensory interactions observed here are modulatory in nature (Carriere et al. 2008; Dehner et al. 2004; Lakatos et al. 2007) as opposed to directly driven responses found in association

cortex (Wallace et al. 1992; Carriere et al. 2007), and may account for the difficulty in detecting multi-sensory influences in primary sensory cortex. Surprisingly, the modulatory influence is present even in normally reared rats and is maximally enhanced when whisker deprivation is coupled with click rearing. Based on this, we propose that in all rearing groups the auditory click stimulation generates sub-threshold changes in the excitability of barrel cortex neurons, and in that way modulate the response firing rates of the neurons to their driving input (whiskers). This hypothesis requires testing with intracellular recording from the S1 neurons in response to auditory stimuli, and preliminary, unpublished intracellular recordings suggest that this indeed may be the case (M. Brecht, personal communication).

The modulatory cross-sensory influence was clearly detectable in the CS stimulus condition when the response magnitude was compared with whisker-only stimulus conditions (CS vs W1 or W2). The neuronal responses to W1, CS and W2 were subdivided in order to separate the entire evoked-response period into time windows assumed to be dominated by thalamocortical inputs (0-15 ms) and a longer latency time window (15-30 ms) driven by strong corticocortical connections. (Armstrong-James et al. 1992). One fundamental difference in the modulation of the two response components in the CS stimulus condition was that the putative thalamocortical responses were always facilitated in all 4 rearing groups, whereas the modulations in the corticocortical component differed significantly among the rearing conditions. For example, click rearing conditions led to the CS stimulus reducing the probability of whisker-driven responses in the 15-30 ms epoch, with the effect being most significant in the WBD/CR+ rats. Without click rearing, rats showed a tendency toward increased

responses to CS stimulus in this epoch. This differential effect of rearing experience on the two post-stimulus epochs suggests that the cross-sensory modulations in each epoch could be caused by different mechanisms. Thus, a two-stage model best fits the cross-sensory response modulations observed under our conditions (Fig. 4-7).

The response modulation observed in the 0-15 ms epoch was always facilitative in nature. What is the best explanation for such facilitation in an early response period dominated primarily by thalamocortical inputs? There is ample evidence that subcortical auditory structures such as the dorsal cochlear nucleus (DCN) (Zhou and Shore 2004; Haenggeli et al. 2005; Shore 2005; Shore et al. 2008), the inferior colliculus (IC) (Jain and Shore 2006; Zhou and Shore 2006), and the medial division of the thalamic medial geniculate nucleus (mMGN) (Poggio and Mountcastle 1960; Blum et al. 1979; Nicolelis et al. 1991) all receive significant somatosensory inputs and show multisensory responses. Thus, these structures, in turn, may influence subcortical somatosensory structures such as the trigeminal nucleus (TN) and the thalamic ventral posterior medial nucleus (VPM). One candidate structure that could mediate such cross-sensory interactions is the brainstem reticular formation (RF), which receives inputs from the DCN (Cant and Benson 2003) and the IC (Kudo et al. 1983), and also projects directly to the TN (Ter Horst et al. 1991) and VPM (Bowsher 1975). Thus, in the CS stimulus condition, when an auditory click is presented first, the subcortical auditory structures could activate the RF projections to subcortical somatic sensory structures to increase their excitability just before the whisker stimulus activates these structures. In addition, the cholinergic components of the RF could increase the excitability of VPM by disinhibiting inputs from the thalamic reticular nucleus (Berry 1986; Lee and Ebner 1994

a,b; Deschenes et al. 2005) (Fig. 4-7A-D dashed line connections). Increased excitability of the VPM neurons would theoretically lead to increased activation by thalamocortical projections, which could explain the increase in spontaneous firing rates for non-click reared rats and facilitation of response magnitude in the 0-15 ms for all groups of animals. This cross-talk between subcortical sensory structures can thus be a probable mechanism for part of the early cross-sensory effect observed here, and is a testable hypothesis which was not directly addressed by the present experiments.

Although there were no significant changes in modulation between rearing groups in the 0-15 ms epoch, percentage analysis of individual neurons suggests that a higher percentage of neurons showed the facilitation in CON animals compared to the WBD animals and in the click reared animals compared to the non-click reared animals (e.g. CON/CR+ over CON/CR- and CON/CR+ over WBD/CR+ rats). The difference between CON and WBD cortex may be due to the basic deficiency in the response magnitude of barrel cortex found after bilateral deprivation due to possible reduction in the synaptic efficacy of the thalamocortical inputs (Popescu and Ebner 2010). The click rearing, in turn, might result in more effective, robust and widespread subcortical multisensory interactions and thus produce an increased percentage of neurons that show the facilitation in the 0-15 ms epoch.

The short latency facilitation is followed by modulation of responses in the 15-30 ms time window. There is little evidence in the literature for strong direct connections between A1 and S1 cortex in the rat that could provide the substrate for such a modulation. However, the dysgranular zone (DGZ) between A1 and barrel cortex could mediate such an influence. The DGZ neurons respond to both whisker and auditory

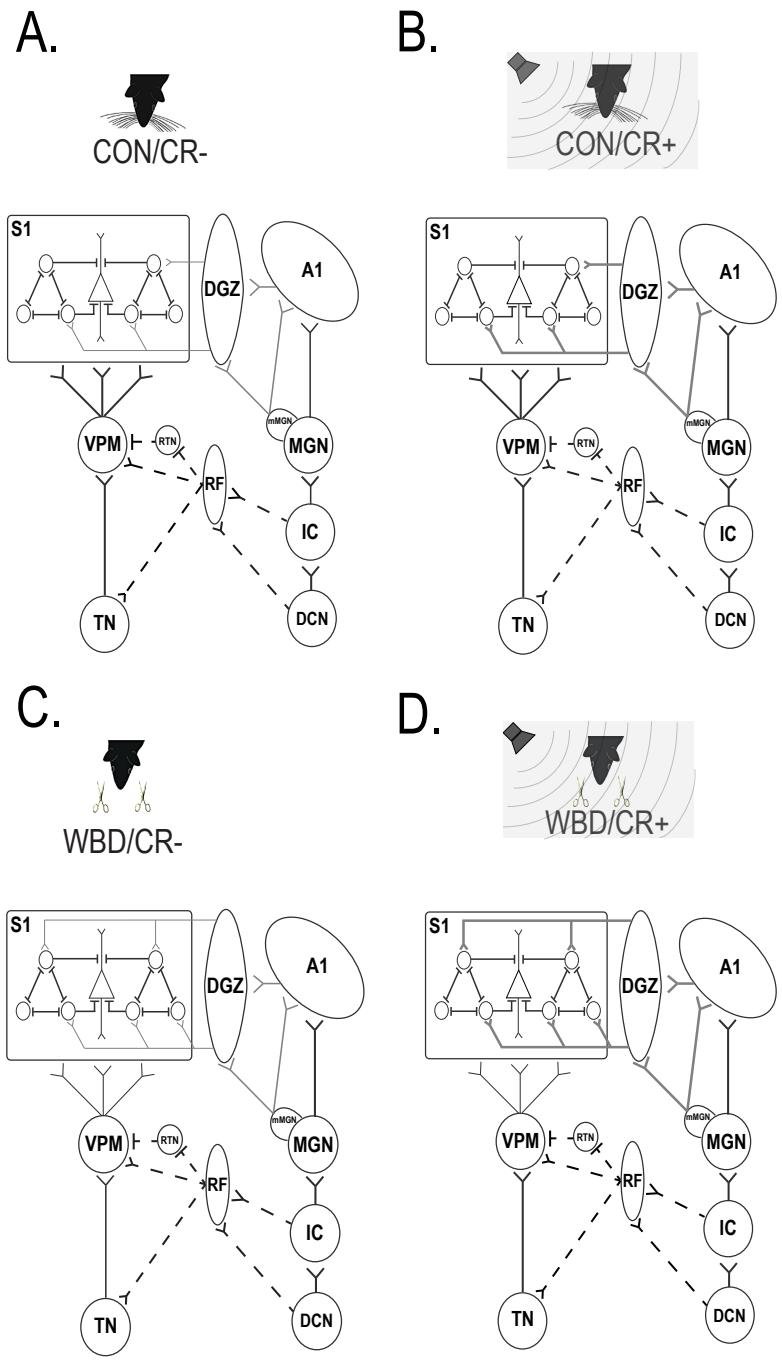

Figure 4-7. Hypothetical model proposing an explanation for the origin of the cross-sensory responses in S1 barrel cortex
The mechanism of the cross-sensory responsiveness in S1 barrel cortex remains to be clarified. Here the 4 rearing conditions are shown with the auditory and touch relay structures projecting to A1 and S1 cortex labeled with the abbreviations listed below. The lines ending with an open fork indicate excitatory connections and those ending with a short bar indicate inhibitory connections. Dashed connections represent the possible anatomical substrates governing cross-sensory effects in the 0-15 ms epoch and solid gray lines indicate connections for potential substrates of the 15-30 ms response epoch. Solid black connections highlight well known sensory pathways. Line thickness reflects the strength of a particular connection with thicker lines representing stronger connections and vice versa. Open circles and triangles in S1 symbolize FSUs and RSUs, respectively. According to this model in all rearing groups the auditory subcortical structures facilitate activity in the somatic sensory subcortical structures via the reticular formation (RF) (black dashed connections) and are responsible for the response facilitation observed in the 0-15 ms response epoch. On the other hand, the connections from the cortical DGZ that interconnect A1 and S1 cortex (gray connections) is postulated as the dynamic structure responsible for the response modulations observed in the 15-30 ms epoch. According to our hypothesis, the sparse and weak excitatory, auditory intracortical inputs onto inhibitory neurons in S1 cortex in the CON/CR- rats (A) is up-regulated with whisker deprivation and simultaneous click rearing (WBD/CR+) both in their number and in synaptic efficacy (D). This is thought to lead to the reported suppression observed in the 15-30 ms epoch of the WBD/CR+ rats. However, click rearing alone (B) may produce stronger, but less numerous, DGZ to S1 connections to barrel cortex FSUs, whereas, bilateral whisker deprivation alone (C) might lead to more numerous, but weak, corticocortical DGZ to S1 connections (gray lines) onto FSUs in barrel cortex. Both these effects in B and C would lead to a slightly higher level of inhibition in barrel cortex that could explain the absence of facilitation in the 15-30 ms epochs after CS stimulation under these rearing conditions. (See text for detailed explanation of the model). S1: Barrel cortex; A1: Primary auditory cortex; DGZ: Dysgranular Zone; VPM: Ventral Posterior Medial nucleus; RTN: Thalamic Reticular Nucleus; MGN: ventral division of the Medial Geniculate Nucleus; mMGN: medial division of the MGN; IC: Inferior Colliculus; DCN: Dorsal Cochlear Nucleus; TN: Trigeminal Nucleus.

stimuli (Brett-Green et al. 2003; Wallace et al. 2004). The DGZ receives auditory input from A1 cortex, from the ventral division of the MGN and also from the multi-sensory neurons in the mMGN (Brett-Green et al. 2003). DGZ also projects directly to S1 barrel cortex (Chapin et al. 1987; Koralek et al. 1990). Moreover, since horizontal connections in cortex including somatosensory cortex have been shown to recruit inhibitory networks under some conditions (Tucker and Katz 2003; Pluto et al. 2005; Keniston et al. 2010) it is possible that the projections from the DGZ to barrel cortex terminate on inhibitory FSU's that could cause sub-threshold changes leading to an increased excitability in inhibitory neurons. Thus, this model predicts that the click component of the CS stimulus would activate the DGZ, which could increase the excitability of the FSUs in barrel cortex via corticocortical connections leading to a suppression of firing in the longer latency component of the barrel field responses. Moreover, since FSUs are interconnected to RSUs as well as each other, the inhibition should be observed in both cell types, which is consistent with our data (Fig. 4-7).

The question remains open as to why there are significant rearing group differences in the response modulations of the 15-30 ms epoch. It is possible that with altered rearing experience that spans through the critical period, the synaptic number or efficacies or both are modified for the DGZ to S1 connection. In normal animals the facilitation observed in the 15-30 ms epoch was much smaller than that observed in the earlier 0-15 ms epoch. This reduction in facilitation could be due to active inhibition from the corticocortical connections by the mechanism described above. However, it is possible that the amount of inhibition is lower in normal animals because of sparse and low efficacy synapses and thus, low-level facilitation is still observed in the 15-30ms

epoch (Fig. 4-7A). In rats that were whisker deprived (WBD/CR-), the deprived state of the S1 cortex might lead to increased retention of the cross-sensory DGZ-S1 connections after maturation, without changing their synaptic efficacies from the normal levels. On the other hand, click rearing may increase the DGZ to S1 synaptic efficacies as opposed to the synaptic numbers. Any increase in inhibition due to the above two mechanisms, could serve to reduce facilitation in 15-30 ms response epoch of both the CON/CR+ and the WBD/CR- rats (Fig. 4-7B and C). The dramatic suppression of the response in the 15-30 ms epoch in WBD/CR+ rats could be a result of strong activation of numerous FSUs and consequently a steep increase in post-excitatory inhibition. By the above assumptions, this increased inhibition would be due to increase in both the synaptic density possibly by whisker deprivation and synaptic efficacy due to simultaneous click rearing (Fig. 4-7D). The absence of increased spontaneous firing rate after the CS stimuli in the click-reared animals could be explained by an increase efficacy of such DGZ to S1 horizontal collaterals maintaining a relatively higher level of inhibition in click-reared barrel cortex. Finally, since the responses in the 15-30 ms epoch following the W2 stimulus remains significantly lower than that of W1 only in WBD/CR+ rats, it is possible that the CS stimulus has a longer acting effect on inhibition in this group of animals. It is also possible that the preceding set of auditory click stimuli alone (A2) could influence such a reduced response to W2.

The model described above is based on assumptions that can only be tested with further experiments. Recording from the subcortical structures under our conditions would be a key experiment that might shed light on whether the dual-mechanism hypothesis is tenable. An increase in excitability of neurons should be accompanied by

an improved timing of response, and if the increase in inhibition is due to selective activation of FSUs, then there should also be an increase in neuronal synchrony and oscillations in barrel cortex after auditory stimuli (Cardin et al. 2009). This result would be similar to another study that showed that somatosensory stimulus-initiated oscillations reset the spontaneous oscillations in macaque A1 so that the phase of these reset oscillations produced enhanced auditory responses (Lakatos et al. 2007). Preliminary results suggest the click stimulus is indeed capable of changing the response timing properties of barrel cortex neurons, such as onset latency, spike synchrony and spike coherence (Ghoshal et al. 2010).

Finally, although the model is consistent with our observed results, other mechanisms cannot be ruled out. For example, activity in the auditory pathways could influence the S1 barrel cortex via the nucleus basalis and/or amygdala (Gao and Suga 1998; Ma and Suga 2001). Further, the changes observed with altered rearing, especially with whisker deprivation, could be attributed to reported retention of direct projections from the mMGN to S1 barrel field (Nicolelis et al. 1991), although the latter study involved peripheral follicle damage (follicle cauterization) in contrast to the non-invasive whisker trimming used in these studies.

In conclusion, our results show that there is a significant auditory modulation of responses in the primary somatosensory cortex, and that this modulation is significantly altered by early postnatal rearing conditions, indicating that cross-sensory responsiveness is developmentally regulated by experience. In addition, the fact that such cross-sensory interactions can be detected even under anesthesia raises the possibility that cross-sensory interactions in primary sensory cortex may be even more

robust in awake, behaving rats, and may facilitate the perceptual capability of the animals.

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CHAPTER V

CROSS-SENSORY STIMULI MODULATE THE TEMPORAL PROPERTIES OF BARREL CORTEX RESPONSES AFTER NORMAL OR ALTERED POSTNATAL SENSORY EXPERIENCE

Introduction

Cross-sensory interactions are commonly observed in higher order cortical areas. Such interactions were considered extremely rare or absent in the lower order areas including the primary sensory cortex. However, in the recent past several studies have reported such multisensory interactions in these areas (Foxe and Schroeder 2005; Ghazanfar and Schroeder 2006; Kayser and Logothetis 2007). These “cross-sensory” interactions have been shown in lower order visual (Allman and Meredith 2007; Allman et al. 2008), somatosensory (Zhou and Fuster 2000; 2004) and auditory areas (Bizley et al. 2006; Lakatos et al. 2007). Cross-sensory interactions also have been reported along the borders between cortical areas representing different sensory systems (Brett-Green et al. 2003; Wallace et al. 2004). Previously, our lab has also shown that S1 barrel cortex of rats is significantly influenced by auditory stimuli (Ghoshal et al. 2010). Typically in most of the studies mentioned above the cross-sensory stimuli have been shown to either increase the firing rate of the neurons in sensory cortex (Zhou and Fuster 2000; 2004; Bizley et al. 2006; Wallace et al. 2004) or to modulate the firing rate of individual cortical neurons to sensory inputs (Allman and Meredith 2007; Allman et al. 2008; Lakatos et al. 2007; Ghoshal et al. 2010). For example, we observed that in

normal rats, an auditory click stimulus, which by itself fails to drive the barrel cortex neurons, significantly facilitated the whisker driven response when preceding a whisker stimulus (Ghoshal et al. 2010; Chapter IV). Here we show that in addition the auditory click stimulus can also influence the temporal properties of barrel cortex neurons namely the response onset-latency, coincident discharge of neurons. We also show, that some of these effects on the temporal properties can be observed independent of the firing rate changes.

It has also been shown previously, that altered rearing experience spanning through the developmental critical period can also alter the nature of such cross-sensory interactions (see Bavelier and Neville 2002; Collignon et al. 2009; Kral 2007 for a review). This also holds true for such interactions observed in barrel cortex neurons (Ghoshal et al. 2010). When rats with or without bilateral whisker deprivation were raised in a background of auditory click environment there were significant changes in both the quality and quantity of cross-sensory interactions observed in barrel cortex (Ghoshal et al. 2010). Here, we investigate further whether the auditory influence on the temporal properties of barrel cortex is also developmentally regulated by carefully studying the differences between different rearing groups. Our results show that with bilateral whisker deprivation or click rearing or both, there is a significant modulation in the amount of cross-sensory influence on the temporal properties of neurons observed in urethane anesthetized barrel cortex.

Methods

Animal Groups/ Bilateral whisker deprivation/ Auditory click rearing/ Surgery and Recording/ Whisker and Auditory Stimulation during recording/ Data analysis: Similar methods were applied as mentioned in chapter IV for rearing the 4 groups of animals (Fig. 5-1A) and a similar stimulus sequence (Fig. 5-1B) and stimulus delivery was used in this study as well. The surgery, recording under urethane anesthesia was also performed similarly as mentioned in the previous chapter.

First Spike Analysis: The first spike occurring between 3-100 ms after each trial of whisker stimulus during the W1, CS and W2 stimulus condition were extracted using a custom script in Neuroexplorer. First spike rasters were computed using the Perivent Raster script in NEX. Latency histograms were created with the first spikes using NEX and MATLAB. The average time for the first spikes to occur for the 100 trials of W1, CS and W2 were calculated and compared among the stimulus conditions in each rearing group.

JPSTH analysis: JPSTHs were computed and used similarly as described in chapter II to compute spike-spike synchrony between a pair of neurons under the different stimulus conditions. The peak correlation coefficients from the JPSTHs were compared between each stimulus conditions.

Statistical Analyses: All measures were compared using a matched sample paired t-test in this study. Bonferroni corrections were employed wherever applicable to correct for multiple comparisons.

Results

Effect of cross-sensory stimuli on onset latency of whisker-driven responses of barrel cortex neurons:

We recorded from the barrel cortex of each group of animals and computed the first spike latency to W1, CS and W2 stimulus conditions. Since there were no detectable increase in firing rate to the A1 and A2 stimulations, these stimulus conditions were not considered for onset latency analysis. Typical single neuron first-spike raster plots for the different stimulus conditions and different rearing groups are displayed in Fig. 5-1C. The raster plots shows that there is a general sharpening of the first spikes across trials in the CS conditions as compared to the W1 stimulus in all rearing groups. This general trend in single cells transpired even when the population measures were compared using a one-tailed t test (p values were corrected for multiple comparisons with the critical p value being 0.167) as seen in Fig. 5-2.

In the normal rats (CON/CR-) the mean onset latency in the CS conditions was significantly lower than W1 ($p< 0.001$). However, there was no such difference between the whisker only conditions (W1 vs W2 $p>0.05$; one-tailed t-test) (Fig. 5-2A). This effect of fastening onset latency was observed when the barrel cortex neurons from CON/CR-rats were further subdivided according to their laminar positions into L-II/III cells ($p<0.0001$) and L-IV cells ($p<0.01$) or according to their waveform type into RSUs ($p<0.0001$) and FSUs ($p<0.0167$).

The animal group that was solely click reared but had intact whiskers (CON/CR+) served as a logical control for click rearing. These group of animals also showed similar

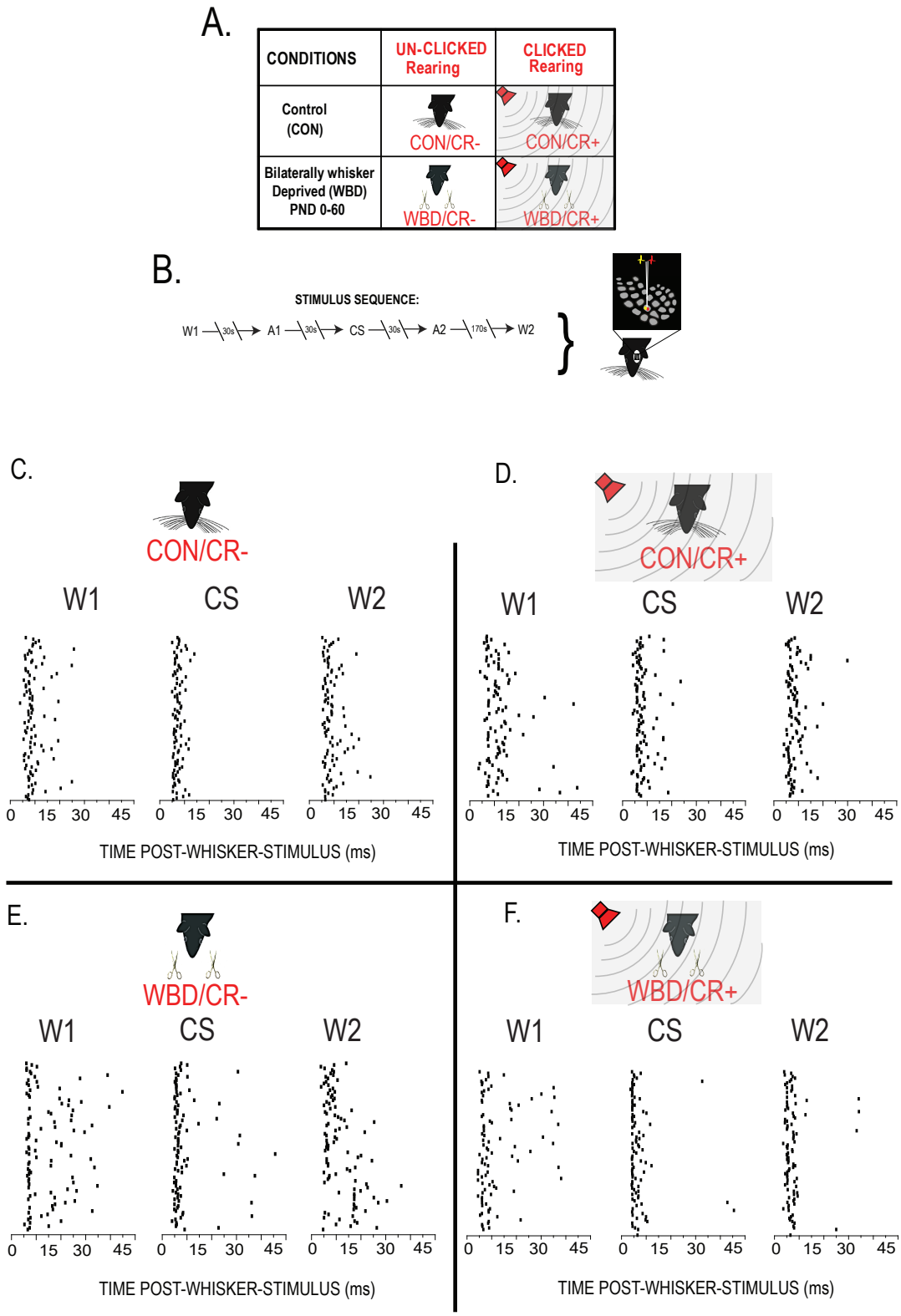

Figure 5-1. Experimental Design and first spike raster plots from representative neurons
A. Experimental design is similar to the one used in chapter IV, with 4 rearing groups consisting of normal (CON) and bilaterally whisker deprived (BD) rats, some of whom were reared with background auditory clicks (CLICKED rearing) from PND 0. B. Neurons were recorded from barrel cortex of each of the 4 rearing groups shown in A in response to the stimulus sequence displayed consisting of principal whisker stimulus alone (W1 and W2), auditory click stimulus alone (A1 and A2) and a paired cross-sensory stimulus with an auditory click preceding the whisker stimulus by 10 ms (CS). C-F. First spike raster plots of representative neurons from each rearing group under the W1, CS and W2 stimulus condition. A sharpening of the first spikes with the CS stimulus is observed for each rearing group.

effects on onset latency with a significant decrease in its means with the CS stimulus ($p<0.001$) but not with W2 stimulus when compared to W1 ($p>0.05$) stimulus condition (Fig. 5-2B). The L-II/III ($p<0.0001$), L-IV ($p<0.0001$), RS ($p<0.0001$) and FS ($p<0.01$) neurons, all show such a significant decrease in onset latency with the CS stimulus when compared to the W1 stimulus. The bilaterally whisker deprived group (WBD/CR-) which serves as a control for whisker trimming, also showed faster onset latencies with the CS stimulus when compared to the W1 stimulus ($p<0.0001$) with the latencies for W2 not being different from that of W1 ($p>0.05$; Fig. 5-2C). This effect of decreased mean latency with the CS stimulus, held true for L-II/III ($p<0.0001$) and L-IV ($p<0.001$) cells as well as for both the RSUs ($p<0.001$) and FSUs ($p<0.01$).

The effect of fastening the onset latency was also observed in the S1 neuronal population of the experimental WBD/CR+ rats. There was a significant decrease in the mean onset latency in the CS stimulus when compared to the W1 stimulus conditions ($p<0.0001$), whereas, the W1 and W2 onset latencies were not significantly different ($p>0.05$; Fig. 5-2D). The onset latency remained significantly lower in the CS stimulus conditions when compared to the W1 for both L-II/III ($p<0.0001$) and L-IV ($p<0.001$) neurons as well as RSUs ($p<0.001$) in this group of animals. However, the FSU's failed to show any significant differences in first spike latency in the CS condition, although there was a strong statistical trend ($p=0.03$) for a decrease in mean onset latency with the CS stimulus when compared to the W1 stimulus.

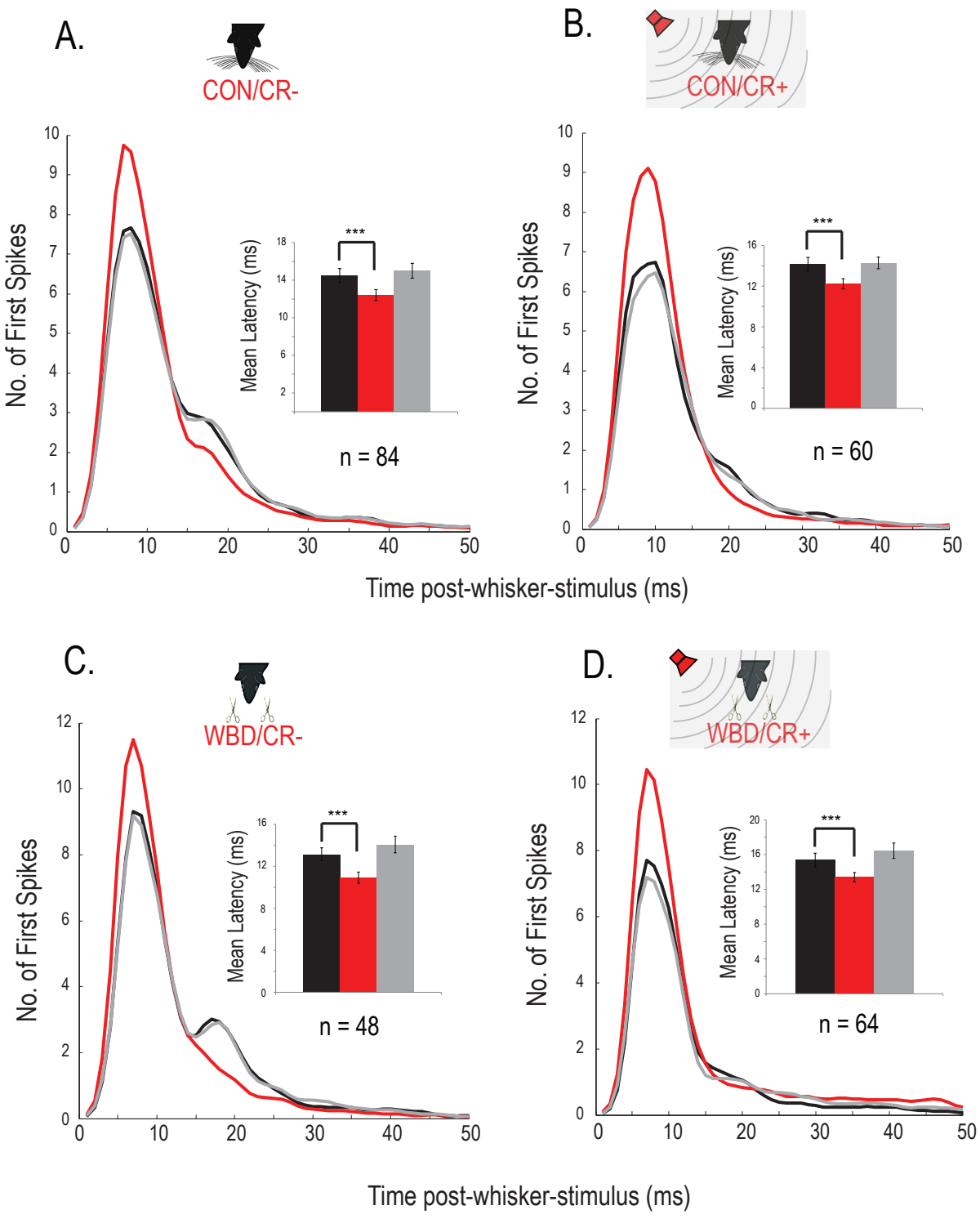

Figure 5-2. Population latency histograms and mean onset latency for each rearing group
A-D. Latency histograms for each rearing group for W1 (black trace), CS (red trace) and W2 (gray trace) stimulus conditions showing an increase in the number of first spikes during the CS stimulus at a shorter post-stimulus-times in each rearing group. Mean latency bar graphs are shown in inset of each latency histogram. The color code for the stimulus conditions is similar to that of the latency histograms. The bar-graphs show that there is a significant reduction in mean onset-latency with the CS stimulus evident in all rearing groups.
Error bars in the bar-graph represent the standard error of means or S.E.M. *** denote $p < 0.0001$.

Effect of cross-sensory stimuli on coincident discharge of barrel cortex neurons:

Coincident spike discharges for pairs of single neurons were calculated using JPSTH analysis under all stimulus conditions and were compared among the conditions for each rearing group of animals. Fig. 5-3 displays representative example JPSTHs computed for single neuronal pairs for each stimulus condition and rearing group. In general, cross-sensory stimuli alone (A1 and/or A2) which failed to drive barrel cortex neurons (as evident from the PSTHs) were able to significantly increase coincident discharges among the same pair of neurons in all animal groups. Population peak correlation coefficients (PCC) for pairs of neurons in W1stimulus condition were compared with that in A1, CS, A2 and W2 respectively using a paired one-tailed t-test. The p values were adjusted for multiple comparisons using Bonferroni's correction with the critical p value being 0.0125. The cumulative distribution and mean bar-graphs for each rearing condition are displayed in Fig. 5-4.

In CON/CR- rats (n = 97 for all stimulus conditions) there were significant increase in the mean PCC for the A1 (18% increase, $p<0.001$) and A2 (31% increase, $p<0.0001$) stimulus conditions respectively. However, there was no significant increase in the mean PCC value in the CS and W2 ($p>0.05$) stimulus condition when compared to W1 (Fig. 5-4A). When the neuron pairs were further subdivided into L-II/III (n = 42) and L-IV (n = 55) groups according to their laminar location the significant increase with A1 ($p<0.01$ for L-II/III and $p<0.0001$ for L-IV) and A2 ($p<0.0001$ for both L-II/III and L-IV) were observed in both groups. There were no significant differences in mean PCC between W1 and CS ($p>0.05$) or W2 ($p>0.05$) for L-II/III neurons. However, L-IV neurons in CON/CR- rats showed a strong trend although non-significant increase in

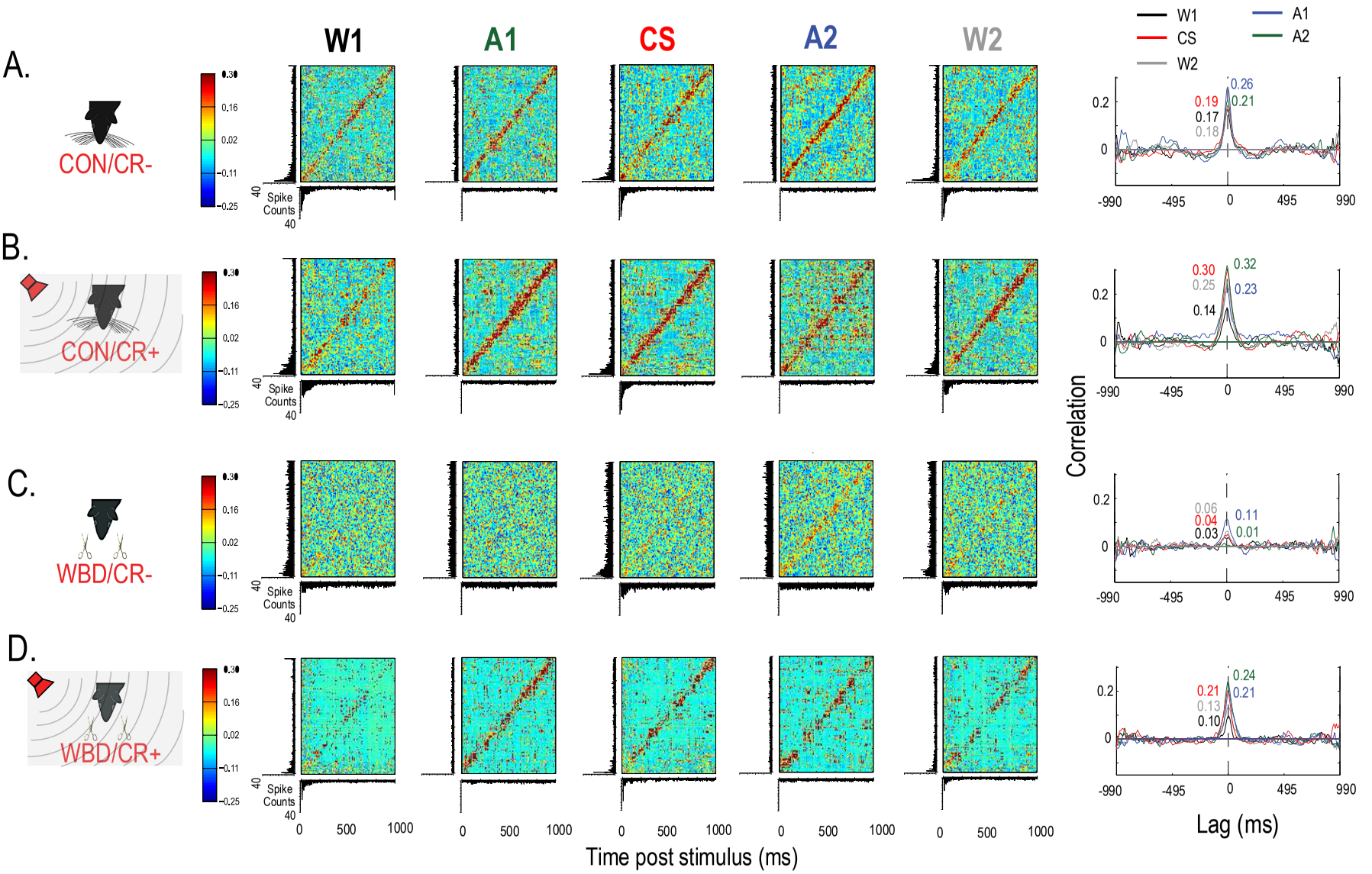

Figure 5-3. Example JPSTHs and cross-correlograms for a pair of representative neurons from each rearing group

A-D. Typical JPSTHs and the corresponding cross-correlograms for a representative neuron pair in each rearing group are shown for the 5 different stimulus conditions, namely, W1 (black trace), A1 (deep green trace), CS (red trace), A2 (blue trace) and W2 (gray trace). The peak correlation coefficients (PCC) for the neuron pair is noted in cross-correlogram with a similar color code. In all rearing groups there is an evident increase in spike synchrony with one or both the auditory alone stimulus (A1, A2) even without a detectable changes in the firing rate as shown in the PSTHs. There is also similar observable increase with the CS stimulus in the click reared groups namely the CON/CR+ (B) and WBD/CR+ (D) groups. The spike synchrony remains elevated for the W2 stimulus condition in these groups.

mean PCC with the CS stimulus ($p=0.02$) and significant increase in PCC with W2 stimulus ($p<0.01$) when compared to the W1 stimulus.

In the click reared control (CON/CR+) rats not all neurons were recorded with the stimulus condition A1 and A2 and therefore there are unequal observations for W1/CS/W2 ($n = 48$) and A1/A2 ($n = 24$). Significant increases in mean PCC were observed for CS and W2 stimulus conditions when compared to that of W1 ($p<0.001$ for both). When the sample for A1 and A2 were compared with matched sample of W1, they also showed significant increases ($p<0.001$ for both). The increase with CS ($p<0.01$) and W2 ($p<0.01$) was also observed when only 24 pairs of neurons that were recorded with A1 and A2 were considered for analysis (Fig. 5-4B). When divided further into L-II/III and L-IV most of these relations remained true except there was a lack of significant increase with A2 ($p>0.05$) in L-II/III. However, since only 12 pairs were involved in the analysis, the lack of significance could be due to a loss of power for a small sample size. In WBD/CR- rats ($n = 45$ for all stimulus conditions) the population mean PCC were significantly higher only in the A2 condition (39%, $p<0.001$) but not in the A1 ($p>0.05$), CS or W2 condition ($p>0.0167$ for both CS and W2) (Fig. 5-4C). However, when the neurons were divided into layers, the L-II/III neurons ($n = 18$) showed a significant increase in coincident discharge with the CS ($p<0.01$) and A2 ($p<0.0001$) and a strong trend albeit non-significant increase with A1 ($p=0.016$). While L-IV neurons ($n = 27$) didn't show any significant increase either with A1, A2 or W2 ($p>0.02$ for A1, A2, W2) but strangely showed a significant decrease with the CS condition ($p<0.01$).

In the extreme experimental condition, rats with simultaneous whisker deprivation and click rearing (WBD/CR+; n = 45 for all stimulus conditions) also showed more pronounced auditory influences on barrel cortex coincident discharges but in the similar direction as that of normal rats. Thus, there was a significant increase in the population mean PCC with A1 (44%; $p<0.0001$), CS (38%; $p<0.0001$), A2 (67%; $p<0.0001$) and W2 (34%; $p<0.001$) when compared to that of the W1 stimulus condition (Fig. 5-4D). This significant increase in mean PCC with all stimulus conditions was also true for L-II/III cells (n = 24; $p<0.0001$ for W1 vs A1, A2 and W2; $p<0.01$ for W1 vs CS). On the other hand, L-IV cells (n= 21) showed increase in mean PCC with A1 ($p<0.0001$), CS ($p<0.01$), A2 ($p<0.001$) but not with the W2 ($p>0.05$) stimulus condition.

Effect of CS stimulus on response and relative spontaneous period coincidence:

The data was further analyzed for changes specifically during the response period and relatively spontaneous periods after the CS stimulation. Neuronal coincidence in each rearing group was measured in 0-50 ms (representing the response period) and 950-1000 ms (representing the relatively spontaneous periods) post-whisker stimulus epoch during the W1 and CS stimulus condition and were compared using paired t-test. There was a significant reduction in coincidence during the response period when compared to the relative spontaneous period for both W1 and CS conditions in all rearing groups ($p<0.001$ for all). In normal rats, with the CS stimulus, there was no difference in coincidence in the response period ($p>0.05$) but there was a significant increase in coincidence in the relatively spontaneous period ($p<0.001$) when compared to those in W1 stimulus condition (Fig. 5-5A). For the CON/CR+ (Fig. 5-5B)

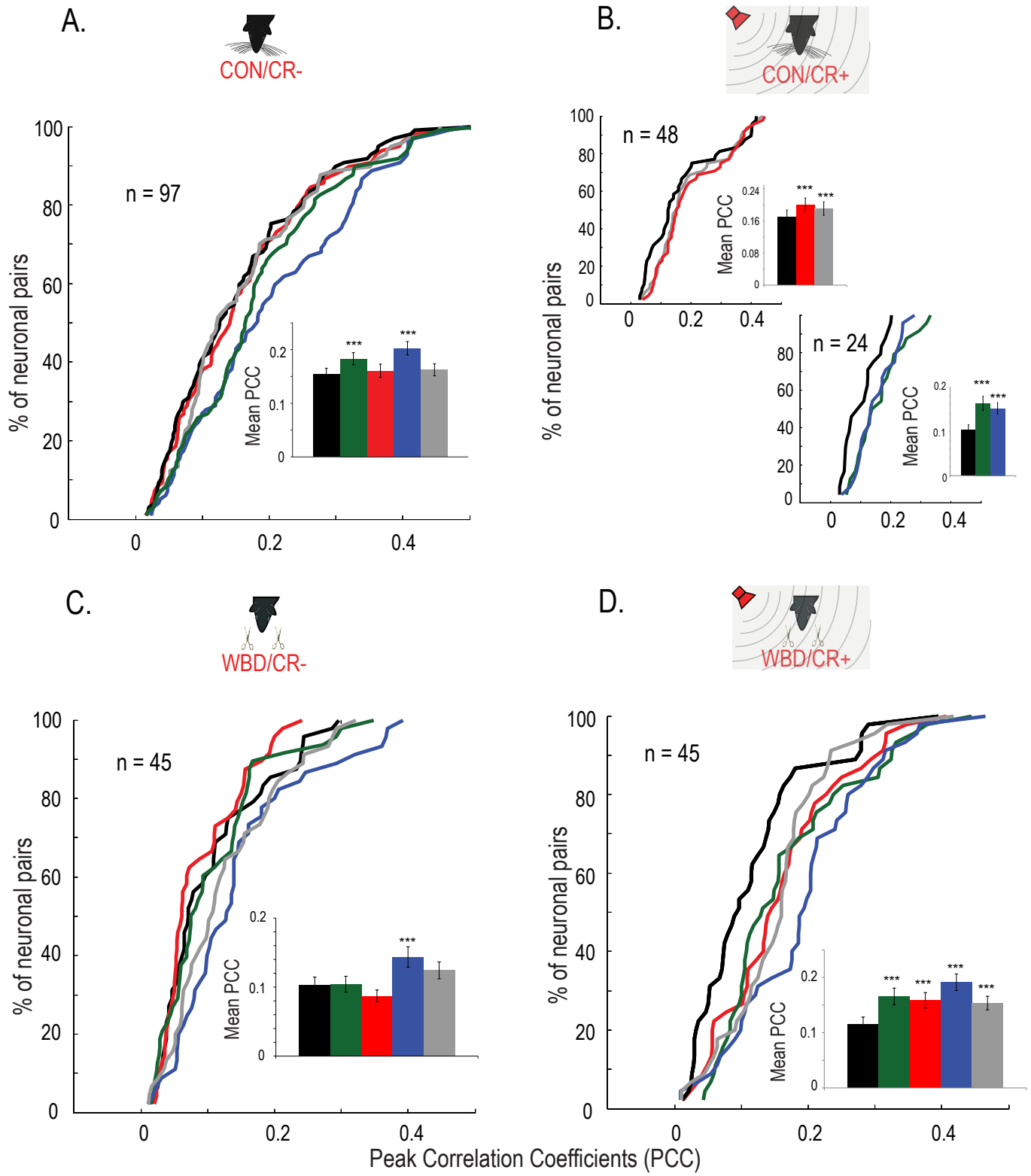

Figure 5-4. Population cumulative distribution and means of peak correlation coefficients in each rearing group

A-D. Cumulative distribution for PCCs displayed with a similar color code as in Fig. 5-3 for the 5 stimulus conditions in each rearing group. The mean bar-graphs are shown as an inset in each distribution, color coded with the same rules. In normal animals there is a significant increase in A1 and A2 PCCs (A) when compared to W1, whereas, in the whisker deprived only group (C) the increase is significant only with A2. On the other hand, all stimulus conditions led to significant increases in PCCs from W1 in the click reared groups (B and D). The CON/CR+ (B) rats have two distributions displayed as not all neurons were recorded with A1 and A2 stimulus conditions in this group. Thus the top graph has the PCC distribution and means bar-graph for 48 neuron pairs under W1, CS and W2 conditions, while the bottom graph has the same for 24 neuron pairs under A1 and A2 stimulus conditions. Error bars represent standard error of mean or S.E.M. *** denote $p < 0.0001$ when comparison were done with W1.

and WBD/CR+ (Fig. 5-5D) rats, the increased coincidence with CS was significant in both the response period ($p<0.001$ for both groups) and the relative spontaneous period ($p<0.001$ for both groups) when compared to W1. For the whisker-deprived only group (WBD/CR-) there was a significant reduction in CS coincidence during the response period ($p<0.001$) but no significant changes were observed during the relatively spontaneous periods ($p>0.05$) as compared to W1 (Fig. 5-5C).

Discussion

The presence of an auditory influence on barrel cortex was described previously (Ghoshal et al. 2010; Chapter IV) where it was shown that auditory clicks presented in conjunction with whisker stimuli significantly modulated the magnitude of whisker-driven responses in normal as well as whisker deprived and click reared animals. Here we show that the auditory clicks can also alter the temporal properties of the barrel cortex neurons following normal sensory rearing conditions as well as after developmentally manipulated rearing experiences. First, the results show that in all rearing groups, the cross-sensory stimulus (CS), produced responses with a significantly shorter onset-latency when compared to a whisker stimulus alone (W1). Such a reduction in onset latency was observed even when the neurons were classified according to their laminar location (L-II/III or L-IV) and waveform type (RSU or FSU). The single exception was for FSUs under WBD/CR+ conditions which produced a strong trend toward a reduction in onset latency, but failed to reach statistical significance. Secondly, there was a significant increase in coincident discharge in the presence of the click stimulus. The click stimuli alone (either A1or A2 or both) failed to initiate spiking responses in any of

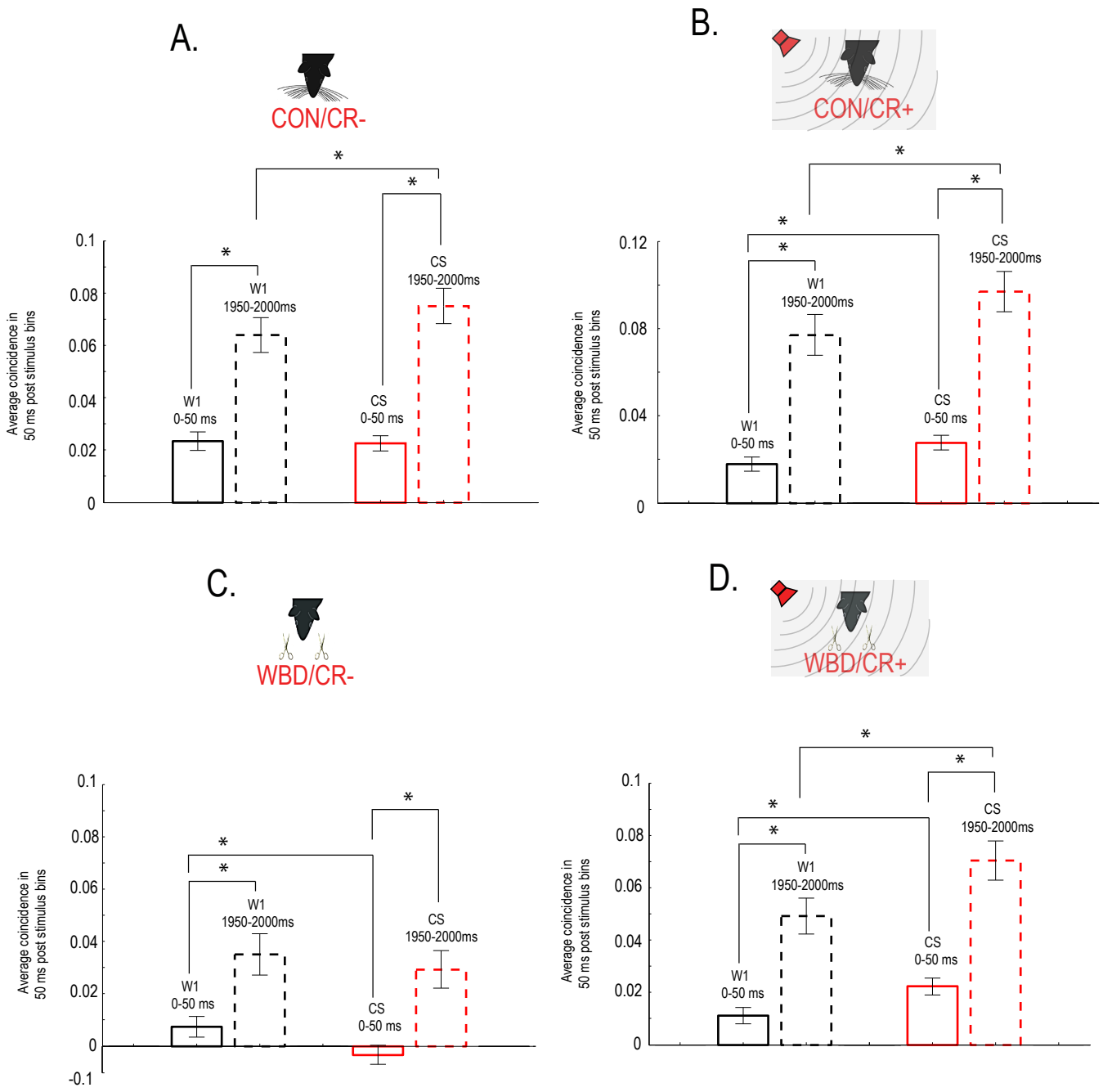

Figure 5-5. Comparison of spike synchrony between response periods and relatively spontaneous periods under W1 and CS stimulus conditions

A-D. Comparison of the average coincidence of spikes in neuronal pairs in the 'first' 50 ms (0-50 ms post-whisker-stimulus, solid bars; response period) and the 'last' 50 ms (950-1000 ms post-whisker-stimulus, dashed bars; relative spontaneous period) after W1 (black bars) and CS (red bars) stimulus in all rearing groups. There is a reduction in correlation strength in the response period when compared with that of relative spontaneous periods in both the W1 and CS stimulus condition. With the CS stimulus there is an increase in relative spontaneous period spike synchrony in all rearing groups except in WBD/CR- rats (C). An increase in spike synchrony during the response period is significant only in the click reared animals (B and D) but not in the normal animals (A). Interestingly, there is a significant decrease in response period synchrony in the WBD/CR- rats (C).

Error bars represent standard error of mean or S.E.M. * denotes $p < 0.001$.

the barrel cortical neurons, but significantly increased coincident discharge in all rearing groups. This surprising separation of driving and modulatory effects indicated that in the primary somatic sensory cortex, neuronal synchrony can be modulated independent of the firing rate of a neuron, at least during such cross-sensory interactions. The paired CS stimuli, in turn, only increased the coincident discharge in the click reared animals (WBD/CR+ and CON/CR+ rats) both in L-II/III and IV. The WBD/CR- animals had increased correlated discharge only in L-II/III and only during the response period, while the normal animals (CON/CR-) only showed a trend toward increased coincidence in L-IV and a significant increase during the relatively spontaneous period with the CS stimulus. Thus, the effect of CS stimuli on SI neuronal coincidence was minimal in non-click reared animals supporting the conclusion that click rearing increased the level of spontaneous and whisker-driven coincident firing in SI cortex.

When the cross-sensory auditory clicks were coupled to the driving whisker stimulus with a short inter-stimulus interval, they produced a decrease in onset latency in addition to the increased magnitude of whisker-driven responses. This faster response onset is seen even in normal rats with intact whiskers. Several mechanisms could produce such a reduction in onset-latency by a preceding auditory click. First, the faster onset response could be a direct consequence of increased excitability of the barrel cortex neurons. It is possible that click stimuli increase the sensitivity of response to an incoming whisker stimulus and thereby cause faster onset in addition to the facilitated magnitude in the short latency response epochs. In a previous hypothetical model we predicted that the facilitation of whisker-driven responses by the cross-sensory auditory stimulus in barrel cortex could be due to a cross-talk between the

somatosensory and auditory structures at a subcortical level via the brainstem reticular formation (Ghoshal et al 2010; Chapter IV). Such a mechanism could also be responsible for the effect observed on onset-latency. Thus click stimuli could increase the excitability of Trigeminal Nucleus (TN) neurons or Ventral Postero Medial thalamic nucleus (VPM) neurons through connections from the Dorsal Cochlear Nucleus or the Inferior Colliculus via the reticular formation (Bowsher 1975; Kudo et al. 1983; Ter Horst et al. 1991; Cant and Benson 2003). This in turn, can lead to sub-threshold increases in sensitivity of barrel cortical neurons as well leading to consistently faster onset in response. Also, the fact that such reduction is consistent across the different rearing groups indicates that, this property of cross-sensory interactions has little if any regulation during development. Another possible mechanism that could influence onset latency is corticocortical interactions either directly between the S1 and A1 or indirectly via the multisensory Dysgranular zone between the A1 and S1. Also an auditory influence could reach S1 via the cholinergic nucleus basalis and/or the amygdala (Gao and Suga 1998; Ma and Suga 2001).

It has been calculated that in the rat whisker system the majority of the information (~90%) is contained in the first spike in cortex following a stimulus (Petersen, et al., 2002). The fact that click stimuli improve the precision of first spike onset in normal cortex suggests that cross-sensory auditory stimuli may facilitate perceptual sensitivity and perhaps acuity in the whisker system. Moreover, bilateral deprivation alone increases the variability of response onset (Popescu and Ebner 2010), a deficit which was suggested to be one of the key deficits leading to the loss of behavioral acuity on sensory discrimination tasks in sensory deprived rats (Carvell and

Simons 1996). The results in this chapter show that the auditory clicks produce a faster and more time-locked response onset even in the deprived animals with or without click rearing. Thus, if faster response onsets improve information processing and generate a more precise temporal code structure, then the production of an auditory signal during or shortly before a whisker contacts an object could improve the behavioral acuity of somatic sensory deprived animals.

In addition to affecting the whisker-driven response onset and magnitude, the cross-sensory stimulus also selectively increases the amount of correlated discharge among barrel cortex neurons. This increase is observed in normal animals, indicating that such interactions occur in primary sensory cortex even without any developmental manipulations. It is still an open question whether increased spike synchrony among neurons in an assembly has a positive or negative effect on sensory information processing. Negative effects would arise from widespread synchronous discharge independent of a sensory stimulus, for example during sleep states or seizures when processing is minimal (Neckelmann et al. 1998). Positive effects have been postulated in the auditory system where a cluster of coincident spike firing neurons has been identified as a coding mechanism in addition to instantaneous firing rates, integrated firing rates, and the mean interspike interval (Eggermont 2001, 2006).

Our results are consistent with the idea that cross-sensory stimuli serve to reset the phase of spontaneous oscillations in primary sensory cortex (Lakatos et al. 2007). Previous studies have also shown that activation of inhibitory interneurons can induce a synchronous state in cortical neurons (Cardin et al. 2009). Thus, as previously postulated (Ghoshal et al. 2010), auditory corticocortical activation of inhibitory

interneurons in barrel cortex could serve as a candidate mechanism for the identified influence on synchrony. However, influences from subcortical structures cannot be ruled out as a plausible mechanism.

Interestingly, the modulatory effect on synchrony had several differences across the rearing groups indicating that such effects could be influenced during postnatal development. The differences across rearing groups could be summarized by the statement that the effect of neuronal synchrony was much more robust in click reared than the non-click reared rats. Even though the auditory stimulus alone produced an increased correlated discharge among all rearing groups, the percent increase was notably higher in the click reared animals, with maximal increase observed in the WBD/CR+ group. Also, during the CS stimulus condition, only the click reared animals showed an overall increase in neuronal synchrony, unlike the non-click reared animals. Strengthening corticocortical interactions between A1 and S1 by click rearing could underlie the robust effect on synchrony in these animals. Alternatively, click rearing might entrain the barrel cortex to a particular frequency within the broadband frequency spectrum and the effect on synchrony with such clicks could be a direct manifestation of such stimulus entrainment.

Another interesting aspect on the effect of synchrony was the differences observed within a group across the different stimulus conditions. For example, in all rearing groups the response magnitude of A2 was consistently greater than the response to A1, except in CON/CR+ rats. Since A2 was delivered after the CS condition, it is possible that the paired stimulus had a sustained effect on cortex. Secondly, although the normal rats failed to show any overall increase in spike

synchrony to the CS stimulus, an increase in correlated discharge was observed during the relatively spontaneous periods, but not during the response period. This is probably due to the fact that whisker stimulus reduces the correlated responses of barrel cortex neurons (Ghoshal et al. 2009; Chapter II). In the other rearing groups, except the WBD/CR- rats, we did observe an overall increase in spike synchrony with the CS stimulus even in the presence of de-correlation by the whisker stimulus and the increase was evident in both the response and spontaneous periods.

Finally, the fact that increased synchronous discharge occurs without a detectable effect on firing rate due to the auditory stimulus alone predicts the likelihood of a temporal code which can operate independently of the firing rate code to synthesize the final neural code of a sensory event. Clear evidence has been provided for a rate code being the central mechanism when the behavioral task is a roughness discrimination in rats (Arabzadeh et al. 2006; von Heimendahl et al. 2007) or distinguishing between flutter stimuli in a primate (Romo and Salinas 2003). However, our results indicate that under some conditions, such as during cross-sensory interactions, the temporal code may operate independently as an overlay of the firing rate code. Modulating the temporal code, even as early as primary sensory cortex, while leaving the rate code untouched could provide a novel mechanism through which cross-sensory stimuli can condition the network rendering it more efficient and effective in processing incoming stimuli.

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CHAPTER VI

SUMMARY AND FUTURE DIRECTIONS

The experiments and findings that are described in the preceding chapters lead to a deeper view of how functional reorganization occurs in barrel cortex after different developmental challenges. First, it can be concluded that deprivation of whisker sensation can affect ensemble neuronal properties such as cortical synchrony, in addition to affecting single neuron properties of barrel cortex as described by Popescu and Ebner (2010). Moreover, the studies provide strong evidence showing that simple trimming of whiskers on one or both sides of the face throughout postnatal development has a huge effect on the way in which neuronal assemblies in cortex develop their typical or atypical mature characteristics. Secondly, the studies described also demonstrate the presence of significant multisensory interactions in primary sensory cortex in normal rats. These multisensory interactions, referred to as ‘cross-sensory’ in this work, were also shown to be developmentally regulated. Finally, the studies involving sensory deprivation and cross-sensory interactions reveal the prime importance of cortical synchrony in sensory processing and unravel the key characteristics of neuronal correlated discharge and how it can be manipulated developmentally.

Functional reorganization following bilateral and unilateral whisker deprivation:

Electrophysiological recordings from the bilaterally and unilaterally whisker deprived barrel cortex pointed out critical abnormalities in neuronal correlated discharge. With bilateral deprivation there was a global reduction of correlated discharge (spike synchrony) in the L-IV barrel cortex but not in L-II/III whereas, with unilateral deprivation, there was a dramatic increase in all areas of barrel cortex except L-IV barrels. These results show that in addition to the single cell abnormalities which were described seminally by Wiesel and Hubel in kitten visual cortex (1963) and Popescu and Ebner in UB and BD barrel cortex (2010), sensory deprivation spanning across the developmental critical period can also alter network properties like cortical synchrony in barrel cortex of rats. Moreover, the changes observed in the sensory deprived cortex depended critically on the methods adopted for the non-traumatic reduction in peripheral sensory activity. On one hand, after bilateral whisker trimming, there was a general depression of the network properties of the overall cortex, unilateral whisker trimming shifted the transmission of sensory processing from barrel-to-above-barrel to septum-to-above-septum. The UD results supported many studies showing that in animals with their whiskers trimmed on one side of the face, the first feed forward intracortical circuit between layer IV and superficial layers is the major target of functional loss (Rema et al. 2003; Allen et al. 2003; Shepherd et al. 2003). An important implication of this observation is that one should be careful about the methods adopted for drawing conclusions about the effects of sensory deprivation: the devil is in the details. Thus, at least in rats, neurophysiological assays conducted in unilaterally

whisker-trimmed animals cannot be used to explain behavioral and perceptual deficits observed in rats lacking all, or all but one, whiskers or any other of dozens of combinations designed to produce critical period peripheral deprivation. This caution applies to any sensory system, as pointed out originally by Wiesel and Hubel in the visual system nearly fifty years ago, and ignored at their risk by the whisker deprivation community.

Another important conclusion from these results is that barrels and septa have distinctly different functional roles in sensory processing. The traditional view is that L-IV barrels are purely sensory receiving connections from the lemniscal input, whereas, the septum has much more heterogeneous anatomical connections and is involved in sensorimotor processing. These results show that even in normal animals there could be different subgroups of L-IV septal cells with different degrees of correlated discharge and presumably connections, whereas the L-IV barrels have a much more uniform distribution of correlated neuron pairs and the homogeneous thalamic inputs. Moreover, the results also point out that the barrel and the septal columns were consistently differently affected with the different developmental manipulations, also indicating the differences in how they acquire their network properties and highlight their different functional roles in sensory processing.

There are likely to be several different molecular mechanisms responsible for the developmental regulation of cortical spike synchrony. It has been shown before that cortical spike synchrony measured as 'noise correlations' have a specific developmental pattern. Noise correlations were shown to have a developmental peak around 9-15 PND after which is reduced to normal adult levels (Ikemoze et al. 2008). It is possible that

such a peak during development is necessary in shaping and characterizing spike synchrony in adult mature cortex. The fact that gap junction proteins like connexin-36 and zinc have a similar developmental peak around PND 9-15 (Liu and Jones 2003; Land and Shamalla-Hannah 2002) makes them potent candidates which play an important role in developing neuronal synchrony during cortical maturation.

Unfortunately, whether the cortical synchrony and single unit deficits observed following bilateral deprivation may underlie the loss of behavioral acuity observed in behavioral studies (Carvell and Simons 1996) remains an open question. Clear evidence has been provided for a rate code being the central mechanism when the behavioral task is roughness discrimination in rats (Arabzadeh et al. 2006; von Heimendahl et al. 2007). Since it has also been shown that neural code depends on correlation between the responses of individual neurons (Eggermont 2007) and correlated activity in a neural assembly can improve the accuracy of neural code (Abbott and Dayan 1999; Rolls et al. 2003), spike synchrony becomes an important candidate for the basis of behavioral loss of acuity following deprivation. An interesting follow-up study, therefore, will be to investigate whether firing rate and cortical synchrony contribute separately to degrade behavioral performance. Alternatively, cortical synchrony may facilitate the formation of a rate code to develop a final neural code. Behavioral experiments after manipulating cortical synchrony alone, keeping the firing rate intact, with the help of drugs such as cannabinoids (Robbe et al. 2006) might help to answer that question and can also unravel whether reversing the deficits in cortical synchrony alone might help improve behavioral performance. The latter then

might explain the increase in septal synchrony in UD as a compensatory mechanism, since behavioral deficits with UD are rarely reported.

Multisensory interactions in primary sensory cortex and its developmental regulation:

In contrast to the traditional belief that primary sensory cortex is purely unisensory, experiments described in previous chapters illustrate some compelling evidence for the presence of multisensory interactions in normal S1 barrel cortex of rats. Such 'cross-sensory' interactions can be defined as influences by a sensory stimulus other than the primary/dominant sensory stimulus in primary sensory cortex. In short, an auditory click stimulus significantly modulated the firing rate as well as the onset-latency of whisker driven responses of barrel cortex neurons. Moreover, even though the click stimulus alone failed to drive the barrel cortex neurons, they could significantly modulate cortical spike synchrony among a pair of neurons in S1. One unanswered question concerns the optimal inter-stimulus interval to achieve the maximum cross-sensory response enhancement. Ten ms was chosen for these studies, but no systematic study of auditory before, simultaneous, or after somatic sensory stimuli were carried out since the highest priority was to communicate the basic finding of cross-sensory influences in barrel cortex. Under our conditions, several characteristics of such cross-sensory interactions were found to be developmentally regulated and the barrel cortex neurons of animals that were bilaterally whisker deprived and simultaneously click reared from birth showed maximum propensity to respond to auditory inputs.

Cross-sensory interactions are more common in higher order integrative sensory areas such as the anterior ectosylvian and suprasylvian sulcus in cats (Wallace et al. 1992; Jiang et al. 2001). However, there is a growing body of literature that describes such interactions even in lower order sensory areas including primary sensory cortex. Our results provide extensive support for the presence of early cross-sensory influences by showing auditory modulatory effects on primary somatic sensory cortex responses to whisker stimulation. This indicates that multisensory interactions can occur even in areas that are considered until now as purely unisensory. One reason, that such interactions have not been identified earlier could be due to the modulatory nature of the influence. Instead of being a ‘driving’ type of influence, multisensory influences in primary sensory cortex as reported here and earlier (Lakatos et al. 2007) modulate the response to the 'natural' predominant inputs. Therefore, influences from other non-dominant sensory stimuli are much more subtle and possibly sub-threshold in nature. This conclusion is supported by the observation that auditory stimuli, that influenced the magnitude as well as the temporal properties of the barrel cortex neurons, failed to directly drive any of the neurons, indicating that auditory stimuli might have influenced the neurons by inducing sub-threshold post synaptic potentials and thereby modulating the response properties within an assembly of neurons. Unpublished intracellular studies from Michael Brecht’s lab indicate that auditory stimuli can indeed activate post synaptic potentials under anesthesia in barrel cortex, thus supporting our hypothesis, but the latency to PSP onset is over 100 ms, so it is difficult to compare these preliminary results with our results without further study.

The cross-sensory interactions influenced several characteristics of the responses to whisker stimulation. First, the interactions could be broadly divided into auditory influences on the magnitude and the temporal properties of individual neuronal responses as described in chapter IV and V. The influence on the magnitude of whisker-driven responses consisted of a facilitation of response in the 0-15 ms post-whisker-stimulus epoch. This facilitation was consistent across all rearing groups, although the percentage of neurons showing cross-sensory effects was different with different rearing experience. Thus, the cortex in whisker-deprived and simultaneously click-reared (WBD/CR+) animals developed the largest number of neurons whose responses were facilitated by the presence of an auditory click. In contrast, the 15-30 ms post-whisker stimulus epoch contained neurons that were modulated differently in the four different rearing groups, with a reduced facilitation in normal animals, and a strong suppression of response in the WBD/CR+ animals. Moreover, the lack of facilitation in the only-deprived and only-click-reared animals indicated that bilateral deprivation or click rearing alone can modulate the responses in this epoch in a less dramatic fashion. Interestingly, the click reared alone group had a strong trend of suppression of response in the 15-30 ms epoch in the low responsive neurons which suggests that click rearing might be a vital factor for the suppressions observed in the WBD/CR+ rats. Also, the fact that response modulations in the 0-15 ms epoch were much less affected by developmental manipulations unlike the 15-30 ms epoch strongly supports the hypothesis that there are multiple mechanisms underlying the overall auditory influence in barrel cortex: one mechanism affecting the thalamocortical inputs and a second mechanism affecting the corticocortical connections within barrel cortex.

Since it is known that whisker driven 0-15 ms response epochs are primarily dominated by activity in the thalamocortical inputs (Armstrong-James and Fox 1987), it is possible that such effects are mediated by an auditory influence that begins in the somatosensory VPM thalamus or even the brainstem trigeminal nuclei. Although our data does not provide direct evidence that separates these two hypotheses, extracellular recordings from the subcortical structures under similar experimental conditions could shed light on these alternatives. Also, the fact that short latency responses are primarily mediated by AMPA receptors (Armstrong-James et al. 1993), makes them a suitable molecular target that could be involved in the facilitative responses observed in the presence of the click stimulus. In contrast, the 15-30 ms response epoch after a whisker stimulus is primarily dependent on corticortical interactions (Armstrong-James and Fox 1987). We postulate that the cross-sensory interactions seen in this epoch could be of cortical origin. Furthermore, since with different sensory experience the interactions in this epoch were distinctly different, it is possible that the malleable nature of the cortical connections during the critical period of development (Keller and Carlson 1999) makes them more responsive to the 'abnormal' critical period sensory experiences. Also, corticocortical connections in barrel cortex (Tucker and Katz 2003; Pluto et al. 2005) or in other somatosensory cortex like in cat SIV (Keniston et al. 2010) often involve inhibitory interneurons. Thus, the reduced facilitation in normal converting to full-blown suppression in the WBD/CR+ rats could be a result of low inhibition by weak corticocortical interactions in normal converting to increased level of inhibition by strong and numerous such connections when the low somatic sensory activity is coupled with the unusually high level auditory inputs. Such

activation of inhibitory interneurons may lead to an oscillatory state in cortex (Cardin et al. 2009), which is exactly what we observed in barrel cortex where in presence of an auditory click stimulus alone, the spike synchrony increases in an oscillatory manner over time. Finally, NMDA receptors are more responsible for the response characteristics observed in the 15-30 ms epoch in normal animals (Armstrong-James et al. 1993). Thus, the interactions observed in this epoch and some of the longer lasting cross-sensory effects especially in the deprived barrel cortex can be mediated by the NMDA receptors.

Temporal properties including onset-latency and spike synchrony were also modulated by the cross-sensory stimulus. Since the majority of sensory information is carried by the first spike post-stimulus in rat cortex (Petersen et al. 2002), the improved temporal profile of the first spikes following cross-sensory stimuli may lead one to hypothesize that it improves information processing and generates a more precise temporal code structure in the cross-sensory stimulus condition. An increase of spike synchrony with the auditory stimulus alone may indicate that the click stimulus serves to reset the phase of the barrel cortical neurons and render them more excitable to an incoming whisker stimulus.

From a developmental perspective, the cross-sensory effect on onset latency was barely affected by abnormal sensory experience, whereas, the effect on spike synchrony was exaggerated with simultaneous deprivation and click rearing. Since deprivation alone failed to modulate the effect on spike synchrony, it can be safely concluded that click rearing was responsible for strengthening the mechanisms governing the changes in spike synchrony in the presence of a click stimulus. Finally,

most of these synchronous changes were observed in the absence of any change in firing rate with the click stimulus alone. The importance of this result lies in the fact that, this reveals a novel mechanism by which any sensory stimulus can affect other cortical neurons even in primary sensory areas. Also, since spike synchrony has not been studied in the context of uni- vs multisensory nature of cortex, it could explain why such multisensory interactions were overlooked in primary sensory cortex.

The work presented in this thesis sheds light on possible circuitry underlying cross-sensory interactions in the primary somatosensory cortex of rats offering directions and hypothesis for future research. Since such subtle interactions were also detectable even under anesthesia can make one strongly predict that such interactions would be exaggerated in the cortex of awake behaving animals. Thus, one future direction from these studies would be to characterize cross-sensory interactions in the cortex of awake animals. Other future directions include characterizing how different inter-stimulus intervals between the auditory and whisker stimulus might affect the cross-sensory interactions. Also, teasing out the role of the nature of auditory stimulus on such cross-sensory interactions could shed light on the underlying mechanisms. Finally, whether, such interactions can be used at the behavioral or perceptual level is another important question and has not been answered by the experiments conducted in this thesis. Behavioral experiments, similar to perceptual tactile discrimination tasks developed by Carvell and Simons in 1996 and modified by the presence of cross-sensory auditory stimulus could solve whether the presence of such stimuli may help in the behavioral outcome of the rats. Furthermore, important temporal response characteristics like onset-latency and spike synchrony, which are abnormal in bilaterally

whisker deprived rats (Popescu and Ebner 2010, Ghoshal et al. 2009; Chapter II), are returned to near normal levels in sensory deprived rats in the presence of the click stimulus. This can make one optimistic about the hypothesis that cross-sensory stimulus may help in compensatory mechanisms in primary sensory cortex after sensory deprivation and may have strong behavioral and therapeutic implications.

Characteristics of cortical synchrony and its possible importance in sensory processing:

The importance of neuronal synchrony in cortex is becoming clearer, but it is in an emergent state. While some claim that synchrony is less important and/or redundant in sensory processing (Neckelmann et al. 1998), others have provided strong evidence that neuronal synchrony plays an important role in the formation of neural code (Eggermont 2001, 2006; Abott and Dayan 2003). The studies described in this thesis makes an attempt to reveal important features of cortical spike synchrony in barrel cortex under different rearing conditions and also provides evidence for its role in sensory processing. Results from the experiments described in this thesis show that correlated discharge among neurons is widespread in S1 barrel cortex of rats. Within barrel cortex, the maximum correlated discharge is observed to be in L-IV barrels and in a subpopulation of L-IV septal neurons. The magnitude of synchrony in the supragranular layers is consistently lower than the granular layers (Fig. 6-1), indicating high synchrony could be a function of reliable and active thalamocortical inputs. Correlated discharge also strictly followed anatomical relations between cells in barrel cortex. Therefore, while L-IV barrel-septal pairs were highly correlated, the same was

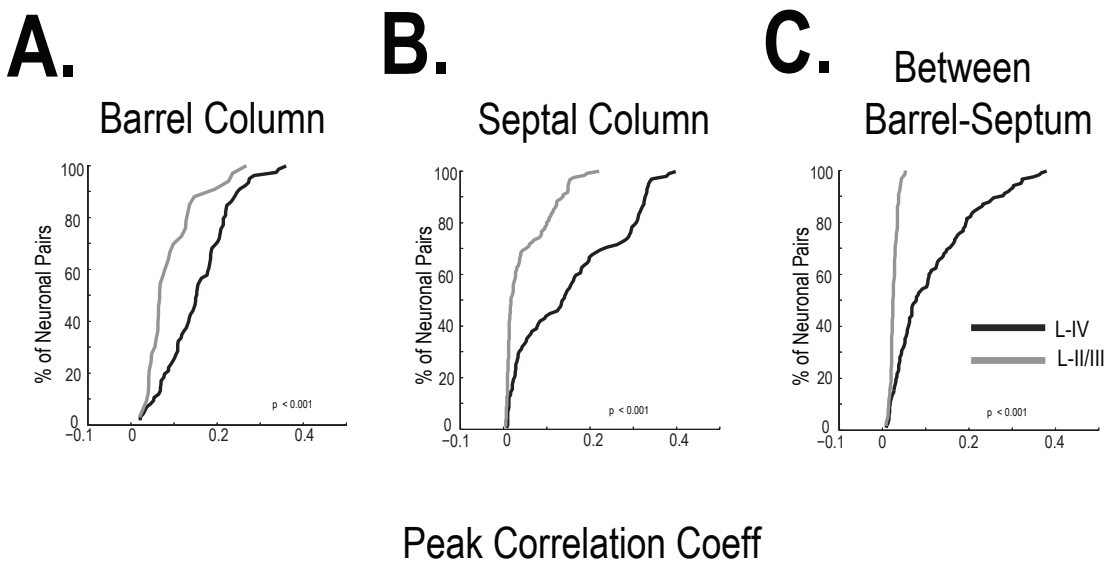

Figure 6-1: Population cumulative distributions of peak correlation coefficients in LII-III and LIV of normal barrel cortex

Cumulative distributions of peak correlation coefficients are displayed for neuron pairs located in LIV (black line) and LII-III (gray line) of a single barrel column (A) or a single septal column (B) or when calculated between a barrel column and the adjacent septal column (C) of a normal rats. Overall, the correlation strength of LIV neurons is significantly higher than the LII-III neuronal pairs (p values for Wilcoxson ranksum test are displayed in each graph) in all areas of barrel cortex.

not true for L-II/III barrel-septal pairs. This could be due to the fact the neurons above a barrel are known to have stronger connections with that of other barrel columns but not necessarily with the neurons above the septum (Alloway 2008), whereas adjacent barrels and septum in L-IV are well connected (Kim and Ebner 1999; Alloway 2008). Moreover, L-IV septal neurons had several subgroups with respect to correlated discharge which could be due heterogeneity in their anatomical connections.

Correlated discharge among the barrel cortex neurons was observed with a near zero lag, indicating that the S1 neurons fire simultaneously. Interestingly, although significant correlated discharge is observed during both the response period and the relatively spontaneous period, the magnitude is dramatically reduced during the response to a whisker stimulus. The latter observation may imply that, at least in barrel cortex, stimulus-driven and spontaneous synchrony could be implemented by separate mechanisms. This is in contrast to the other sensory systems such as the visual system where similar mechanisms have been hypothesized to govern both aspects of synchrony (Jermakowicz et al. 2009). It is possible that spontaneous synchrony in barrel cortex is a feature of the functional grouping of neurons and may serve to keep an ensemble of neurons (e.g. belonging to a single barrel column) in phase in anticipation of an incoming whisker stimulus. The advent of the whisker stimulus in turn desynchronizes the neurons, which might help in channeling the information for specific features of the stimulus, like direction, angle, etc to subsets of neurons within a barrel. A single barrel column receiving information from a single whisker, has been shown to have anatomical sub-barrel patterns (Land and Erickson 2005; Louderback et al. 2006; Ermentrout et al. 2009). It is possible that the neurons in a sub-region of a barrel form

local circuits according to their specificity for stimulus features. Thus encoding a whisker stimulus in L-IV of a single barrel column would require the information to be channeled to neurons having similar stimulus feature specificity. This, in turn, might explain de-synchronization of a pair of neurons in a barrel column which may not encode the same stimulus features of a single whisker stimulus. It has also been shown that too high positive correlations may limit signaling capacity of a pool of neurons (Zohary et al. 1994), which may also provide an explanation for the reduction of synchrony with a whisker stimulus to achieve faithful information transmission.

Another important aspect of cortical synchrony that could be concluded from these results is that it is highly activity-dependent. With bilateral deprivation, there is radical reduction in thalamocortical activity and there is a paucity of activity in L-IV barrel cortex, and accordingly we observed that there is also a considerable reduction in correlated discharge in L-IV. When whiskers were clipped only on one side of the face, which may up-regulate the importance of commissural fiber activity innervating the septal and supragranular areas of the deprived hemisphere, there was a predictable shift in the level of correlated discharge to the presumably more active septal column and supragranular layers. Interestingly, the de-synchronization observed in normal barrel cortex during the response period was absent in bilaterally sensory deprived (BD) cortex. This loss of correlated discharge could be indirect evidence for the presumptive role of this de-synchronization in active perception, as BD animals have a severe loss of behavioral acuity. Also, the fact that such a loss was not observed in UD animals, which are rarely reported to have any behavioral deficits, strengthens the hypothesis.

Synchronous spike discharges in barrel cortex were also modulated by cross-sensory stimuli. During such cross-sensory interactions with auditory click stimuli a very interesting aspect of synchrony was revealed. It was observed, that synchrony can be affected between pairs of neurons without affecting their firing rate, which predicts the likelihood of a temporal code which can operate independently of the firing rate code to synthesize the final neural code of a sensory event (Fig. 6-2). Modulating the temporal code, even as early as primary sensory cortex, while leaving the rate code unaltered could provide a novel mechanism through which cross-sensory stimuli can condition the network, rendering it more efficient and effective in processing incoming stimuli. This mechanism alone could be used for processing certain aspects of sensory events and it would be interesting to see if other non-dominant or weak sensory stimuli such as stimulation of adjacent (surround) whiskers in barrel cortex would have a similar effect on synchronous discharge. Thus, this novel demonstration of firing rate and synchrony dissociation in primary sensory cortex, which supports a similar phenomenon observed in cat S1 by Roy and Alloway (1999), emphasizes the importance of synchronous discharge in sensory processing. In addition to opening up various research directions this implicates the importance of studying the effect of manipulating synchronous discharge alone on neuronal physiology and behavior.

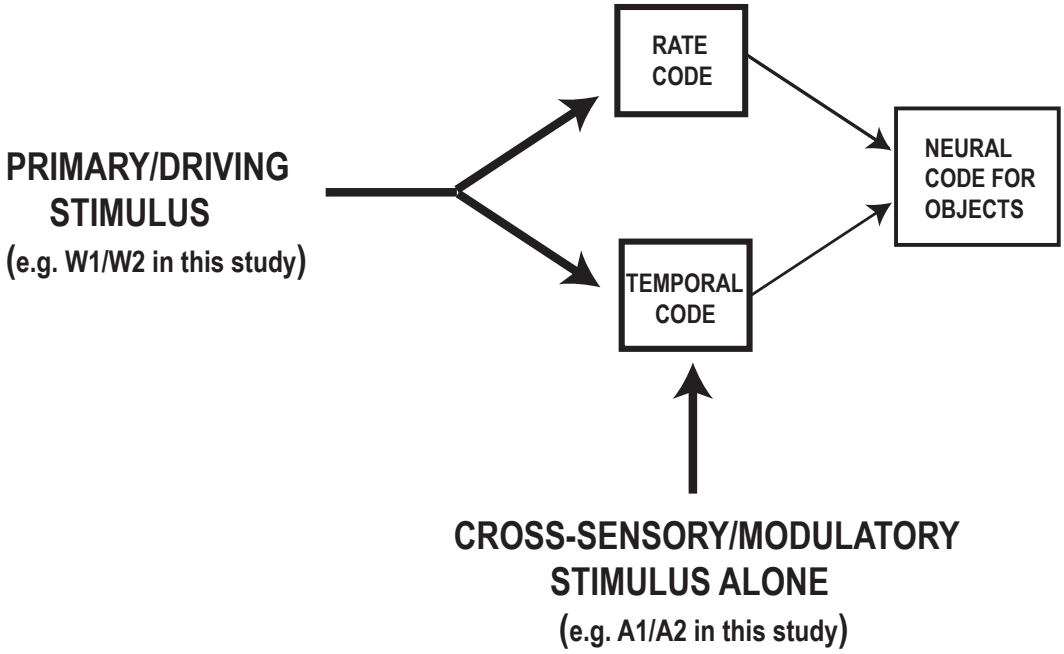

Figure 6-2. Contribution of synchrony on the neural code: interpretation of the results
According to this hypothetical model a rate code comprising of firing rate information and temporal code comprising of temporal profiles of responses like synchronous discharge of neurons can independently contribute to the final neural code. As per our results, a primary stimulus like the whisker stimulus can affect both the rate and the temporal component of the neural code. However, a modulatory stimulus like the cross-sensory A1 or A2 stimulus in our case is also likely to affect the neural code of primary sensory cortex by affecting the temporal code independently of the rate code.

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