# Integration of peripheral and central activity patterns in the developing visual cortex

Dissertation zur Erlangung des
Doktorgrades der Naturwissenschaften

der Fakultät für Biologie der
Ludwig-Maximilians-Universität München

Vorgelegt von

Friederike Siegel

München, 20. September 2010

Erstgutachter: Prof. Dr. Tobias Bonhoeffer
Zweitgutachter: Prof. Dr. Rainer Uhl
Tag der mündlichen Prüfung: 08. November 2010

**Ehrenwörtliche Versicherung:**

Ich versichere hiermit ehrenwörtlich, dass ich die Dissertation mit dem Titel "Integration of peripheral and central activity patterns in the developing visual cortex" selbständig und ohne unerlaubte Hilfe angefertigt habe. Ich habe mich dabei keiner anderen als der von mir ausdrücklich bezeichneten Hilfen und Quellen bedient.

**Erklärung:**

Hiermit erkläre ich, dass ich mich nicht anderweitig einer Doktorprüfung ohne Erfolg unterzogen habe. Die Dissertation wurde in ihrer jetzigen oder ähnlichen Form bei keiner anderen Hochschule eingereicht und hat noch keinen sonstigen Prüfungszwecken gedient.

München, den 20. September 2010

Friederike Siegel

Die vorliegende Arbeit wurde zwischen Juli 2006 und September 2010 unter Anleitung von Dr. Christian Lohmann am Max-Planck-Institut für Neurobiologie in Martinsried sowie am Netherlands Institute for Neuroscience in Amsterdam, Niederlande durchgeführt.

# Table of contents

# List of Figures

# List of Tables

# Abbreviations

| | |
|---|---|
| ACh | Acetylcholine |
| AM | Acetoxymethyl |
| β2nAChR KO | KO for the 2β subunit of nAChR |
| $Ca^{2+}$ | Calcium |
| CAM | Cell adhesion molecule |
| cAMP | Cyclic adenosine monophosphate |
| CCD | Charge-coupled device |
| DIV | Days in vitro |
| dLGN | Dorsal lateral geniculate nucleus |
| DMSO | Dimethyl sulfoxide |
| DSCAM | Down syndrome cell adhesion molecule |
| ENO | Early network oscillation |
| EPI | Epibatidine |
| F | Fluorescence |
| g | Gram |
| GABA | γ-aminobutyric acid |
| GDP | Giant depolarizing potential |
| GFP | Green fluorescent protein |
| Hz | Hertz |
| IgSF | Immunoglobulin superfamiliy |
| KO | Knockout |
| µg | Microgram |
| µl | Microliter |
| µm | Micrometer |
| MΩ | Mega Ohm |
| MCBL | Multi-cell bolus loading |

| | |
|---|---|
| MHC | Major histocompatibility complex |
| min | Minute |
| mm | Millimeter |
| mM | Millimolar |
| n | Number |
| NA | Numerical aperture |
| nAChR | Nicotinic acetylcholine receptor |
| NKH477 | cAMP activator |
| nm | Nanometer |
| NMDA | N-methyl-D-aspartate |
| ODC | Ocular dominance column |
| OGB-1 | Oregon Green BAPTA-1 |
| p | Probability |
| P | Postnatal day |
| PSI | Pounds per square inch |
| RGC | Retinal ganglion cell |
| ROI | Region of interest |
| rpm | Rounds per minute |
| s | Second |
| SAT | Slow activity transient |
| SEM | Standard error of mean |
| SC | Superior colliculus |
| SynCAM | Synaptic cell adhesion molecule |
| V1 | Primary visual cortex |

# 1 Abstract

In the visual system, key structures of the ascending pathway are formed before the onset of vision. The concerted interplay between molecular guidance cues and activity-dependent mechanisms guides these developmental processes. While molecular factors set up coarse topographic structures, neuronal activity drives the refinement of these structures into functional networks. During this time, spontaneous waves of activity are generated in the retinal network that regulate the formation of the basic connectivity from the retina to the visual cortex. However, how retinal inputs contribute to network activity in the developing visual cortex is poorly understood.

In the present study, I used two-photon calcium imaging to unravel spontaneous activity patterns in the visual cortex of the developing mouse in vivo. The labeling of large populations of cortical neurons enabled me to follow the behavior of many cells at the same time. Spontaneous cortical activity comprised two distinct patterns of network events that could be separated by neuronal participation rate, synchronicity and amplitude. In addition, the different classes of network activity were differentially regulated in the second postnatal week. The perturbation of spontaneous retinal activity revealed that specifically cortical network events with a low to medium participation rate are triggered by retinal inputs. In contrast, cortical network events that comprised the majority of imaged neurons remained unaffected by retinal manipulations, suggesting that these network events are centrally generated. Thus, before the onset of vision, distinct classes of network events of different origins coexist in the visual cortex and are likely to regulate various aspects of network development.

# 2 Introduction

The ability of organisms to adapt to changes in the environment is virtually unlimited. The hub for these adaptations is the nervous system with its billions of interconnected neurons which in turn form innumerable connections. This way, the brain of higher animals is able to perform complex actions and reactions and thus meets the demands of an ever changing environment. This complexity of the brain develops early during the first stages of life and is required for proper brain functioning.

The formation of the brain is an intricate process. Because neuronal networks are not built from a master plan, their formation requires countless rearrangements to establish the complex mesh of neuronal connections. Moreover, to guarantee the ability to adapt to changes in the surrounding, the plastic properties of neuronal networks are maintained throughout adulthood. To meet these requirements, both the developing and the mature brain are in a state of constant structural and functional change. Given these complex modifications, it is not surprising that many open questions remain to be answered regarding the mechanisms that lead to the formation of mature neuronal networks.

On the next pages, I will outline the hallmarks of what is currently known about the mechanisms of neuronal network formation and in particular concentrate on the development of the visual system.

## 2.1 Nervous system development: The concerted interplay of activity-dependent mechanisms and molecular guidance cues

The development of neuronal networks is a highly specific process during which neuronal projections find the appropriate target area and make contact with the right partners. This specificity is critical for the formation of neuronal networks and for proper functioning of the mature brain. The miswiring of the neuronal network can cause severe defects and, in the worst case, result in neurodevelopmental diseases (Levitt et al., 2004; Van Quyen et al., 2006).

The communication between neurons in the central nervous system is mainly achieved by chemical signals. In order to exchange information in a timely manner, the neurons are in close contact and form specialized connections with each other. These connections, called synapses, serve as a basis for information exchange that is mainly achieved by the release of chemical signals, so-called neurotransmitters. Synapses are primarily formed between the presynaptic axon, the part of a neuron that dispatches the signal, and the postsynaptic dendrite, which receives and processes the information. To find the correct synaptic partners during network formation, neurons send out axonal processes that navigate through various brain regions and come to a halt when the axons encounter the right postsynaptic structures. The challenge to form synapses with the appropriate partners in the target area is largely regulated by two mechanisms: molecular cues and activity-dependent mechanisms. It is widely accepted that the concerted interplay of these two mechanisms is sufficient to build up the mature brain (Lee and Sheng, 2000; Katz and Crowley, 2002; Cline, 2003). Molecular factors, either secreted or bound to cells guide the navigating axon and lure it to its final destination. These positional cues act either as repellents or attractors and lead to the directed outgrowth of neuronal processes into the designated area or layer (Huberman et al., 2010). The encounter with appropriate postsynaptic structures will ultimately lead to the formation of neuronal networks and an initial coarse projection map is established.

Once neurons are brought into close contact with each other, activity-dependent mechanisms come into play. The sources of activity are diverse. Early during develop-

ment, neuronal activity arises spontaneously and without sensory stimuli. Later, inputs from the periphery, predominantly elicited by sensory stimulation trigger the activation of neuronal networks. Activity-dependent mechanisms mediate signaling cascades that lead to the maturation and refinement of neuronal networks. Among these mechanisms are activity-dependent gene expression, structural rearrangements and mechanisms involved in synapse stabilization (Cline, 2003)

Spontaneous activity-dependent processes during early development involve a variety of mechanisms ranging from the simultaneous activation of the entire network (Allene and Cossart, 2010) down to the specific activation of confined stretches of neuronal processes (Lohmann, 2009). According to Donald Hebb's postulate (Hebb, 1949), the correlated firing of cells results in the strengthening of mutual connections: "Cells that fire together wire together" (coined by C. J. Shatz). Conversely, synapses of cells that fire in an un-correlated manner are weakened and eventually eliminated: "Cells that fire out of sync lose their link". These principles provide a rule for the formation of neuronal networks based on activity-dependent cues. Hence, spontaneous network activations that recruit large populations of neurons at the same time are thought to sculpt neuronal networks on a large scale. This form of activity is common in the developing brain and connects co-active cells to form functional units (Huberman et al., 2008; Lopez-Bendito and Molnar, 2003). Similarly, the simultaneous activation of sites on dendrites of a neuron could strengthen connections on a subcellular scale (Kleindienst et al., submitted).

Since the pioneering work of Donald Hebb, numerous studies engaged in these theories and it became clear that activity-dependent mechanisms are indispensable for the wiring of most parts of the brain. One of the best studied systems in this context is the developing visual system of mammals. Within the scope of this study, I will first give an overview of the role of large-scale network oscillations during nervous system development in general (chapter 2.2). Secondly, I will focus on the developing visual system and emphasize the importance of the concerted interplay of activity-dependent mechanisms and molecular guidance cues for the formation of the mature visual system (chapter 2.3).

## 2.2 Spontaneous activity patterns guide the formation of neuronal networks

The first postnatal weeks are a period of extensive structural changes and rearrangements in the cortex of developing rodents (Feller and Scanziani, 2005; Bence and Levelt, 2005). Numerous new connections are formed whereas exuberant connections are removed from the neuronal network. These changes are largely regulated by activity-dependent mechanisms. Even before sensation, the developing brain generates patterns of spontaneous activity that control the formation of neuronal networks (Khazipov and Luhmann, 2006; Blankenship and Feller, 2010). Such activity patterns have been described in numerous in vitro and in vivo preparations. Among the most extensively studied structures are the retina (Meister et al., 1991; Wong et al., 1993), the spinal cord (ODonovan et al., 1998; Hanson and Landmesser, 2004), the hippocampus (Ben Ari, 2001; Leinekugel et al., 2002) and the neocortex (Khazipov et al., 2004; Adelsberger et al., 2005; Allene et al., 2008). Within the scope of this study, I will focus on spontaneous activity patterns in the developing cerebral cortex during the first two postnatal weeks.

Despite the vast literature on spontaneous network activity in the developing cortex, the mechanisms underlying the different activity patterns are not clear (Allene and Cossart, 2010). Already before birth, neurons in the neocortex are activated in a synchronous but non-synaptic fashion (Owens and Kriegstein, 1998; Corlew et al., 2004). These activity patterns, largely mediated by gap junctions that couple neurons in the immature network, are thought to promote early steps in development, such as neurogenesis and neuronal migration (Owens and Kriegstein, 1998; Weissman et al., 2004). However, the activation only recruits few cells and is restricted to relatively small areas of the cortex (Figure 2.1 A). Around birth, early network oscillations (ENOs) emerge which engage the majority of cells in the cortical structure (Garaschuk et al., 2000; Corlew et al., 2004; Adelsberger et al., 2005). In contrast to embryonic activity patterns, ENOs are driven by synaptic inputs and are sensitive to the blockade of glutamatergic transmission (Figure 2.1 B) (Garaschuk et al., 2000; Allene et al., 2008).

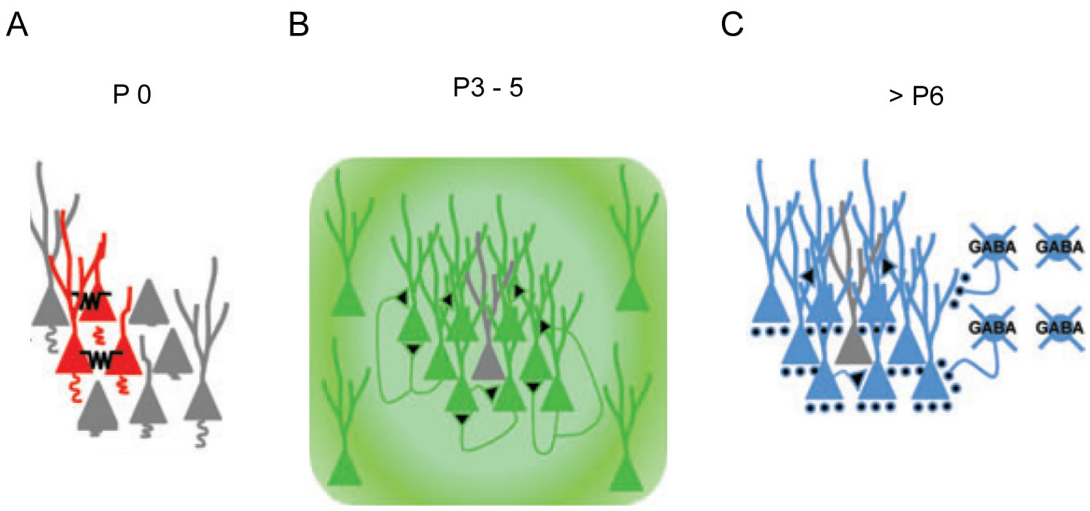


**Figure 2.1 Different patterns of spontaneous network activity shape the neuronal network of the developing cortex.** (**A**) Around birth, the synchronous activation of small ensembles of neurons is non-synaptic and mediated by gap junctions. (**B**) In the first week of life, ENOs emerge which are characterized by the simultaneous activation of large neuronal populations of the network through glutamatergic transmission. (**C**) GABAergic GDPs develop at the end of the first postnatal week. (Adapted from Allene and Cossart, 2010)

It has been shown recently that ENOs are triggered by the activation of extrasynaptic NMDA receptors, glutamate receptors that are thought to regulate the maturation of functional synapses. Glutamate spillover from neighboring synapses is thus responsible for the generation of ENOs in the developing cortex (Allene et al., 2008). ENOs are recurrent activity patterns that travel across large areas of the cortex (Garaschuk et al., 2000). They act as a synchronizing element that regulates the development over large cortical regions. ENOs have also been described in the newborn mouse cortex in vivo where they predominantly occur during the quiescent state of the animal, similar to sleep (Adelsberger et al., 2005). This early network activity is soon replaced by more complex activity patterns, commonly described as giant depolarizing potentials (GDPs). GDPs have first been described in the hippocampus (Ben-Ari et al., 1989) but are also present in the cortex during early stages of development (Figure 2.1 C) (Allene et al., 2008; Rheims et al., 2008). GDPs arise several days after birth and differ from ENOs in both spatial and temporal parameters and the transmitter involved. While ENOs occur at low frequencies and activate the entire cortical network with slow kinetics, GDPs resemble fast oscillations that occur frequently and are restricted to smaller ensembles of neurons. In contrast to ENOs, cortical GDPs are most likely

driven by GABAergic transmission (Allene et al., 2008). At this time of development, GABA functions as excitatory transmitter and important mediator of activity in the developing network. Both patterns of correlated activity, ENOs and GDPs, are likely to control the refinement of neuronal maps by means of Hebbian-like mechanisms and strengthen the connections between co-active neurons (Song and Abbott, 2001). What drives these network oscillations in the developing cortex? Evidence is accumulating, that subtypes of GABAergic neurons serve as initiators of network oscillations. These so-called pacemaker-like neurons or hub cells have been described in the hippocampus (Bonifazi et al., 2009) as well as in the cortex (Voigt et al., 2001; Le Bon-Jego and Yuste, 2007). This class of GABAergic neurons initiates network activation in the developing brain and serves as integrating element in the synchronization of the developing network.

ENOs and GDPs have both been described first in slice cultures of various brain regions (Ben-Ari et al., 1989; Garaschuk et al., 1998; Garaschuk et al., 2000). Recently, in vivo recordings of spontaneous network activity have been reported by several laboratories. For instance, Yang and colleagues have demonstrated with electrophysiological field recordings a similar developmental sequence of activity patterns in the developing cortex of rats, terming the corresponding phenomena long oscillations and spindle bursts (Yang et al., 2009). The transition from a highly correlated state of spontaneous network events to sparse activity patterns around the second week after birth has been demonstrated in the visual and somatosensory cortex of developing mice (Rochefort et al., 2009; Golshani et al., 2009). This transition is thought to be a prerequisite of activity patterns in the adult animal in which a sparse coding is thought to be preferable for more efficient information processing (Olshausen and Field, 2004; Kerr et al., 2007).

How do inputs from the periphery contribute to the generation of cortical network activity during early stages of development? It is thought that information processing in the mature brain is achieved by the sparse firing of only few neurons and not by the correlated activation of large neuronal ensembles. In vivo calcium imaging studies have shown that the process of de-correlating spontaneous network events in the cortex is not dependent on sensory experience. Neither the deprivation of visual (Rochefort et al., 2009) nor tactile information (Golshani et al., 2009) could prevent

the transition from highly synchronous activity patterns in the developing cortex to the sparse firing in the adult. If pivotal developmental processes are largely independent of sensory information, what could be the mechanisms that shape the developing neuronal network? One prime candidate could be patterned activity that arises spontaneously from peripheral inputs before sense organs are fully developed. For instance, electrophysiological recordings revealed that spontaneous network activity in the somatosensory cortex is altered but not abolished after spinal cord transection, suggesting that peripheral activity shapes network activity in central brain structures (Khazipov et al., 2004). Such activity patterns are likely to strengthen or eliminate connections between neurons through Hebbian-like mechanisms. The significance of patterned activity arising from the periphery and its role in the development of mature neuronal networks has been widely studied in the visual system.

## 2.3 Visual system development

How does activity from the periphery influence the functioning of central structures, such as the neocortex? Since the pioneering work of Hubel and Wiesel (Hubel and Wiesel, 1959; Wiesel and Hubel, 1963), the mammalian visual system is a prominent field of research to study the development of the nervous system. Furthermore, the interplay of specific guidance cues and activity-dependent mechanisms has been studied in the developing visual system of mammals and has provided important insights into the formation of functional network.

### 2.3.1 The mouse visual system

The visual system of the mouse has attracted the attention of neuroscientists only recently. In contrast to other mammals such as primates and carnivores, the mouse does not primarily rely on vision. Because it rather uses olfactory and tactile cues for orientation, the visual system of the mouse is less sophisticated. However, most principal features that characterize the better developed visual system of higher mammals, such as orientation and direction selectivity, are present in rodents (Drager, 1975; Metin et al., 1988). Due to the availability of genetic tools that are not suitable for other mammals, the study of the mouse nervous system became highly favored recently. Transgenic technologies, such as knockout mice or reporter lines that express a fluorescent protein in specific cell types have been generated and are now being used for the study of various aspects of the visual system (Rossi et al., 2001; Cang et al., 2005a; Hofer et al., 2009).

The visual system of the mouse, such as that of other mammals, is constructed in a hierarchical manner (Figure 2.2 A). The retina receives sensory stimuli and serves as a first post of neuronal processing. The axons of the retinal ganglion cells (RGCs) leave the retina via the optic nerve and project to the dorsal lateral geniculate nucleus (dLGN). Neurons in the dLGN establish connections with layer 4 neurons of the primary visual cortex (V1). From there, the signal is distributed to other cortical layers and further processed in higher visual areas (Figure 2.2 B). RGCs also send projections to the superior colliculus (SC) in the midbrain that mediates visual reflexes, such as eye or head movements. Nevertheless, the majority of RGC axons from one eye leave the retina via the optic nerve, cross the hemisphere at the optic chiasm and project to the contralateral dLGN (Figure 2.2 A). A small proportion of RGCs, however, send their axons to the ipsilateral side of the brain. While the inputs from both eyes are completely separated in the dLGN and the SC, the primary visual cortex contains a binocular region, where inputs from both eyes converge (Figure 2.2 A). Due to the lateral location of the eyes in the mouse, stereoscopic vision is poorly developed and the area of binocular projections is relatively small. However, like in other animals, the mouse visual system is constructed in a topographic order.

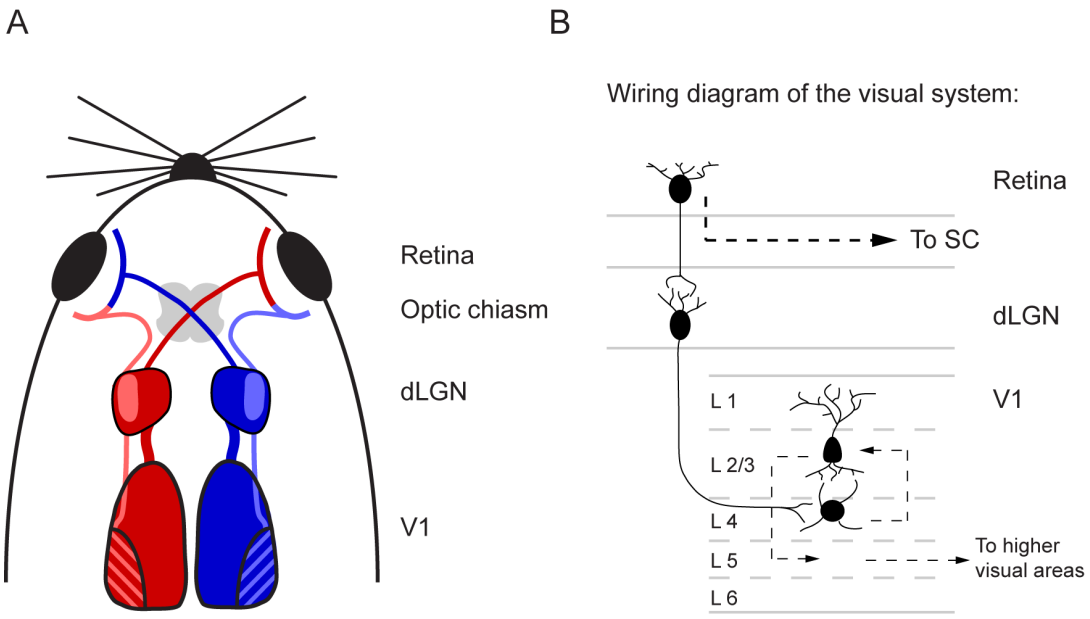


**Figure 2.2 Layout of the visual system of the mouse.** (**A**) Schematic of the mouse visual system. Visual information is perceived by the retina and transferred to upstream targets, the dLGN and primary visual cortex (V1). While most inputs from the retina (dark colors) cross at the optic chiasm (gray structure), a small fraction of RGC axons projects to the ipsilateral side (light colors). (**B**) Simplified wiring diagram of the visual system from the retina to V1. RGC synapse onto dLGN neurons. Layer 4 neurons of V1 receive projections from the dLGN and the information is further distributed to other cortical layers as well as higher visual areas. A small fraction of RGC axons projects to the SC.

Adjacent points in the animal's visual field are perceived by neighboring retinal neurons and this topographic map is maintained in upstream targets of the visual system. The so-called retinotopic map preserves spatial information across the different brain structures and ensures a continuous representation of visual space. Owing to its scarcely developed vision, the visual cortex of the mouse lacks a highly ordered columnar organization that is characteristic for other mammals such as primates and carnivores. These structures contain neurons that share similar response properties. For instance, neurons that preferentially respond to one eye are grouped into ocular dominance columns that span all layers of the cortex (Hubel et al., 1977; LeVay et al., 1978). Neurons that prefer similar stimulus properties, such as orientation or direction, are likewise clustered in a highly ordered, columnar fashion (Bonhoeffer and Grinvald, 1991; Weliky et al., 1996). To date, none of these structures have been found in the mouse visual system. In contrast, in vivo imaging of the rodent visual cortex

revealed a salt and pepper like distribution of neurons with similar response preferences (Schuett et al., 2002; Ohki et al., 2005). Nevertheless, individual neurons of the rodent visual cortex show sharply tuned responses to specific stimulus properties such as direction and orientation (Metin et al., 1988; Ohki et al., 2005).

How does the specificity of the neuronal network come about during visual system development? While there is general consent that interactions of molecular cues and activity-dependent mechanisms play a critical role during network formation, the exact mechanisms are still largely unknown. In the next chapters, I will give an overview of what is currently known about the two mechanisms during visual system development. First, I will focus on molecular factors that primarily regulate the first steps of network formation. Second, I will demonstrate how neuronal activity is involved in the refinement of neuronal projections, especially during later stages of network formation.

### 2.3.2 Molecular cues and visual system development

Once newly generated neurons have settled in the appropriate environment, they start sensing the environment for the right synaptic partners. For example, RGC axons traverse long distances from the retina into the dLGN where they form synapses with adequate postsynaptic dendrites of dLGN neurons. The specificity in synaptic partner finding is largely accomplished with the help of molecular guidance cues. This concept was established over 40 years ago by the neurobiologist Roger Sperry. He postulated in his chemoaffinity hypothesis that the graded expression of specific guidance molecules can act as attractive or repulsive signal for developing nerve cells (Sperry, 1963). Thus, pre- and postsynaptic partners find their match through specific expression patterns of various receptors and ligands. However, no class of molecules has been found to date that meets all requirements to overcome the restrictions imprinted in the number of genes available. Instead, the concerted interplay with activity-dependent mechanisms could provide the specificity required for the formation of the visual system.

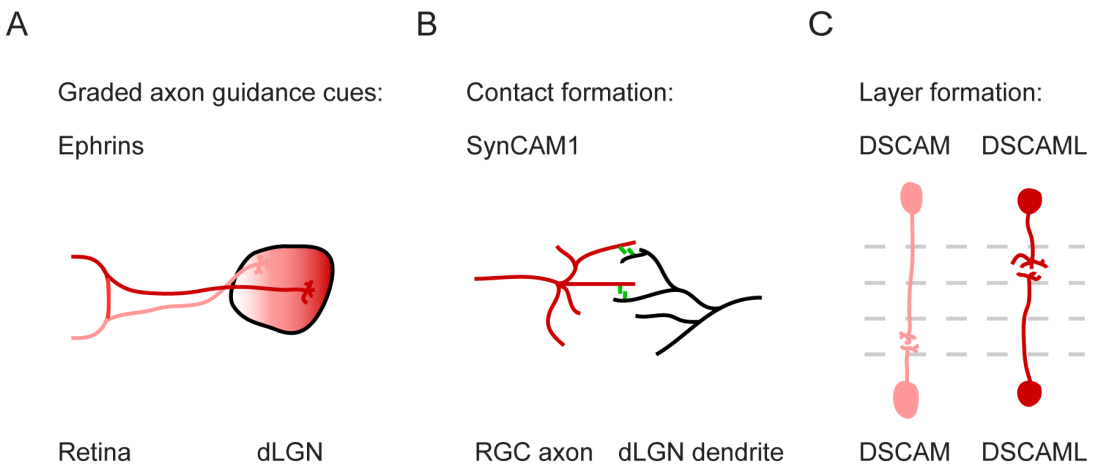


**Figure 2.3 Molecular factors serve different roles during network development.** (**A**) Axon guidance cues, such as ephrins, are expressed in a graded fashion to regulate the ingrowth of RGC axons into the dLGN. (**B**) Cell adhesion molecules (e.g. SynCAM1, green) provide a mechanism to bring neuronal partners from the retina (red) and dLGN (black) into close contact. (**C**) Cells that express the same variants of DSCAM establish connections with each other in the appropriate layer.

One of the best studied examples of topographic map formation by means of molecular gradients is the retinotectal projection of the visual system (Mclaughlin and O'Leary, 2005; Flanagan, 2006). Here, the binding partners ephrin-A and the corresponding receptors EphAs, a set of specific tyrosin kinases, were identified to mediate retinotopic map formation in the nasal-temporal axis in various parts of the visual system, such as the SC and the dLGN (Figure 2.3 A) (Cheng et al., 1995; Drescher et al., 1995; Feldheim et al., 1998). Conversely, the receptor/ligand pair ephrin-B and EphB control the development of the dorsoventral axis of the retinotectal projections (Mann et al., 2002; Hindges et al., 2002). These positional labels are expressed in a complementary and graded fashion and regulate the formation of a coarse retinotopic map. Overexpressing specific EphAs in RGCs disrupts the correct patterning of axonal projections in the dLGN of newborn ferrets, resulting in intermingled patches of eye-specific projections (Huberman et al., 2005). These findings implicate an important role for ephrins and their receptors for axonal guidance and topographic map formation.

Once the neurites have reached their target area, axons and dendrites make synaptic contacts with each other. In order to form functional synapses, the pre- and postsy-

naptic neurons have to come in close physical contact with each other. Several classes of cell adhesion molecules (CAMs) have been identified as mediators of the first steps of synaptogenesis (Figure 2.3 B) (Dalva et al., 2007). CAMs not only connect neurons with each other, but they are also thought to be involved in much more complex functions of synaptogenesis, such as receptor recruitment to the nascent synapse and mediating synaptic specificity (Waites et al., 2005; Dalva et al., 2007). Several adhesion molecules involved in synapse formation belong to the immunoglobulin superfamily (IgSF). For instance, SynCAM1 (Synaptic cell adhesion molecule 1) has been identified to initiate contact formation between neurons and to regulate excitatory synapse number (Biederer et al., 2002; Fogel et al., 2007). In the visual cortex, the expression of SynCAM1 is downregulated after a certain period of development, suggesting that this molecule is required for synaptogenesis (Lyckman et al., 2008). Another member of the IgSF that has been found to be involved in visual system development is DSCAM (Down syndrome cell adhesion molecule). A specific variant of the molecule, DSCAM2, has first been described in the visual nervous system of Drosophila where it mediates repulsion upon homophilic binding in lamina neurons (Millard et al., 2007). Due to its vast number of splice isoforms, DSCAM2 is prone to contribute to the neuron's ability to distinguish between own and foreign neurites (Matthews et al., 2007). This self-avoidance mechanism is thought to establish lamination in the visual system of the fly. Similarly, recent work has provided evidence that the homophilic interactions between members of the IgSF, including DSCAMs and sidekicks, serve as important mediators of layer formation during the development of the vertebrate visual system (Figure 2.3 C) (Yamagata et al., 2002; Yamagata and Sanes, 2008; Fuerst et al., 2009; Fuerst and Burgess, 2009).

One exciting line of research that evolved over the last years studies the role of immune proteins in topographic map formation (Boulanger and Shatz, 2004). Even though the central nervous system is immunologically privileged, recent work has suggested that various components of the immune system play an important role in the healthy brain. For instance, MHC (major histocompatibility complex) class I, a key mediator of the immune response, is expressed in normal, non-infected neurons in various brain structures (Neumann et al., 1995; Corriveau et al., 1998; Lidman et al., 1999). By reducing the amount of MHCI expression on the cellular surface, eye-specific layers fail to form in the developing dLGN (Huh et al., 2000; Boulanger and

Shatz, 2004). Another group of molecules that is closely related to key factors of the immune system are neuronal pentraxins. Similarly to MHCI, the loss of neuronal pentraxins results in perturbed eye-specific map formation in the dLGN (Bjartmar et al., 2006) and prevents the elimination of aberrant connections in the retinogeniculate projection (Koch and Ullian, 2010). A third group of immune proteins are components of the classical complement cascade. These small molecules are expressed at the developing retinogeniculate synapse and are involved in synaptic pruning of aberrant connections that occurs during refinement (Stevens et al., 2007).

Although there is extensive experimental evidence that supports the role of molecular guidance cues during visual system development, it becomes clear that molecules alone cannot set up the specificity of neuronal connections in the mature brain. The demand for rearrangements of neuronal projections during development and later during learning and memory cannot solely be accomplished by molecular factors and must therefore be generated by additional mechanisms that provide specificity.

### 2.3.3 Activity-dependent mechanisms and visual system development

As already pointed out in chapter 2.1, molecular cues and activity-dependent mechanisms go hand in hand during nervous system development. Spontaneous activity, that means activity triggered by intrinsic sources, has been studied in various structures of the developing visual system. Before the onset of visual experience, activity patterns have been reported in the retina (Galli and Maffei, 1988; Meister et al., 1991), the dLGN (Mooney et al., 1996; Weliky and Katz, 1999) and the visual cortex (Schwartz et al., 1998; Rochefort et al., 2009). These patterns with their spatiotemporal properties are requisite features for the formation of networks based on Hebbian rules (Butts et al. 2007). Activity patterns that are generated in the retina are transferred to upstream targets, such as the dLGN and V1 (Mooney et al., 1996; Hanganu et al., 2006). This occurs before the retina becomes light-sensitive and is able to process visual cues. The disruption or elimination of spontaneous activity patterns in the retina leads to severe effects in the formation of functioning networks in upstream targets. There-

fore, spontaneous activity, not driven by sensory inputs, is likely to play a key role in the development of mature neuronal networks.

**Spontaneous network activity in the developing visual system**

Spontaneous activity patterns in the developing visual system are best described in the retina. Waves of activity sweep across the retina and cause the correlated activation of neighboring RGCs before the retina is capable of phototransduction (Meister et al., 1991; Wong et al., 1995). In the mouse retina, waves are already present before birth and persist until after eye-opening (Figure 2.4). Distinct stages of retinal waves can be distinguished based on the transmitter system, frequency and travel velocity (Wong, 1999; Firth et al., 2005). In the mouse retina, stage I waves emerge before birth, occur relatively infrequently and are largely mediated by acetylcholine (ACh) (Bansal et al., 2000; Huberman et al., 2008). During this period, non-synaptic mechanisms are likely to contribute to the generation of activity patterns in the prenatal retina (Bansal et al., 2000; Syed et al., 2004). Stage II waves take over around birth, are likewise evoked by ACh and occur infrequently (Bansal et al., 2000). In contrast to stage I waves, the proportion of cells that is recruited to stage II retinal waves is considerably larger. During the second postnatal week, glutamatergic stage III waves emerge and persist until around the first week after eye-opening (Bansal et al., 2000; Demas et al., 2003). These waves are driven by glutamatergic inputs from bipolar cells onto RGCs. While RGCs are activated relatively unspecifically during waves of the first two stages, stage III waves selectively recruit RGCs from either the On- or the Off-subtype (Myhr et al., 2001). This ensures the transfer of functional differences between RGCs to upstream targets.

In the developing visual system, patterned activity is generated in the retina and relayed to the dLGN before the onset of vision (Figure 2.4). It has been shown in in vitro preparations (Mooney et al., 1996) as well as in vivo (Weliky and Katz, 1999) that retinal activity has the ability to drive highly correlated firing in the dLGN.

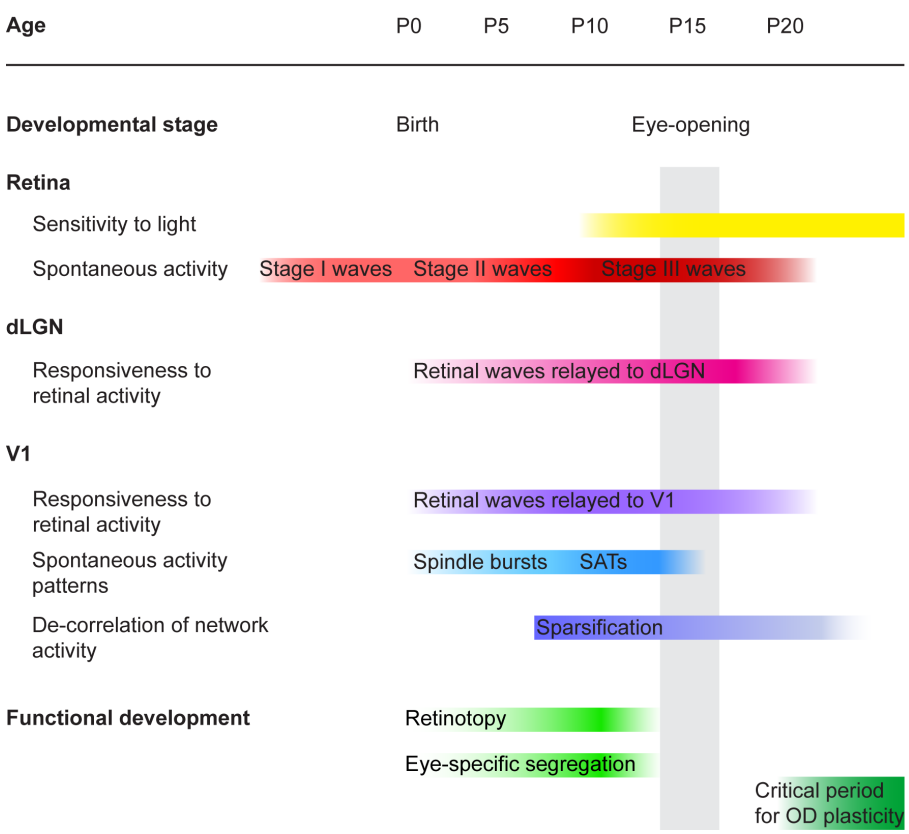


**Figure 2.4 Timeline of major developmental processes in the mouse visual system.** Sensitivity to light is achieved shortly before eye-opening (yellow gradient). The gray bar depicts eye-opening around the end of the second postnatal week. Retinal waves (red bars) are relayed to upstream targets, the dLGN and V1, shortly after birth (pink and purple bars). Distinct patterns of network activity, such as spindle bursts and SATs, exist in the visual cortex before eye-opening (light blue bars). The de-correlation of cortical network events ('sparsification', dark blue bar) starts at around eye-opening. Retinotopy and eye-specific segregation (light green bars) are completed before eye-opening. The critical period for ocular dominance (OD) plasticity starts around the beginning of the third postnatal week (dark green bar).

Likewise, retinal activity is relayed to the visual cortex as early as P2 where it drives spindle bursts, a specific form of network oscillations (Hanganu et al., 2006; Colonnese and Khazipov, 2010) and perturbations of retinal activity have profound effects on the frequency of network activity in the visual cortex (Hanganu et al., 2006). Later during development, slow activity transients (SATs) which are composed of several spindle bursts emerge in the visual cortex of unanesthetized rats (Figure 2.4) (Colonnese and Khazipov, 2010). SATs are likely to be triggered by stage III retinal waves and thought to mediate circuitry formation in the visual cortex (Colonnese and Khazipov, 2010). In summary, in most structures of the visual system spontaneous activity provides a general Hebbian-based mechanism to set up a functional network before visual experience sets in.

**Retinotopic map formation**

What is the significance of the specific patterns of spontaneous activity in the developing visual system? It is conceivable that the spatiotemporal information of spontaneous activity patterns is used to set up neuronal networks in upstream targets before the onset of sensory experience. For instance, due to their spatiotemporal properties (short duration and long interburst intervals), stage II retinal waves are optimally suited to establish the retinotopic map of upstream targets. During retinal waves, neighboring RGCs are likely to be activated together which results both in the strengthening of mutual connections and connections with neurons in upstream targets (Wong, 1999). On the other hand, the simultaneous activation of distant cell pairs or cells from the two eyes is unlikely which in turn leads to the weakening and elimination of those connections.

The development of retinotopy is a good model to highlight the importance of the interplay between activity-dependent mechanisms and molecular guidance cues for topographic map formation. The outgrowth of RGC axons to higher areas of the visual system is not directed to the final location but rather characterized by extensive overshoot of their projections. This is followed by the pruning of aberrant protrusions and the stabilization of functional connections (Ruthazer et al., 2003; Mclaughlin et al., 2003). Due to expression gradients of ephrins and their receptors, a coarse retinotopic map is established whereby axonal projections of neighboring RGCs occupy the

same areas and distant pairs will cover different regions. It has been shown that this refinement process requires patterned activity from the retina by means of Hebbian mechanisms. When retinal activity is blocked pharmacologically, the axonal terminals occupy larger areas in the dLGN and fail to refine to their characteristic patterns (Kobayashi et al., 1990; Simon et al., 1992). The generation of mice that are deficient for the β2 subunit of the nicotinic acetylcholine receptor (β2nAChR KO) proved very useful to study the role of spontaneous retinal activity during topographic map formation in the visual system (Bansal et al., 2000; Cang et al., 2005b; McLaughlin et al., 2003). These animals specifically lack the spatiotemporal properties of stage II retinal waves whereas the overall firing activity of RGCs is not altered. The uncorrelated firing of RGCs results in perturbed retinotopic maps in the dLGN (Grubb et al., 2003) as well as in the SC and V1 (Mclaughlin et al., 2003; Cang et al., 2005b). Interestingly, mice deficient in both the expression of certain ephrins (ephrin-A2/5 KO) and retinal stage II waves (β2nAChR KO) have topographic cortical maps that are more severely distorted that in either knockout mouse (Cang et al., 2008). These experiments highlight the importance of the interplay between molecular factors and activity-dependent mechanisms during retinotopic map formation (Mclaughlin and O'Leary, 2005).

**Eye-specific segregation**

In the mammalian visual system, RGC axons from both eyes project to both sides of the brain and are organized in eye-specific territories in the dLGN and V1 (Figure 2.2 and Figure 2.5). The eye-specific segregation of RGC axons develops well before the onset of vision (Figure 2.4) and is thus another excellent model to study the interplay between molecular cues and spontaneous activity-dependent mechanisms during the formation of topographic maps. The growth of RGC axons from both eyes into the dLGN and the SC is initially unspecific and axonal terminals are intermingled, resulting in overlapping inputs from both eyes (Linden et al., 1981). This refinement process is regulated by competitive interactions because after selectively eliminating the activity in one eye, the territory in the dLGN occupied by the intact eye is increased dramatically (Sretavan and Shatz, 1986; Morgan and Thompson, 1993).

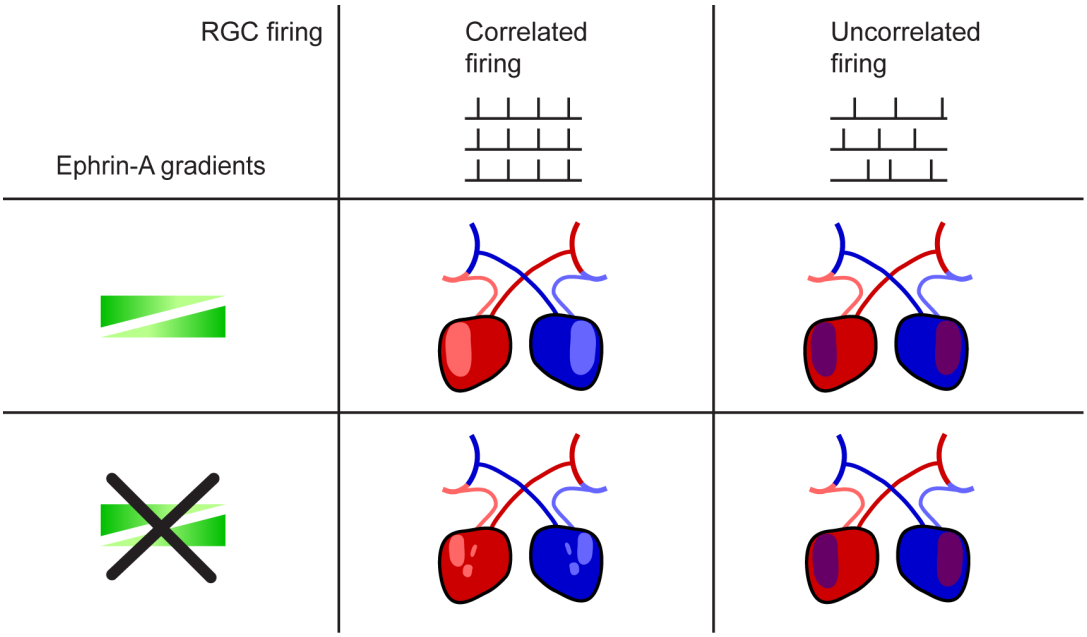


**Figure 2.5 The concerted interplay of ephrin gradients and spontaneous retinal activity regulates eye-specific segregation in the dLGN.** Normal eye-specific segregation in the dLGN (upper left quadrant). The disruption of the ephrin-A gradients with normal spontaneous retinal activity results in patchy eye-specific territories in the dLGN (lower left quadrant). Perturbing normal activity patterns during stage II waves (β2nAChR KO or chronic epibatidine application) leads to intermingled projections of retinal axons from both eyes, independent of whether ephrin-A gradients are intact (upper right quadrant) or perturbed (lower right quadrant).

To study whether activity per se or relative activity levels between the two eyes are relevant, the cAMP activator forskolin was chronically injected into one eye of ferrets which resulted in different levels of stage II waves in the two eyes (Stellwagen et al., 1999; Stellwagen and Shatz, 2002). Consequently, axons from the more active eye occupied larger areas in the dLGN suggesting that relative levels of activity control the segregation of retinal inputs by means of competition-based mechanisms. Pharmacological disruption of the spatiotemporal characteristics of stage II waves with epibatidine (Penn et al., 1998; Rossi et al., 2001) or the use of β2nAChR KO mice (Rossi et al., 2001; Muir-Robinson et al., 2002) revealed that RGC axons fail to segregate in the absence of stage II waves (Figure 2.5). Even though β2nAChR KO animals specifically lack stage II waves and exhibit normal stage III waves, these mice were never able to establish normal eye-specific regions in the dLGN. Instead, after stage III retinal waves ceased, eye-specific segregation was arranged in random and patchy territories (Muir-

Robinson et al., 2002). This implies that, in addition to stage II retinal waves, other mechanisms that are only present during this developmental period are required to control the location of retinal inputs in the corresponding area. Ephrin-As are known to guide the spatial patterning of retinal inputs in the dLGN. In triple knockout mice for ephrins (ephrin-A2/3/5 KO), the segregation of projections into contra- and ipsilateral eye territories is disturbed and, instead, a patchy eye-specific pattern is established (Figure 2.5) (Pfeiffenberger et al., 2005). In addition, if stage II retinal waves are eliminated in ephrin-A2/3/5 KO animals, retinal inputs stay intermingled and fail to form eye-specific territories (Figure 2.5) (Pfeiffenberger et al., 2005).

In conclusion, similar to retinotopic map formation, the cooperation of molecular factors together with activity-dependent mechanisms leads to the transformation of an initially coarse map to a functional network that is able to process eye-specific information. Moreover, spontaneous activity patterns are not only required for the formation but also for the maintenance of topographic maps because the elimination of stage III retinal waves resulted in the de-segregation of previously sorted eye-specific layers in the dLGN (Chapman, 2000; Demas et al., 2006). This suggests that spontaneous retinal activity patterns serve several purposes during the development of the visual system.

**Development of ocular dominance**

Eye-specific segregation which is first established in the dLGN is maintained in the visual cortex. The visual cortex of most carnivores and primates is composed of alternating, non-overlapping stripes that span all cortical layers, so called ocular dominance columns (ODCs) (Hubel et al., 1977; LeVay et al., 1978). In contrast to higher mammals, the visual cortex of rodents lacks an organized columnar pattern. The division of visual inputs in the mouse V1 is rather distributed to monocular and binocular regions (Figure 2.2) (LeVay et al., 1978; Antonini et al., 1999; Hofer et al., 2006).

ODCs are established well before vision occurs (Wiesel and Hubel, 1974; Crair et al., 1998) but the exact mechanisms that underlie ODC formation are unclear. Several studies suggest that the outgrowth of dLGN axons to the correct target area in the visual cortex is direct and thus activity-independent (Crowley and Katz, 1999;

Crowley and Katz, 2000; Crowley and Katz, 2002). On the contrary, a recent study demonstrated that the blockade of stage II retinal waves prevents ODC formation in the ferret visual cortex (Huberman et al., 2006). This report and other studies (Stryker and Harris, 1986) point to an important role of spontaneous activity during ODC formation. Based on these partly contradictory findings, it is not possible to conclude whether ODC formation is solely regulated by molecular factors, by activity-dependent mechanisms or by a combination of both.

### 2.3.4 Visually evoked activity and critical period

Although the layout of the visual system is largely established before the animal perceives visual information, the refinement process continues well after eye-opening. With the maturation of the visual system, visual stimuli become more important for further refinement. At around P11, the mouse retina becomes light-sensitive, marking the time point when photoreceptors have established synapses with the retinal network and visually induced activity is transferred to higher visual areas (Blanks et al., 1974). Even through closed eyelids, the retina and upstream targets respond to visual stimulation (Krug et al., 2001; Colonnese et al., 2010) which, for example, drives the segregation of On- and Off-layers in the dLGN (Akerman et al., 2002).

**Eye-opening**

Eye-opening occurs at around P14 in the mouse and triggers a variety of developmental processes that contribute to the maturation of the visual system. Shortly after eye-opening, stage III retinal waves cease (Demas et al., 2003) and visual stimuli take over. During this period, visually induced activity patterns further refine the responses of the visual system. This includes the sharpening of receptive fields (Tavazoie and Reid, 2000) and the increase in acuity (Prusky and Douglas, 2004). The refinement of visual responses is accompanied by significant structural changes in the retina (Tian and Copenhagen, 2003) as well as in the dLGN (Chen and Regehr, 2000) and the visual cortex (Bence and Levelt, 2005). The opening of the eyes is accompanied by the transformation of network activity from initially highly synchronous network events, re-

sembling GDPs, to sparse activation patterns of few cells after eye-opening (Figure 2.4) (Rochefort et al., 2009). This process of sparsification is thought to contribute to the maturation of visual information processing in the developing animal.

**Critical period**

Once the initial circuitry is formed, a period of plasticity and further refinement sets in. In their pioneering work, Hubel and Wiesel discovered that the binocular representation of inputs to the visual cortex is highly plastic during a specific time window and that changes in visual experience alter this ordered representation drastically (Wiesel and Hubel, 1963; Hubel and Wiesel, 1970). The deprivation of one eye from visual inputs results in significant functional and structural changes in favor to the open eye. This so-called critical period of visual plasticity has been described in a variety of animals (Shatz and Stryker, 1978; LeVay et al., 1980; Gordon and Stryker, 1996) and sets in after eye-opening (Figure 2.4). The maturation of the inhibitory circuitry in the visual cortex seems to be the key determinant for the onset of the critical period (Hensch, 2005; Hooks and Chen, 2007). In contrast to the classic notion that the critical period for ocular dominance is restricted to a certain time span during development, it becomes clear that – at least in the mouse – the capability for visual system plasticity is maintained into adulthood (Sawtell et al., 2003; Hofer et al., 2006; Sato and Stryker, 2008). However, the degree of plasticity observed in juvenile animals cannot be reestablished. Taken together, the ability of the visual system to adapt to changes in the environment is not only restricted to a certain period of development but is maintained throughout life.

## 2.4 Objective of the study

Some years ago, it was hypothesized that the development of the cortical network is driven by distinct activity-dependent mechanisms (Khazipov and Luhmann, 2006). For instance, in the somatosensory cortex of neonatal rats, sensory deafferentiation results in a decrease but not complete loss of network activity (Khazipov et al., 2004). This led the authors to suggest that activity patterns are endogenously generated and shaped by peripheral inputs. However, to date it is not clear to what degree peripheral inputs and centrally generated inputs contribute to network dynamics in the developing cortex. More specifically, whether peripheral inputs shape centrally generated network activity or whether they elicit cortical network activity independently is not known. In addition, due to poor spatial resolution of electrophysiological recording techniques, the activity patterns of identified neurons during ongoing network activity have not been characterized. This thesis aims to investigate the hypothesis that distinct sources of network generation exist and contribute to network dynamics in the developing cortex. I characterized spontaneous activity patterns that are present in the visual cortex of the neonatal mouse before eye-opening. In addition, manipulating retinal inputs, I dissected the contributions of both retina-driven and centrally driven activity to cortical network dynamics. I chose to address these questions in the visual system for several reasons. First, it is a well established model system to study the role of activity-dependent mechanisms during brain development. Second, the visual system is hierarchically structured and the circuitry from the detection site of the stimuli to higher areas is relatively straightforward. Last, the possibilities to manipulate peripheral inputs are manifold and a variety of well documented techniques are available.

To investigate network dynamics of the developing cortical network on the level of individual cortical neurons in vivo, I established two-photon calcium imaging techniques suited for neonatal mice. The labeling of large proportions of the developing cortical network with calcium-sensitive dyes enabled me to describe the behavior of identified neurons of the visual cortex in an intact neuronal environment. To identify possible sources of cortical network activation, I manipulated spontaneous retinal

activity. This revealed contributions of both spontaneous retinal activity and centrally generated activity to ongoing network dynamics in the developing cortex in vivo.

# 3 Material and Methods

## 3.1 Material

### 3.1.1 Chemicals

| Chemical | Supplier |
|---|---|
| NaCl | Sigma |
| KCl | Sigma |
| Glucose * $H_2O$ | Merck |
| Hepes | Merck |
| $CaCl_2$ * 2 $H_2O$ | Sigma |
| $MgSO_4$ * 7 $H_2O$ | Sigma |
| $NaHCO_3$ | Merck |
| DMSO | Sigma |
| Trolox | Sigma |
| HBSS | Invitrogen |
| 20% pluronic in DMSO | Invitrogen |
| OGB-1 (AM) | Invitrogen |
| High electroendosmosis agarose | Biomol |

## 3.1.2 Buffers and solutions

**Recording solution for in vitro experiments**

| | |
|---|---|
| $H_2O$ | 450 ml |
| HBSS 10x (with $CaCl_2$ and $MgCl_2$) | 50 ml |
| $NaHCO_3$ | 0.175 g |
| $CaCl_2$ | 0.147 g |
| Trolox | 100 µl |

**Preparation of Trolox**

| | |
|---|---|
| Trolox | 250 mg |
| DMSO | 2 ml |
| Vortex until dissolved | |

**Dye buffer**

| | |
|---|---|
| $H_2O$ | 500 ml |
| NaCl | 4.383 g |
| KCl | 0.093 g |
| Hepes | 1.192 g |
| Adjust to pH 7.4 with 1 N NaOH | |

**Cortex buffer**

| | |
|---|---|
| $H_2O$ | 500 ml |
| NaCl | 3.653 g |
| KCl | 0.186 g |
| Glucose * $H_2O$ | 0.991 g |
| Hepes | 1.192 g |
| $CaCl_2$ * 2 $H_2O$ | 0.147 g |
| $MgSO_4$ * 7 $H_2O$ | 0.246 g |
| Adjust to pH 7.4 with 1 N NaOH | |

## 3.1.3 Pharmacological agents

| Chemical | Supplier |
|---|---|
| Atropin (0.1 mg/ml) | Eifelfango |
| Dental cement | Paladur, Heraeus Kulzer |
| Epibatidine (1 mM, in cortex buffer) | Tocris |
| Glucose/electrolyte solution (Sterofundin®) | Braun |
| Isoflurane | Abbott |
| Lidocain crème (2%) | Astra Zeneca |
| Lidocain solution (0.1 mg/ml) | Astra Zeneca |
| NKH477 (10 mM, in cortex buffer) | Sigma |
| Saline (0.9%) | Braun |

## 3.1.4 Equipment

| Instrument | Specifications | Supplier |
|---|---|---|
| Balance | AB 204-S | Mettler Toledo |
| PH meter | | Sartorius |
| Peristaltic pump | Minipuls 3 | Gilson |
| Vortex mixer | Vortex-Genie 2 | Scientific Industries |
| Sonicator | Emmi-5 | EMAG |
| Centrifuge | Model 5418 | Eppendorf |

| Instrument | Specifications | Supplier |
|---|---|---|
| Centrifuge tube filters | Pore size: 0.22 µm | Costar |
| Isoflurane evaporator | | Penlon |
| Balance | AB 204-S | Mettler Toledo |
| Anesthesia induction chamber | | Custom-built |
| Animal monitor software | | Custom-built |
| Syringes | | Braun |
| Heating blanket and temperature controller | | CWE |
| Head bar | Diameter: 4 mm | Custom-built |
| Super glue | | Pattex |
| Dissection scope | Stemi DV4 Spot | Zeiss |
| Surgical instruments | | FST |
| Sugis | | Kettenbach Medical |
| Cannulas | Size: 20 G and 27 G | BD |
| Electrode puller | P-97 | Sutter Instrument |
| Glass capillaries | GB 150 TF-8P | Science Products |
| Micromanipulator and controller | LN Mini25 | Luigs and Neumann |
| Picospritzer | Pressure System IIe | Toohey |
| Cover slips | Diameter: 4 mm | Electron Microscopy Sciences |
| Hamilton syringe | Volume: 5 or 10µl | Hamilton |
| Hamilton syringe pump | | WPI |
| Lab jack | | Thorlabs |
| Vaseline | | Unilever |
| Heating plate | MR Hei-Tec | Heidolph |

### 3.1.5 Custom-built two-photon microscope (based on the MOM two-photon microscope, Sutter Instruments)

| Instrument | Specifications | Supplier |
|---|---|---|
| MaiTai laser | | Spectra Physics |
| Shutter | | Uniblitz |
| Pockel's cell and modulator | Model 350-80 | Conoptics |
| Scanner | Model 6215H | Cambridge Technologies |
| Photomultiplier tube | Model R6537 | Hamamatsu |
| I/O boards | Models: PCI-6229 and PCI6110 | National Instruments |
| Objectives | 20x / 0.50 NA and 40x / 0.80 NA | Olympus |
| CCD camera | Evolution QEi | Media Cybernetics |
| Epifluorescence unit | Model BX-RFA | Olympus |
| Photo diode | Model PDA100A-EC | Thorlabs |
| Power meter | Model 407A | Spectra Physics |

### 3.1.6 Software

For image acquisition, I used custom-written software based on LabVIEW (version 2008 and 2009). For analysis and statistical tests, I used the following programs: ImageJ (V 1.41), Matlab (2008a), MS Excel (2007) and PASW Statistics 18 (see chapter 3.7).

## 3.2 In vitro study to investigate the effects of anesthesia on spontaneous network activity in the developing cortex

The in vivo experiments described in this study were all performed in the visual cortex of anesthetized neonatal mice. Due to previous studies that argue for or against profound effects of anesthetics on spontaneous activity in the brain (Adelsberger et al., 2005; Potez and Larkum, 2008; Golshani et al., 2009; Niell and Stryker, 2010; Colonnese and Khazipov, 2010), I addressed these questions first in an in vitro preparation of the developing cortex. I therefore designed a pilot study in cortical slice cultures to establish the relationship between spontaneous network activity and the anesthetic isoflurane. I prepared cortical slice cultures from C57BL/6N pups, postnatal day 1 – 3 (P1 – 3), according to the method of Stoppini et al. (Stoppini et al., 1991). After 2 – 4 days in culture (DIV 2 – 4, 37° C, 7% $CO_2$), I stained the cortical slices with OGB-1 (AM) according to a method which is described in chapter 3.4 and in previous reports (Stosiek et al., 2003; Lang et al., 2007). After the labeling, the slice was transferred to a heated imaging chamber (35° C) that was constantly superfused with recording solution. Spontaneous network activity of cortical slice cultures was recorded with a custom-built two-photon microscope (see chapter 3.5). After baseline recordings, I superfused the imaging chamber with isoflurane solution (0.2 mM) and resumed imaging. The isoflurane solution was prepared by first adding a surplus of the anesthetic to the superfusion solution to obtain a saturated solution (15 mM). The mixture was then stirred for at least three hours under airtight conditions at room temperature (Simon et al., 2001). To obtain a solution with physiologically relevant concentrations, the saturated solution was further diluted to a final concentration of 0.2 mM. The acquired image stacks before and after the addition of the isoflurane solution were analyzed in ImageJ and Excel (see chapter 3.7).

## 3.3 Surgery of neonatal mice to expose the visual cortex

To investigate activity patterns in the developing visual cortex in vivo, a craniotomy above the region of interest was made prior to neuronal labeling and calcium imaging. A clean surgery was crucial to maintain a good health status of the animal and to achieve good imaging quality. The damage of the dura and big blood vessels could impair the well being of the animal and the release of a large number of blood cells could occlude optical access to deeper structures within the brain. Thus, a clean surgery decreases the variability of the data and is a prerequisite for reproducible results.

In this study, I used neonatal mice from the strain C57BL/6N. I chose to study calcium dynamics in the visual cortex in animals after the first postnatal week, because the projections from the retina to the visual cortex are well – albeit immaturely – established. Pups were kept with their mother until the day of the experiment (P8 – 10). The animal was then immediately transferred to an anesthesia induction chamber in which the anesthetic isoflurane in pure $O_2$ was delivered to the animal (2% isoflurane at 1.7 l/min). After anesthesia had become effective, atropine (0.1 µg/g body weight) and lidocaine (0.5 mg/g body weight) in electrolyte/glucose solution (Sterofundin®, absolute volume approximately 50 µl) were injected into the neck muscle to prevent pain and to stabilize the heart beat. After an additional 20 minutes the animal was in a deeply anesthetized state. This period of time was required to completely anesthetize neonatal mice and to abolish reflexes, such as tail and hind limb withdrawal reflexes. The animal was then transferred to the imaging setup and placed on a heating blanket (35.5° C) to maintain a constant body temperature. The temperature was measured with a temperature probe that was placed below the animal's body. Throughout the entire experiment, critical physiological parameters such as heartbeat and body temperature were monitored with a custom-build animal monitor (LabVIEW based). Prior to the surgery, a thin layer of lidocain crème, a local anesthetic, was applied to the area of the craniotomy to minimize pain sensation. The scalp above the skull was carefully but amply removed with a pair of scissors and forceps. The bone underneath was then freed from debris, such as muscle and connective tissue. For stability reasons, it

was crucial that the bone was clean and dry before the head bar was placed onto the skull surface. The head bar with the opening (diameter: 4mm) above the visual cortex was attached to the skull with superglue. A thick layer of dental cement was applied for additional support and allowed to dry for approximately 15 minutes. When the dental cement completely solidified, a small craniotomy (approximately 1 – 2 mm) was made above the visual cortex (lambda: 0.5 – 2.5 mm, midline: 1 – 3 mm). Since the developing skull is very fragile, the use of a dental drill was not advisable. Instead, the bone was first thinned with a fine dissecting knife under visual guidance of a dissection microscope. After that, debris was rinsed off with cortex buffer and the bone was removed with surgical instruments. The first cut was made with a small cannula (27 G) and a rectangular region of bone above the visual cortex was removed with spring scissors, forceps and a scalpel. In any circumstance, the exposed cortex had to be kept moist with cortex buffer. In case blood vessels were damaged, the brain surface had to be cleaned from blood cells as completely as possible in order to keep the access to the brain surface clear and to accomplish good imaging quality. If bleeding occurred, it had to be stopped before the labeling could start. To maintain a good health status of the animal and to prevent it from dehydrating, the pup was given an injection of atropine (0.05 µg/g body weight) dissolved in electrolyte/glucose solution every other hour.

## 3.4 Multi-cell bolus loading in the cortex of neonatal mice

In vivo imaging of calcium dynamics in vertebrates is a well established technique and has been used to study various phenomena in the brain of living animals (Ohki et al., 2005; Mrsic-Flogel et al., 2007; Bollmann and Engert, 2009; Grewe and Helmchen, 2009). The so called multi-cell bolus loading (MCBL) technique (Stosiek et al., 2003; Garaschuk et al., 2006b), is an excellent method to stain large populations of cells and the surrounding neuropil of the desired brain region (up to 0.5mm$^3$ per injection). Here, I used the $Ca^{2+}$-sensitive dye Oregon Green 488 BAPTA-1 (OGB-1) in the acetoxymethyl (AM) ester form. The non-fluorescent dye is taken up by surrounding cells due to its uncharged, lipophilic nature, and is intracellularly cleaved by non-specific esterases to its charged, fluorescent form. MCBL is a relatively straightforward technique to label hundreds of neural cells and has been employed in various preparations of the living brain as well as in slice cultures (Garaschuk et al., 2006a; Lang et al., 2007). However, there are only few studies that investigated network activity in the brain of developing animals with this technique (Adelsberger et al., 2005; Rochefort et al., 2009; Golshani et al., 2009).

To reliably achieve good labeling quality, it was crucial to completely dissolve the dye and remove all residua from the solution. OGB-1 (AM) was first dissolved in 4 µl centrifuged (rpm 14,000; 10 minutes) 20% pluronic in DMSO and vortexed for approximately 1.5 minutes. The dye was then diluted 1:10 in dye buffer and vortexed for another 15 minutes. To completely dissolve the dye mixture, it was sonicated for 5 minutes, filtered through a 0.22 µm filter and kept in the dark until use. After successful surgery, the dye was backfilled into a standard patch electrode (3 – 5 MΩ) and attached to an electronic micromanipulator. The electrode was carefully advanced to the visual cortex (lambda: 1.5 – 2.5 mm, midline: 2.0 – 2.5 mm; Figure 3.1 A) with the help of a dissection microscope and slowly inserted through the dura into the brain. To avoid clogging of the pipette positive pressure (10 – 13 PSI) was applied throughout the procedure.

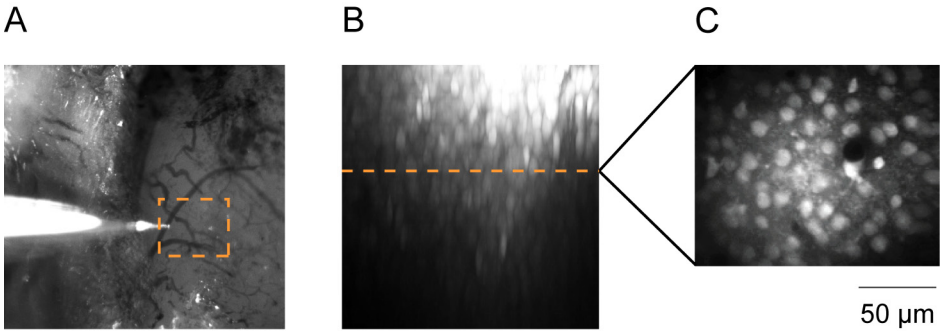


**Figure 3.1 The MCBL technique labels large populations of neural cells in the visual cortex of the developing mouse.** (**A**) View of the surface of the visual cortex after the craniotomy. Left: Borders of remaining bone after the surgery. The pipette filled with OGB-1 (AM) ready to be inserted into the brain. Right: View of the cortical surface with intact dura and blood vessels. (**B**) 3D reconstruction of the cells labeled with MBCL from a region depicted by the orange dashed box in (**A**). Note that primarily cells and neuropil in layers 1 – 2/3 of the visual cortex are labeled. (**C**) Image of labeled cells in layer 2/3 of the visual cortex, delineated with an orange dashed box in (**A**) and an orange line in (**B**).

With the control of a CCD camera and low magnification objective (20x, 0.5 NA), the electrode was advanced approximately 150 – 200 µm into the brain to target layer 2/3 cells of the visual cortex. To achieve the labeling of large populations of neurons, the dye was pressure-ejected for approximately 12 – 14 minutes at 12 PSI. This was necessary because the cortex is densely populated with neurons and diffusion rates are relatively high in the developing cortex. Short ejection periods would therefore wash out the dye very quickly which resulted in insufficient labeling. During the labeling procedure, the anesthesia level was decreased to 1.4% isoflurane. The electrode was then retracted and the anesthesia further decreased to 0.8 – 1% isoflurane. Sometimes, a second injection was placed in a neighboring region. After approximately one hour, the cells that were targeted by the MCBL injections had taken up the dye (Figure 3.1. B and C). To achieve additional stability of the preparation and good imaging quality, a thin layer of 2% high electroendosmosis agarose was applied to the cortical surface and covered with a round cover slip (diameter: 4mm). Once the agarose had solidified, two-photon imaging of calcium dynamics in the visual cortex could commence.

## 3.5 In vivo imaging of spontaneous calcium dynamics in the visual cortex of the neonatal mouse

In this study, time-lapse imaging with a custom-built two-photon microscope was used to monitor spontaneous calcium dynamics in the neuronal network of the visual cortex of developing mice. The femtosecond pulsed laser with an optimal excitation wavelength for OGB-1 (λ = 810 nm) was coupled into the scan head, equipped with two galvanometric scan mirrors, that generated the two-photon image (Figure 3.2). Laser intensities were controlled by a Pockel's cell and adjusted to values that were suitable for long-term imaging (typically 4 – 8% of the maximal laser power). The power of the laser was measured with a photo diode that collected stray light from the laser beam (Figure 3.2). A 40x water-immersion objective (0.8 NA) was used to simultaneously collect information about calcium dynamics of about 50 – 70 cells. The photons emitted by the excited fluorophore OGB-1 were collected by a photomultiplier tube. Images and time-lapse stacks were acquired with custom-written imaging software (LabVIEW-based). To monitor spontaneous network activity over time, consecutive xyt-stacks were generated (typical scan parameters: pixel size: 400 – 600 nm; field of view: x direction 200 – 280 µm, y direction 140 – 180 µm; frame rate: 3 – 6 Hz). It was important to keep a good balance between the pixel size and the acquisition speed to achieve sufficient temporal and spatial resolution. Particular attention was paid to record calcium dynamics from the same neurons across the course of the entire imaging session. Occasionally, an overview stack of the labeled network was created at the end of the imaging session to estimate the extent of the OGB-1 (AM) labeling (Figure 3.1 B). For this, xyz-stacks (typical scan parameters: pixel size: 200 – 300 nm; field of view: 300 x 300 µm; frame rate: 0.1 – 0.3 Hz) were acquired with a z-step distance of 1µm.

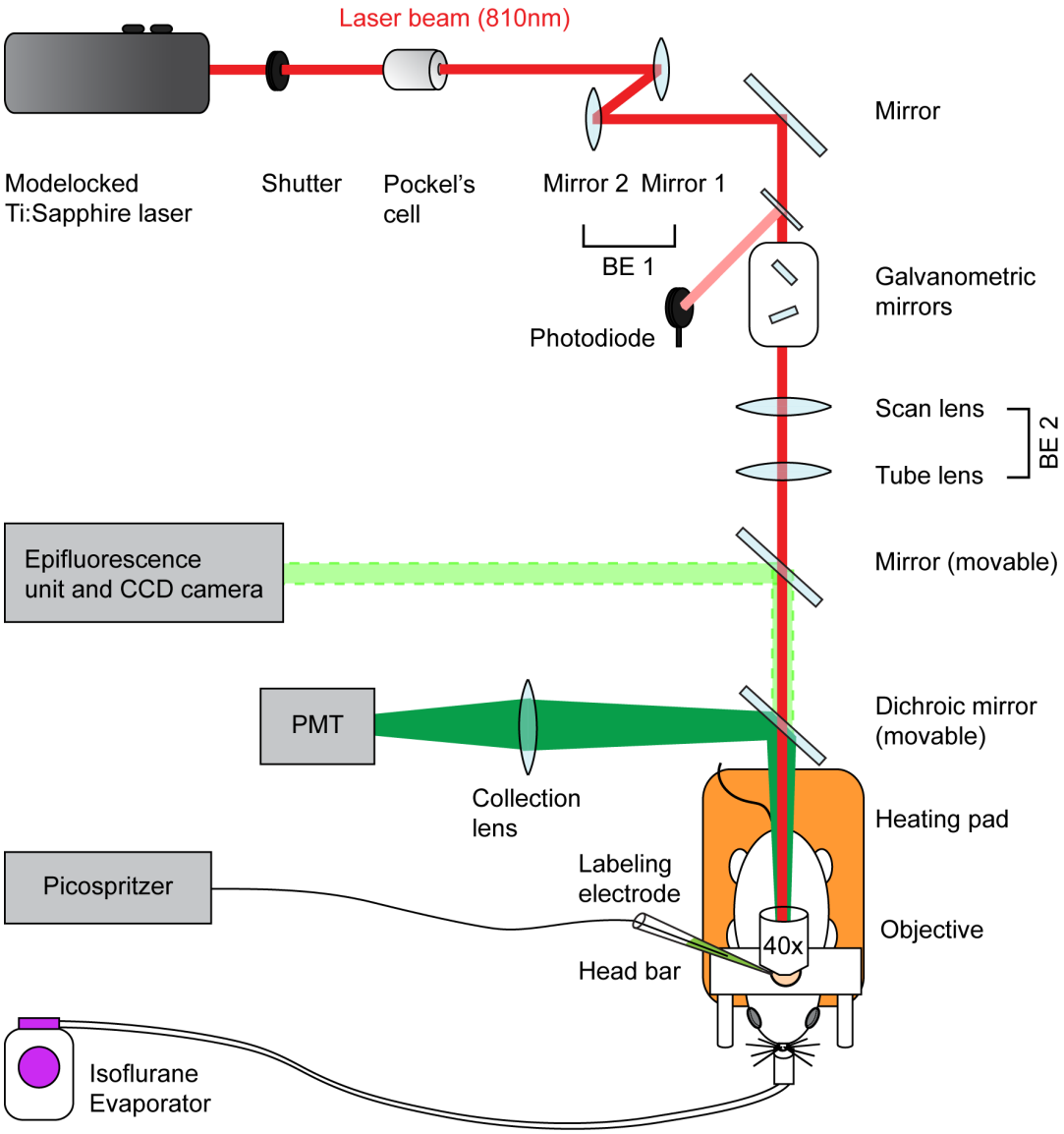


**Figure 3.2 Schematic of a custom-built two-photon microscope setup.** Excitation side: The pulsed wo-photon beam (λ = 810 nm) is generated by a Ti:Sapphire laser (red beam). The intensity and the beam width are regulated by a Pockel's cell and a beam expander (BE1), respectively. The laser power is measured by a photodiode. The fast movements of two galvanometric mirrors create a scanning image. The tube and scan lens serve as a second BE (BE2) and broaden the laser beam to optimally fill the objective's back aperture. A dichroic mirror directs the excitation beam into a high magnification objective. Emission side: Two-photon excited fluorescence (dark green beam) is collected with a collection lens and detected with a photomultiplier tube (PMT). Epifluorescence side: The setup is equipped with an epifluorescence unit and a CCD camera to optionally visualize the labeling electrode and labeled tissue (light green beam). Specimen: The anesthesia level is maintained with an isoflurane evaporator. The labeling electrode is inserted into the brain of the animal that is attached to the head bar. The calcium-sensitive dye is pressure-injected with a picospritzer.

## 3.6 Manipulations of spontaneous activity patterns in the retina of developing mice

The aim of this study was to dissect the different patterns of spontaneous network activity in the developing cortex and to establish their interdependence with retinal inputs. To study this relationship, I chose three different approaches to manipulate spontaneous retinal activity in neonatal mice before eye-opening. First, all inputs arising from the retina were removed by means of binocular enucleation. After recording baseline activity of the animal (approximately 1.5 – 2 hours), the animal was put into a deeply anesthetized state (1.5% isoflurane) to reduce stress symptoms elicited by pain. After opening the eyelids with a pair of spring scissors, the eyeballs were removed with fine forceps and spring scissors. Care was taken not to damage large blood vessel because this resulted in a rapid decline of the animal's well-being. After the animal recovered from the deep anesthesia, imaging resumed at low isoflurane concentrations (0.8 – 1%). Second, to study the behavior of cortical activity related to augmentation of spontaneous activity in the developing retina, 10 mM NKH477, a cAMP activator, was applied to both eyes (Hanganu et al., 2006). NKH477 is a forskolin analogue and known to increase the frequency and amplitude of spontaneous cholinergic waves in the developing retina (Stellwagen et al., 1999). After acquiring baseline recordings of cortical activity, the drug was injected with a Hamilton syringe pump attached to a lab jack into both eyes of the animal. The eyelids were opened with spring scissors and the syringe was advanced to one eye. To better penetrate the eyeball with the Hamilton syringe (volume: 5 or 10 µl), the eyeball was stabilized with fine forceps. A small volume of the drug (~ 0.75 µl) was slowly injected into each eye (~ 1µl / min). After the injection, Vaseline was applied to the opened eyes to prevent them from drying-out. Third, to de-correlate patterned activity in the retina (Cang et al., 2005b; Sun et al., 2008a), binocular injections of 1 mM epibatidine were performed in the same way as for NKH477. Saline solution was injected under the same conditions as a control to ensure that the effect observed in the visual cortex is due to the injected drug and not due to the injection procedure itself.

## 3.7 Analysis of spontaneous network activity in the developing cortex in vivo

To analyze spontaneous network activity recorded in the developing mouse cortex in vivo, the acquired stacks were mainly analyzed by custom-written analysis algorithms in Matlab. For optimal analysis results, the image stacks were pre-processed. This included the removal of large movement artifacts of the animal that occurred during the recordings. Additionally, due to the fast scanning, the pixels in each image of the stacks were shifted against each other and realigned with a custom-written algorithm in ImageJ. To decrease the file size, the image stacks were scaled down in ImageJ to half the pixel size. This decreased the file size of the stacks by about eight times but left sufficient information about the recordings. In addition, to make the stacks suitable for the use with the custom-written Matlab codes, rectangular image stacks had to be brought into squared stacks with ImageJ. Once the stacks were pre-processed, they were fed into the Matlab codes designed by K. Ohki and colleagues (Ohki et al., 2005). This algorithm automatically detected the contours of the cells based on the average of the stack. Then, it computed the corresponding cell mask (Figure 3.3 A) and assigned a region of interest (ROI) to each detected cell. Based on this cell mask, the average gray values within the ROIs were calculated and the corresponding $F/F_0$ traces were generated (Figure 3.3 B, left). $F/F_0$ traces represent changes in the intracellular calcium ($Ca^{2+}$) concentration in relation to the average fluorescence of all frames ($F_0$). The fluorescence of each frame of the image stack (F) is divided by $F_0$, which resulted in normalized traces of changes in intracellular $Ca^{2+}$ concentrations of all cells identified in the image stack. Next, the $F/F_0$ traces were used to detect activity events of individual cells. To reliably determine activity events and to prevent the detection of false positive events through noise, both the $F/F_0$ trace and its differential were used for signal detection (calculated by $F/F_{0\ \text{frame n}} - F/F_{0\ \text{frame n-1}}$). The peak intensity value, when both traces reached the threshold, was considered as an event of an individual cell (Figure 3.3 B, middle). The threshold was determined by 1- to 4-times the standard deviation.

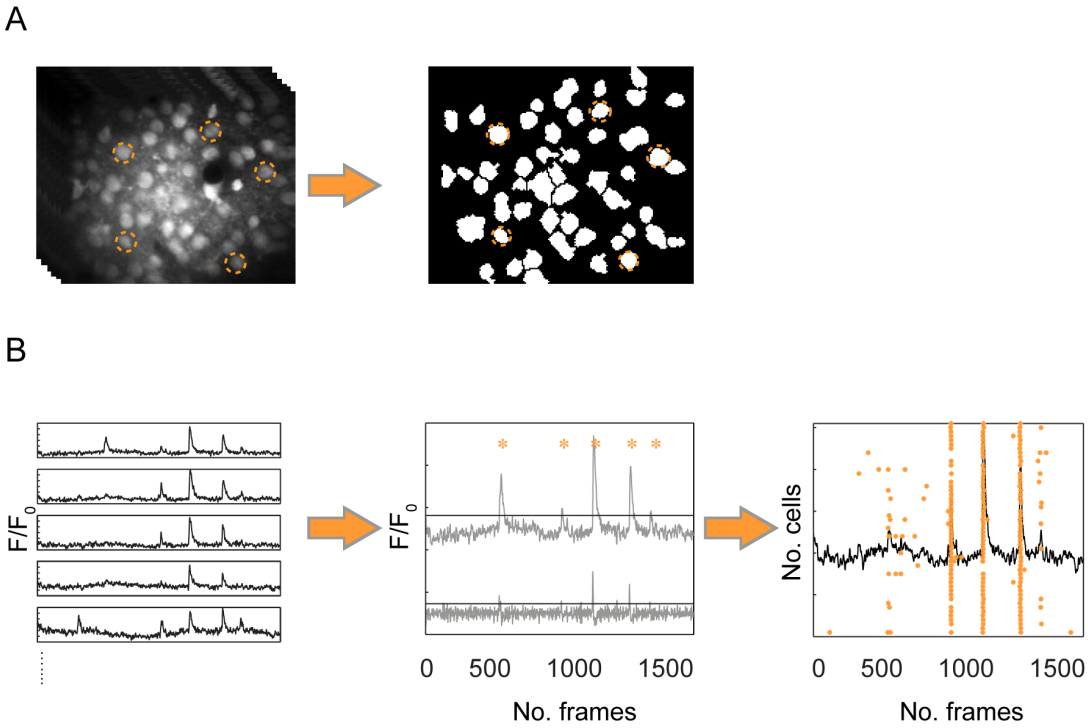


**Figure 3.3 Overview of the analysis routine of spontaneous network activity.** (**A**) Left: Graphic stack of layer 2/3 cells of the visual cortex of developing mice. Right: Cell mask derived from the stack left, generated by a custom-written algorithm. (Note that the white contours resemble the cells in the stack to the left as depicted by the orange dashed circles.) (**B**) Left: Example $F/F_0$ traces of spontaneous calcium dynamics of individual cells for one recording. Middle: The $F/F_0$ trace (top) and the corresponding differential trace (bottom) for an individual cell. If both traces reach the threshold (solid black lines) at the same time, the time point is considered as an activity event of the cell. Right: Network activity of all analyzed cells (black trace) and the corresponding activity of individual cells (orange bullets).

The number of the standard deviations used as threshold was adjusted from experiment to experiment but remained the same within an experiment. A network event was defined by a minimum participation rate of 6% of the imaged cells (Figure 3.3 B, right). Only network events with a participation rate of more than 20% of the cells were generally included in the quantification analyses. For instance, to quantify the synchronicity of network events, the jitter of individual cellular events within a network event was determined. This value was calculated as the standard deviation of the time in which individual cells showed maximal activation. In addition, the mean peak amplitude of the calcium signals of individual cells was calculated and used as another parameter for the characterization of spontaneous network events in the vis-

ual cortex. All graphs and quantification analyses were generated in Matlab and Excel. Data are reported as means ± SEM. Statistical significances were calculated using a two-tailed, paired t-test for comparison of before and after conditions in the retinal manipulation studies, and ANOVA statistics with post-hoc Bonferroni correction otherwise.

# 4 Results

In the visual cortex of rodents, the second postnatal week is a period of continuous activity-dependent rearrangements of neuronal connections. In the present study, I investigated the different patterns of spontaneous network activity that contribute to these rearrangements in the visual cortex of the developing, live mouse. Two-photon calcium imaging of populations of neurons revealed spontaneous network events that recurred intermittently. Detailed analysis showed that these events differed in several parameters. For instance, the participation rate, i.e. the fraction of cells that participated during individual cortical network events, ranged from local populations to all cells in the field of view. Moreover, the jitter, a measure of the synchronicity of the activation of cells as well as the mean amplitude of the cellular calcium signal was different during individual network events.

Cortical network events with a high participation rate (> 80%) showed higher synchronicity and higher mean amplitude than events with a low to medium participation rate (20 – 80%). By manipulating retinal activity, I demonstrated the contributions of peripheral inputs to the generation of cortical network activity. Interestingly, while most cortical activity with low to medium cellular participation rates was affected by retinal manipulations, the network events with a high participation rate were not affected by any retinal manipulation. These results suggest that large parts of spontaneous activity in the developing visual cortex are driven by retinal inputs. However, cortical network events with a high participation rate are independent of retinal inputs and presumably triggered by central mechanisms. These different modes of network activation might provide a mechanism to differentially wire the neuronal circuitry in the developing visual cortex.

## 4.1 Network activity of organotypic cortical slice cultures is not affected by the anesthetic isoflurane

In vivo experiments have striking advantages over studies that use in vitro preparations. Studying live animals reflects the mechanisms that are present during normal brain function or during learning and memory much better than experiments in organotypic slice cultures. Despite the many benefits of in vivo preparations, some constraints have to be considered. For instance, anesthetics have considerable effects on the physiological properties of the neuronal network (Potez and Larkum, 2008; Kuhn et al., 2008) and since the first in vivo imaging experiments, the impact of anesthetics on cortical function has been controversially discussed (Adelsberger et al., 2005; Murayama et al., 2009; Golshani et al., 2009). The impairing effects of anesthesia on brain functioning would alter experimental results and make their interpretation difficult.

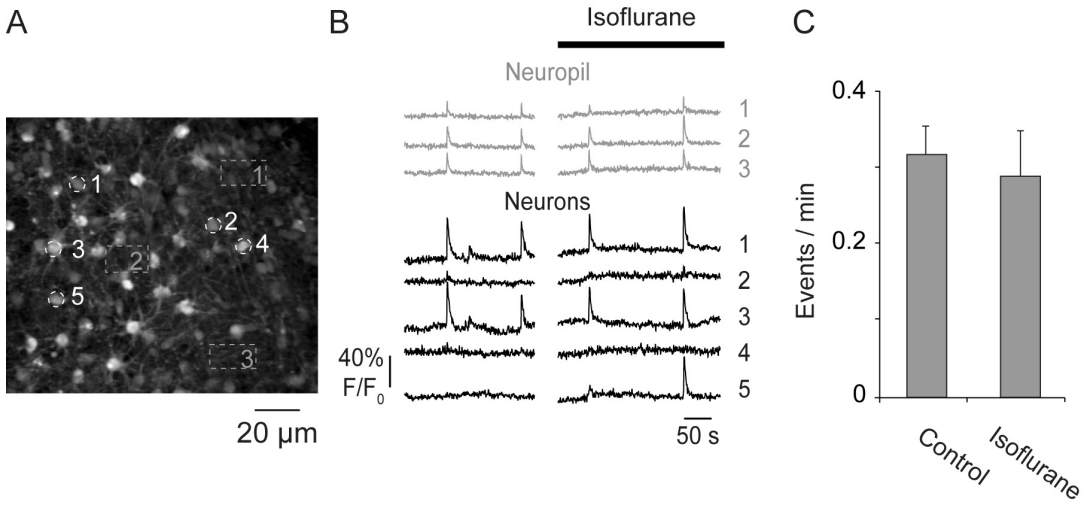


**Figure 4.1 The frequency of spontaneous network events in cortical slice cultures is not altered by isoflurane.** (**A**) Populations of cells labeled with the calcium-sensitive indicator OGB-1 (AM) in a cultured cortical slice of a neonatal mouse (P2, DIV2). (**B**) Example traces of spontaneous network dynamics in the neuropil (gray traces) and in neurons (black traces) depicted in (**A**) before and after the superfusion of isoflurane (0.2 mM, black bar). (**C**) The frequency of spontaneous network events is not changed after the application of the anesthetic isoflurane.

To investigate whether the anesthetic isoflurane has significant effects on spontaneous network activity in the developing mouse cortex in vivo, I designed an in vitro pilot study prior to the actual research project (Figure 4.1). Large populations of cells in organotypic cortical slice cultures were labeled with OGB-1 (AM) and spontaneous network dynamics were monitored with two-photon imaging. After recording baseline activity, the recording chamber was superfused with an isoflurane solution (0.2 mM). Interestingly, the anesthetic isoflurane did not affect the occurrence of spontaneous activity events in cortical slice cultures (control: 0.32 / min ± 0.04; n = 12 slices; isoflurane: 0.30 / min ± 0.06; n = 4 slices; $p > 0.05$; Figure 4.1 B and C).

## 4.2 The developing visual cortex generates distinct patterns of spontaneous network activity in vivo

The study of spontaneous activity patterns in slice cultures of the visual cortex is limited. The majority of neuronal circuits are severed after slice preparation, leaving only a small fraction of connections between neurons intact. Additionally, numerous ectopic connections between neurons are formed during the incubation time. Thus, the circuitry in organotypic cultures is partially artificial and does not reflect neuronal connectivity of live animals. To study spontaneous activity patterns of the developing visual cortex in a natural setting, it is crucial to interfere as little as possible with the preparation and leave all inputs to the visual cortex intact. Therefore, I established in vivo calcium imaging techniques to monitor spontaneous activity of neuronal populations in the intact visual cortex of the neonatal mouse. The multi-cell bolus loading technique (Stosiek et al., 2003) was adapted to gain optimal labeling quality in the visual cortex of developing mice (i.e. higher pressure and longer ejection time). This resulted in the labeling of large populations of neurons and the neuropil (Figure 4.2 A). Two-photon calcium imaging of layer 2/3 cells of the visual cortex revealed synchronous network events that recurred intermittently (frequency: 1.3 / min ± 0.16; n = 23 animals; Figure 4.2 B). Likewise, spontaneous network dynamics could be monitored in the neuropil. Not all cells in the field of view participated in each network event, but nearly all cells showed spontaneous activation over the course of the experiment (Figure 4.2 B). This suggests that the vast majority of neurons in the visual cortex are connected to neighboring cells and are recruited to spontaneous network events at this stage of development.

It has been argued in the last few years that spontaneous network events in the developing rodent cortex are highly sensitive to anesthetics (Adelsberger et al., 2005; Colonnese and Khazipov, 2010). To study this phenomenon in more detail, I established the relationship between the concentration of the anesthetic inhaled by the animal and the frequency of spontaneous cortical network events.

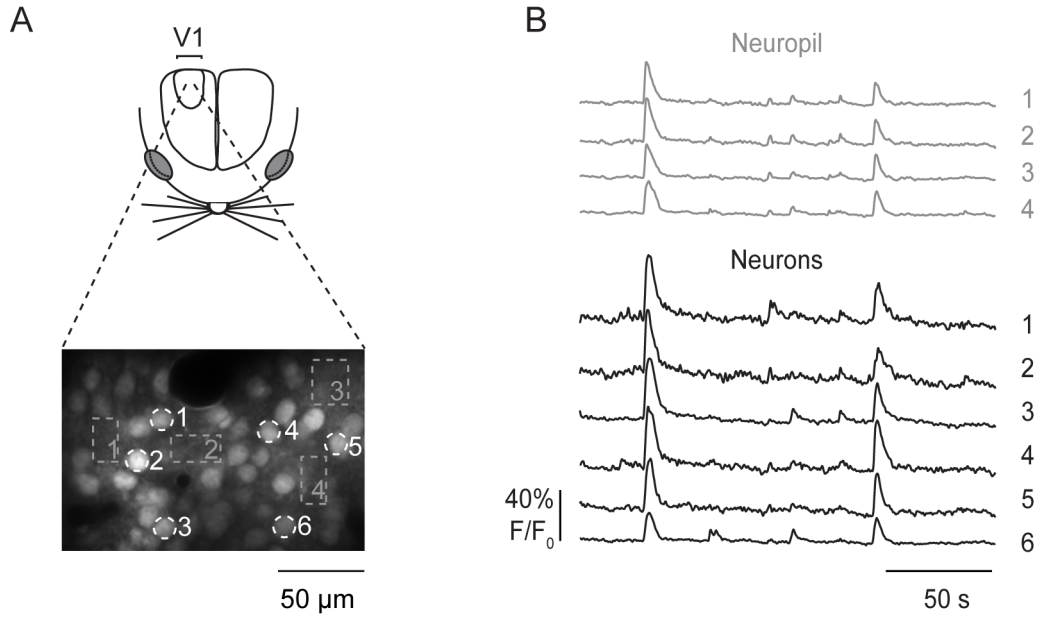


**Figure 4.2 In vivo spontaneous network activity in the visual cortex of the neonatal mouse.** (**A**) Top: Schematic of the neonatal mouse and the approximate location of the primary visual cortex (V1). Bottom: Layer 2/3 cells in the visual cortex of a P10 animal labeled with OGB-1 (AM). (**B**) Example traces of spontaneous network dynamics in the neuropil (gray traces) and in neurons (black traces) depicted in (**A**).

While the levels of isoflurane were gradually shifted from a nearly awake state (0.4% isoflurane) to a medium anesthetized state (0.7% isoflurane) and to a deeply anesthetized state (1.5% isoflurane), spontaneous network activity in the visual cortex was recorded at the same time. Consistent with previous observations (Potez and Larkum, 2008; Greenberg et al., 2008), the occurrence of spontaneous network events in the visual cortex was dependent on the concentration of the anesthetic inhaled by the animal (Figure 4.3). The frequency of spontaneous network events decreased with increasing levels of isoflurane (0.4% isoflurane: 2.2 / min ± 0.22; n = 6 animals; 0.7% isoflurane: 1.6 / min ± 0.21; n = 4 animals). Cortical network events were almost absent at high anesthesia concentrations (1.5% isoflurane: 0.1 / min; n = 2 animals; $p < 0.05$, one-way ANOVA). It should be noted that extending the time of exposure to high isoflurane concentrations (1.5 – 2% isoflurane) far beyond the time required for surgery abolished all spontaneous network activity in the visual cortex of developing mice.

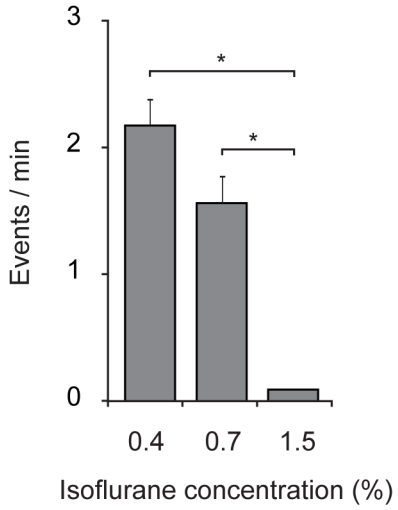


**Figure 4.3 The frequency of spontaneous network dynamics in the developing mouse visual cortex is decreased with increased levels of anesthesia.** The frequency of spontaneous network events is dependent on the concentration of isoflurane inhaled by the animal. Increased levels of anesthesia decrease the occurrence of spontaneous network activity events.

In contrast, experiments in awake animals revealed an increase in the frequency of spontaneous network events compared to the lightly anesthetized state (Figure 4.4). After the acquisition of baseline recordings at anesthesia levels of 0.8 – 1%, isoflurane ceased and the same cells in the cortex were imaged in the awake animal (Figure 4.4 A). As expected, the frequency of network events that recruited more than 20% of the imaged cells increased in the awake condition (anesthetized: 2.1 / min ± 1.2; awake: 3.2 / min ± 1.0; n = 2 animals; Figure 4.4 B and C). Other parameters of network dynamics, such as participation rate and jitter of spontaneous network events were essentially not changed (participation rate: anesthetized: 61.1% ± 5.2; awake: 57.6% ± 9.4; jitter: anesthetized: 0.5 s ± 0.1; awake: 0.5 ± 0.1; n = 2 animals; Figure 4.4 C). In the awake condition, numerous imaging artifacts such as elevated breathing rates or movements of the animal were observed and complicated the analysis. Taken together, the properties of network events barely differed between the anesthetized (0.8 – 1% isoflurane) and awake conditions. In addition, motion artifacts were almost absent in the lightly anesthetized animal which improved the image quality significantly. Consequently, all subsequent in vivo recordings were performed under light anesthesia. This provided replicable results of spontaneous network activity that are nevertheless comparable to the awake state.

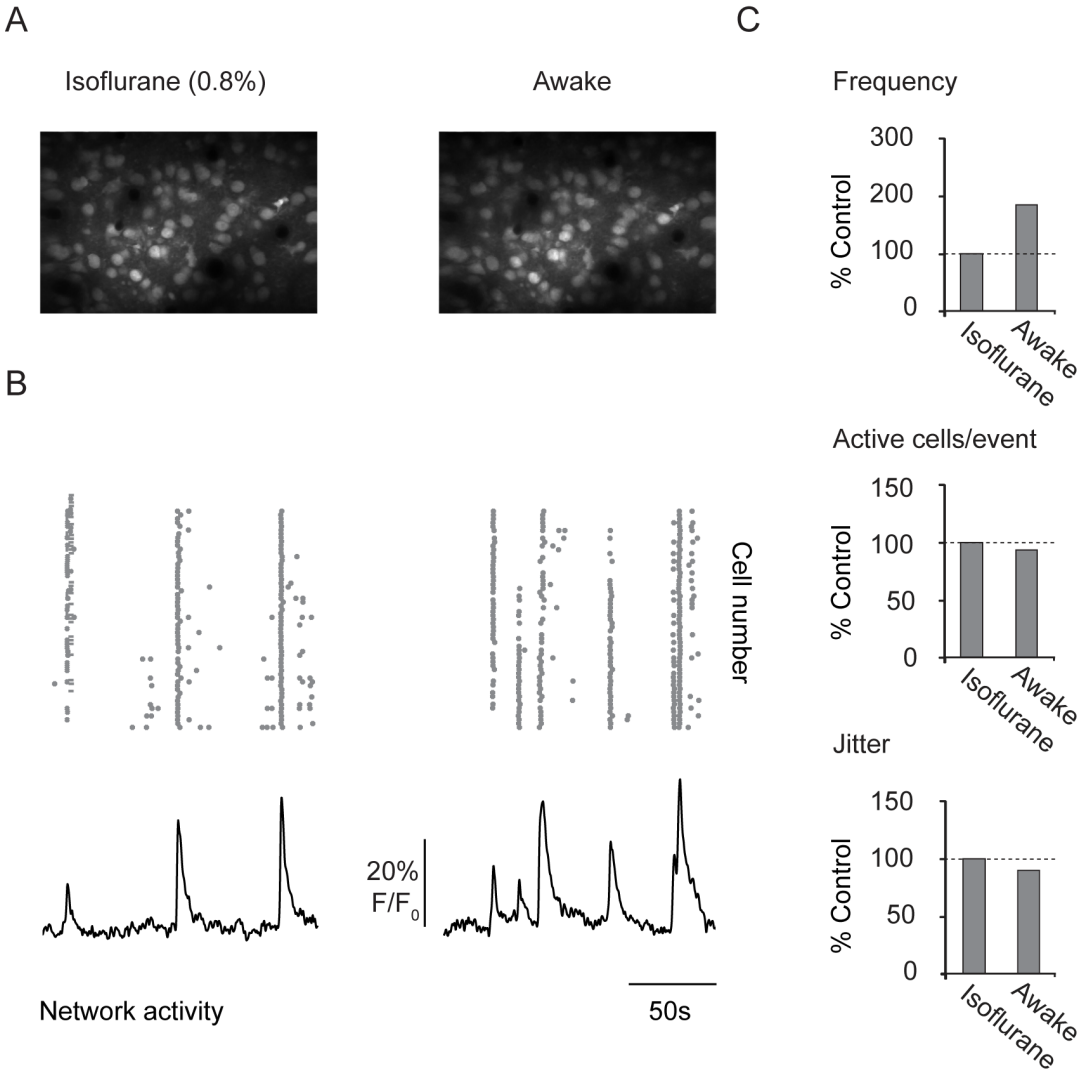


**Figure 4.4 The frequency but not the overall characteristics of spontaneous network events are changed by the anesthetic isoflurane.** (**A**) Example of OGB-1 (AM) labeled layer 2/3 cells in the visual cortex of a P10 mouse in the anesthetized (left) and awake (right) state. Note that the same cells were monitored over the course of the experiment. (**B**) Top: Active cells are depicted as gray bullets over time in the anesthetized (left) and awake (right) condition. Bottom: Network activity plotted as the mean activity of all imaged cells over time. (**C**) Quantification of basic parameters of spontaneous network activity in the anesthetized and awake animal. Top: The frequency of spontaneous network events is increased after removal of isoflurane. Middle: The fraction of co-active cells is not changed. Bottom: The jitter not significantly changed in awake animals.

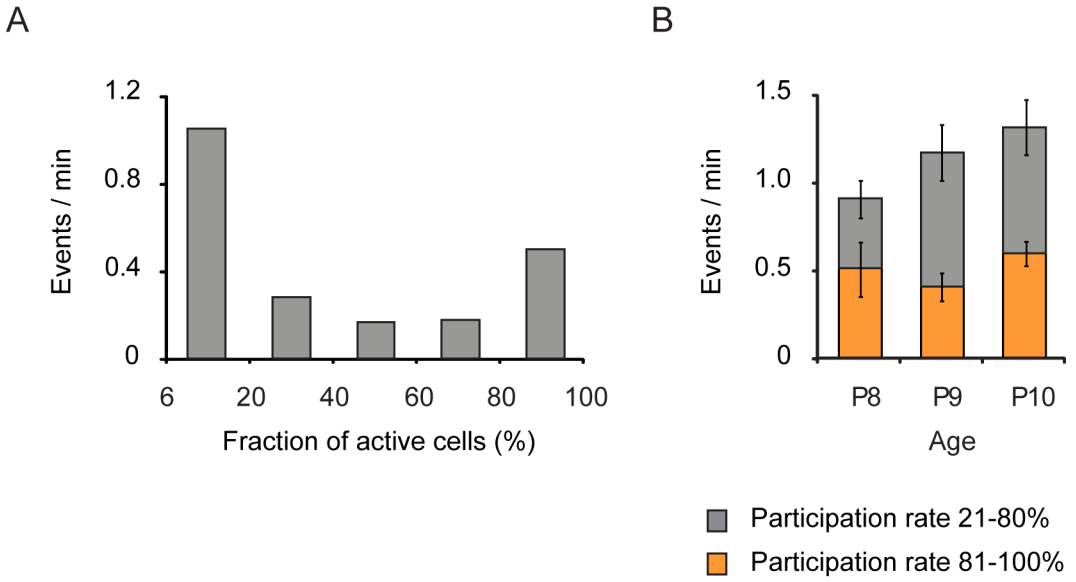


**Figure 4.5 Spontaneous network events recruit various proportions of cells from P8 to P10.** (**A**) The fraction of active cells per spontaneous network event is not evenly distributed. Most events comprise either few cells (6 – 20%) or the majority of cells (> 80%) in the field of view. (**B**) The overall frequency of spontaneous network events increased from P8 to P10. Note that the frequency of large network events (80%, orange bars) does not change with age.

A more detailed analysis of the recordings of spontaneous network activity in the developing visual cortex demonstrated that individual cells were only active in a fraction of the network events (Figure 4.2 B and Figure 4.5 A). The participation rate per spontaneous network event varied from the simultaneous activation of only a few cells (6 – 20%) to events with a medium participation rate and to highly synchronous events in which almost all cells in the field of view were activated at the same time (>80%; Figure 4.5 A). The mean participation rate per network event was 62.3% ± 12.1 (n = 23 animals).

Although the maximum age difference of the animals used in this study was only two days (P8 – 10), relevant changes in the properties of spontaneous network activity over the course of development could be observed. The frequency of spontaneous network events increased from P8 to P10 by almost 60% (P8: 0.8/min ± 0.1; n = 4 animals; P10: 1.3/min ± 0.1; n = 7 animals; $p > 0.05$ for all three age groups, one-way ANOVA; Figure 4.5 B). Detailed analysis revealed that the increase in the frequency of

spontaneous network events was specifically limited to events with a participation rate lower than 80% (Figure 4.5 B). In contrast, the frequency of network events that recruited all cells in the field of view was not changed over the course of this developmental period. The fact that network events with a participation rate of more than 80% are not changed as the animal matures, prompted me to study whether events with a low participation rate (20 – 80%) and events with a high participation rate (> 80%) are qualitatively different.

The temporal alignment of individual cells during network events with a high participation rate is more precise than for the other events. The synchronicity, defined by the jitter of network events, is smaller in events with a low to medium participation rate (20 – 80%) than in large network events with more than 80% co-active cells (20 – 80%: 0.5 s ± 0.01; 81 – 100%: 0.4 s ± 0.01; n = 1,108 events in 23 animals; Figure 4.6 A). Another parameter that is significantly different in large network events is the mean amplitude of the cellular calcium signal during a network event. On average, the cellular calcium signal has a lower amplitude when 20 – 80% of the imaged cells were co-active than in high participation events (20 – 80%: 1.12 $F/F_0$ ± 0.003; 81 – 100%: 1.24 $F/F_0$ ± 0.005; n = 1,023 events in 23 animals; Figure 4.6 B). This sharp increase in amplitude suggests that individual cells get more presynaptic inputs during large network events.

Taken together, the properties of spontaneous network events in the visual cortex of neonatal mice differ in at least three independent parameters. The synchronicity is significantly higher in network events with a high participation fraction than in the other events. Similarly, the mean amplitude of the cellular calcium signal, an indirect measure for presynaptic inputs, is significantly higher in events with a high participation rate compared to events with low to medium participation rates. In addition, the frequency of large network events does not change during development whereas the frequency of events with a low participation rate increases during development.

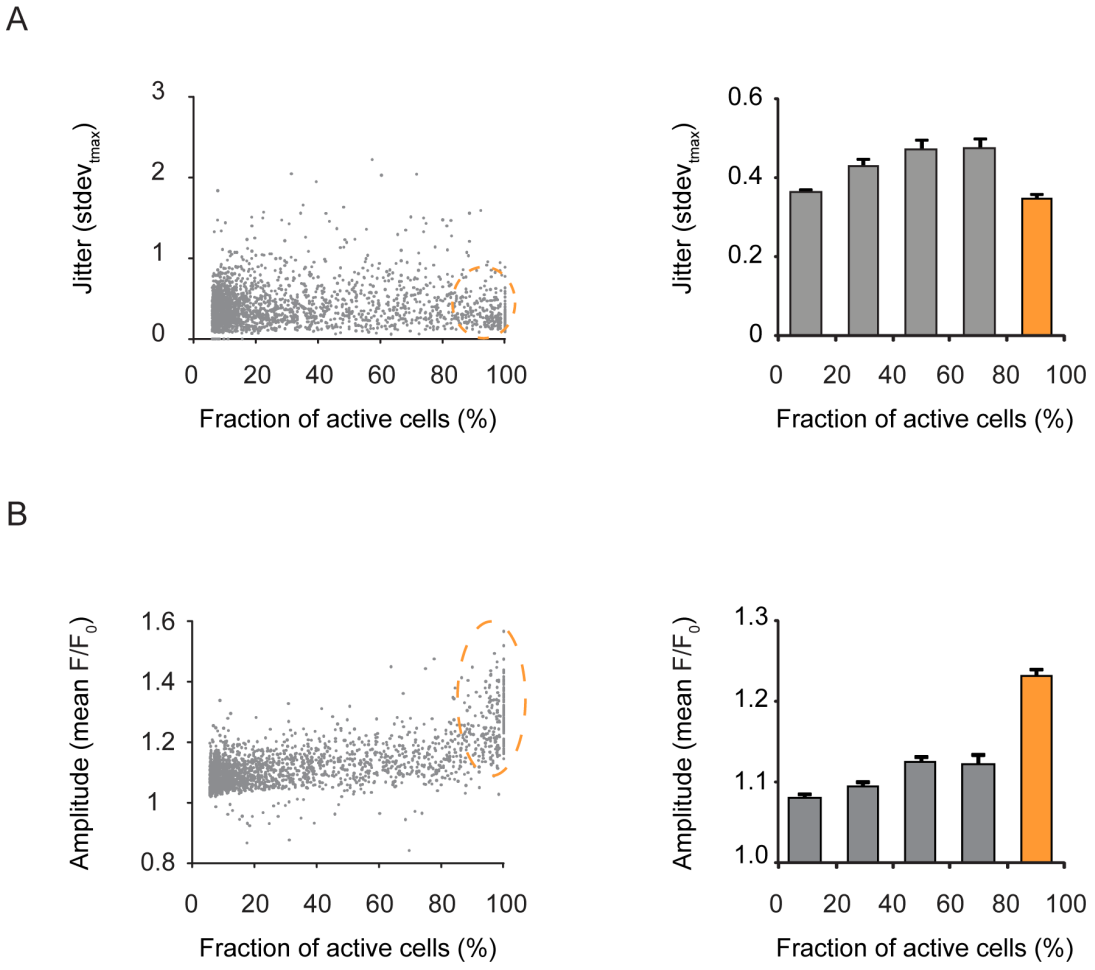


**Figure 4.6 Spontaneous network events with a high participation rate differ in jitter and amplitude from events with lower participation.** (**A**) Left: The scatter plot of the jitter of all network events reveals that the synchronicity is smaller in events with a low to medium participation rate compared to events with high participation (> 80%, dashed orange circle). Right: Bar graph of the mean jitter for various participation fractions. (**B**) Left: Scatter plot of the mean amplitude of the peak $F/F_0$ signal during network events. The dashed orange circle marks events with a high participation rate that show higher amplitudes than the remaining events. Right: Quantification of the mean amplitude for various participation rates.

To determine whether these two distinct patterns of activation are qualitatively different, I examined the effect of manipulation of spontaneous peripheral activity on network activity in the visual cortex. To study the different patterns of network activity in the developing visual cortex and their dependence on retinal inputs, I used three different paradigms to determine the contributions of retinal inputs to the generation of spontaneous activity. First, all retinal inputs were removed by means of binocular enucleation. Second, retinal waves were augmented by means of binocular NKH477 injections. Last, patterned retinal activity was pharmacologically disrupted by epibatidine which is known to de-correlate the firing patterns of retinal neurons.

## 4.3 Binocular enucleation reduces network activity in the developing visual cortex

After the first postnatal week, the mouse retina is still insensitive to light but generates spontaneous waves of activity that simultaneously activate large proportions of retinal cells (Wong, 1999). The complete removal of retinal inputs in the first weeks of development affects the development of upstream targets of the visual system (Crair et al., 1997; Wallace and Bear, 2004; Hanganu et al., 2006).

In the present study, retinal inputs were removed by means of binocular enucleation to determine the impact of retinally driven activity on cortical network events before eye-opening. To gather information about the same cells before and after binocular enucleation, identical cells were continuously monitored during the course of the experiment (Figure 4.7 A). After recording baseline activity, all inputs arising from the retina were eliminated. Both eyes were surgically removed under increased anesthesia and imaging resumed after recovery. Interestingly, residual network activity was observed after the animal had been deprived of retinal inputs (Figure 4.7 B). This is in agreement with our in vitro experiments (chapter 4.1) and with previous reports in organotypic slice cultures (Yuste et al., 1992; Garaschuk et al., 2000; Corlew et al., 2004). Here, spontaneously generated network oscillations were observed although the majority of connections were severed. After the deprivation of retinal inputs, the frequency of cortical network events that comprised at least 20% of the imaged cells significantly decreased (control: 1.6 / min ± 0.3; enucleation: 1.0 / min ± 0.2; n= 6 animals; $p < 0.05$; Figure 4.7 B and Figure 4.8 A). The effect of binocular enucleation on cortical network activity was not age-dependent because a similar decrease of activity was observed at both ages examined (P9: control: 1.7 / min ± 0.6; enucleated: 1.1 / min ± 0.3; P10: control: 1.5 / min ± 0.2; enucleated: 0.9 / min ± 0.1; n = 3 animals in each age group; Figure 4.8 A). Intriguingly, after depriving the animal of visual inputs, only network events with a significantly higher participation fraction than during baseline recordings were observed (control: 62.0% ± 3.4; enucleation: 72.7% ± 4.4; n = 6 animals; $p < 0.05$; Figure 4.7 B and Figure 4.8 B).

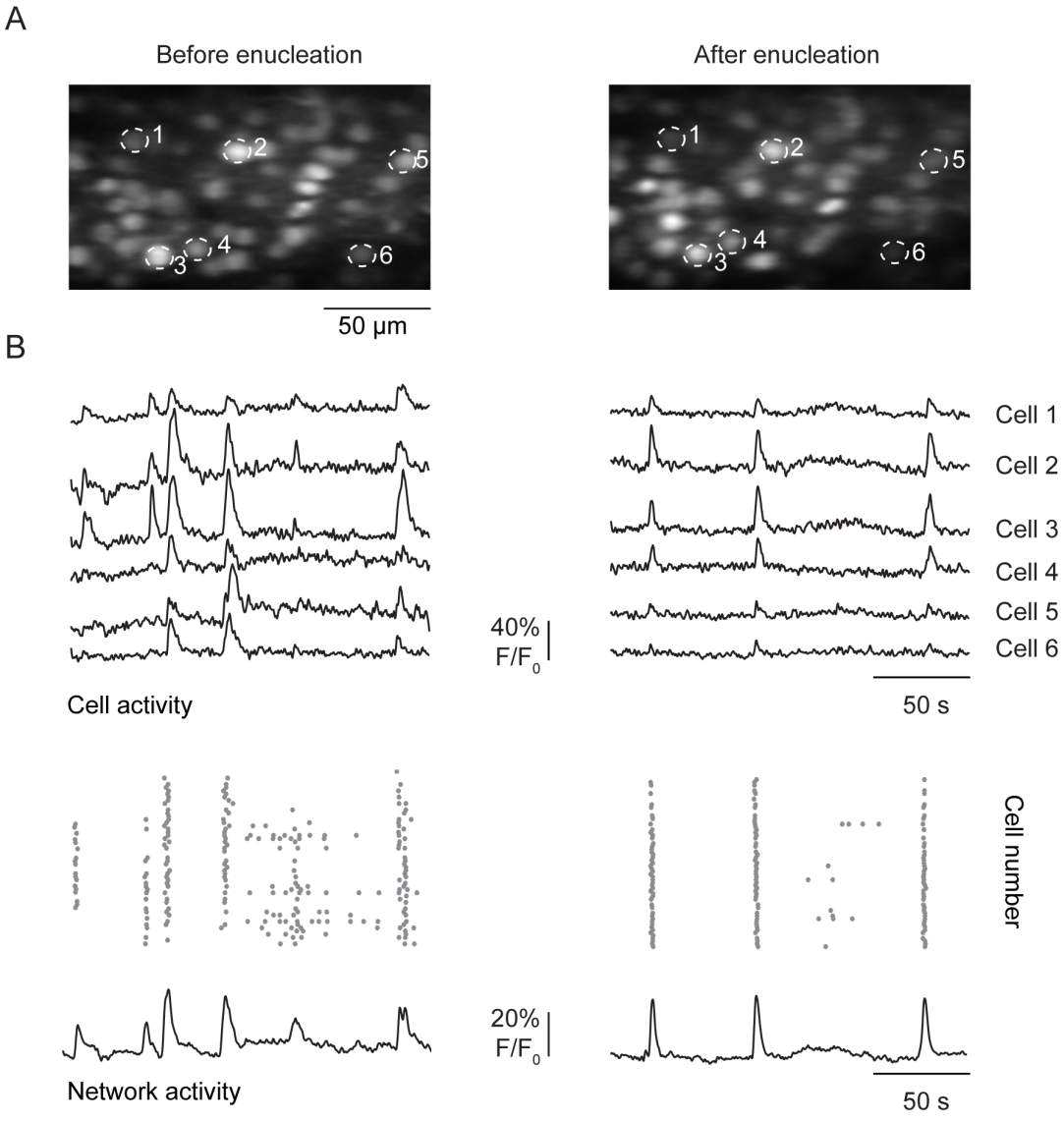


**Figure 4.7 The removal of retinal inputs decreases the frequency of cortical network events.** (**A**) Image of OGB-1 labeled layer 2/3 cells of the visual cortex of a P9 mouse before (left) and after (right) binocular enucleation. Note that the same cells were monitored throughout the experiment. (**B**) Top: Example traces of neurons marked in (**A**) before and after removal of retinal inputs. Bottom: Network activity before and after binocular enucleation (black traces) and corresponding cellular activation patterns (gray bullets).

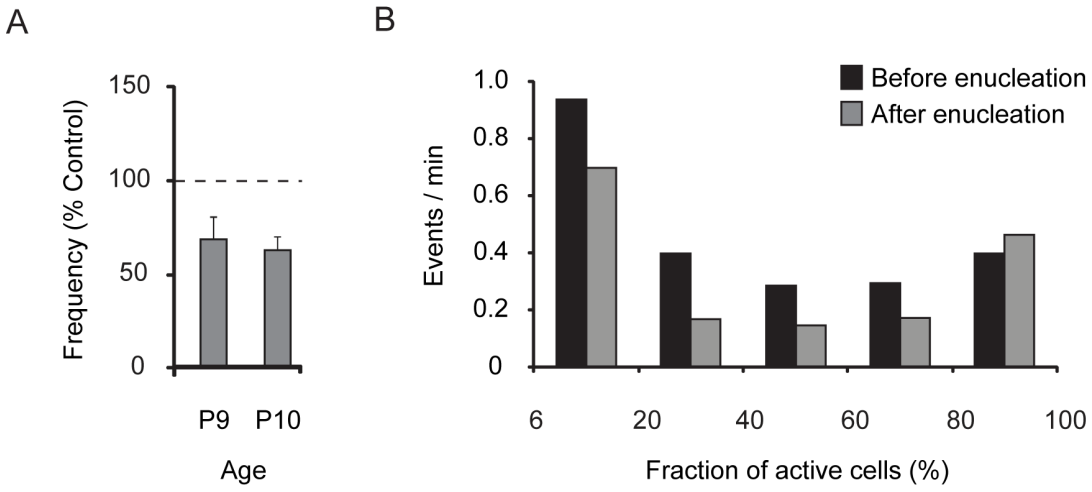


**Figure 4.8 The effect of binocular enucleation is not age-dependent, but specifically eliminates cortical network events with a low to medium participation rate.** (**A**) In both ages studied, the frequency of network events is decreased after removal of retinal inputs compared to baseline activity. (**B**) The network events with a low to medium participation rate (6 – 80%) are specifically affected by binocular enucleation (gray bars) compared to baseline activity (black bars). In contrast, events with a high participation rate (> 80%) were unaffected.

In summary, depriving the animal of retinal inputs decreased the overall frequency of spontaneous network events in the visual cortex by about 40%. Interestingly, the reduction of event frequency is specifically caused by a selective decrease in cortical network events with low to medium participation rates. Events with a participation rate of 80% and more, however, were not affected by deprivation (Figure 4.7 B and 4.8 B). This differential effect after depriving the animal of spontaneous retinal activity suggests that two or more sources of generation of cortical network activity exist. At least one of these mechanisms is independent of retinal inputs and has the ability to evoke synchronous firing in large populations of neurons in the visual cortex.

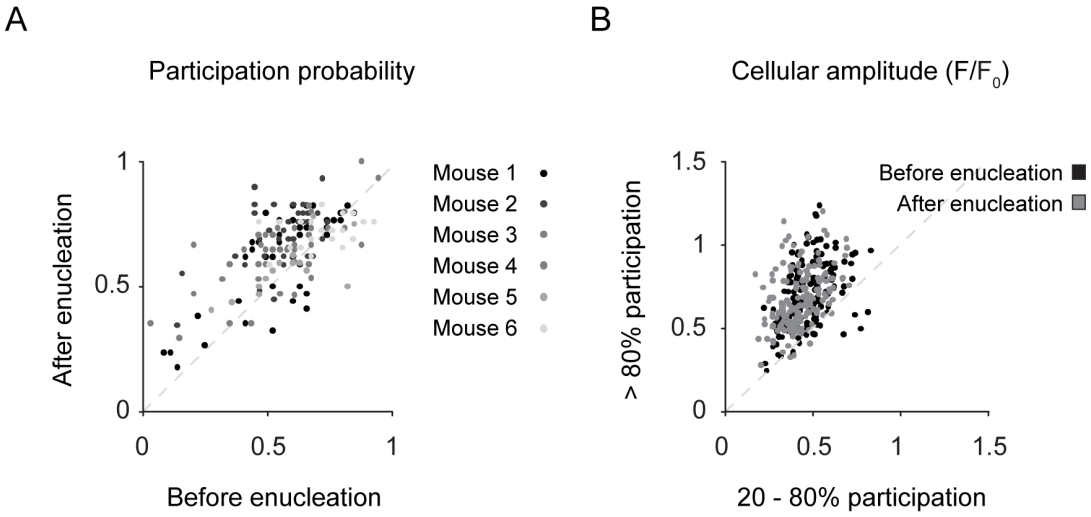


**Figure 4.9 The complete removal of retinal inputs affects participation probability of individual neurons but leaves the amplitude unaffected.** (**A**) The probability to participate in a network event with more than 20% participation is increased in the majority of the neurons after binocular enucleation. Each dot represents an identified layer 2/3 neuron in individual animals (gray shades) that could be followed over the entire experiment. (Dashed line: regression line) (**B**) The amplitude of network events with a participation rate of more than 80% is generally higher than of events with low to medium participation rates. Each dot represents an individual neuron that was imaged before (black dots) and after binocular enucleation (gray dots; dashed line: regression line).

Intriguingly, all analyzed neurons in the developing visual cortex participated in at least one network event with a participation rate of 20% and more (Figure 4.9 A). For the enucleation study, the activity patterns of the same identified cells were analyzed before and after the removal of retinal inputs, and the participation probability for each cell was calculated. In all animals, the probability of individual cells to participate in a network event was significantly higher after the removal of all retinal inputs (control: 0.6 ± 0.01; enucleated: 0.65 ± 0.01; n = 161 cells in 6 animals; $p < 0.05$; Figure 4.9 A). This is in line with the observation that the majority of cortical network events after binocular enucleation were comprised of events with a high participation rate (Figure 4.8 B).

As mentioned in chapter 4.2, the mean amplitude is generally higher in network events with a high participation rate than in events with low to medium participation rates (Figure 4.6 B). To investigate whether this relation is also true on the level of

individual cells, the mean amplitude of identified cells was calculated before and after binocular enucleation. Binning the events into classes with low to medium participation rates (20 – 80%) and high participation rates (> 80%) revealed that the majority of analyzed neurons act in a similar manner (Figure 4.9 B). The amplitude for all neurons during events with high participation was generally significantly higher than during events with low to medium participation rates (high participation: 1.2 $F/F_0$ ± 0.01; low to medium participation: 1.1 $F/F_0$ ± 0.01; n = 161 cells in 6 animals; $p < 0.05$). This figure held true whether retinal inputs contributed to cortical activity or whether they were eliminated after binocular enucleation (Figure 4.9 B).

## 4.4 Binocular NKH477 injections increase the frequency of cortical network events

To further characterize the impact of spontaneous retinal activity patterns on cortical network dynamics, retinal activity was pharmacologically augmented by injections of the forskolin analogue NKH477 into both eyes. Forskolin increases the activity of the enzyme adenylyl cyclase which in turn elevates cAMP levels (Seamon and Daly, 1986). In the developing retina, forskolin potentiates spontaneous activity by increasing the frequency and amplitude of cholinergic waves (Stellwagen et al., 1999). More recently, it has been shown that the injection of forskolin into both eyes increases cortical network activity in neonatal rats (Hanganu et al., 2006). To study the effects of spontaneous retinal activity on network dynamics in the developing visual cortex, I used the drug NKH477, a water-soluble analogue of forskolin. NKH477 has thus the same effects as forskolin but is less harmful to the tissue. To ensure that the injection itself is not causing the observed effects, I injected saline under the same conditions as NKH477 into both eyes. Saline injections did not have significant effects on basic parameters of cortical network activity. The values for saline injections are presented in chapter 4.7.

After recording baseline activity of spontaneous activity patterns in the visual cortex, NKH477 was delivered to both retinas by means of binocular injections of 10mM of the drug with a Hamilton syringe (~ 0.75 µl, 1 µl/min). To follow individual cells before and after injections, the same cells were monitored in each recording (Figure 4.10 A). Immediately after binocular NKH477 injections, the frequency of cortical network events increased significantly (control: 0.8 / min ± 0.2; NKH477: 1.7 / min ± 0.3; n = 11 animals; $p < 0.05$; Figure 4.10 B). In contrast to binocular enucleation, the effect of NKH477 injections on cortical network dynamics was age-dependent (Figure 4.11 A). At P8, a more than 2.5-fold increase in cortical network activity in the visual cortex was observed after NKH477 injections into both eyes (control: 0.6 / min ± 0.04; NKH477: 2.3 / min ± 0.3; n = 2 animals).

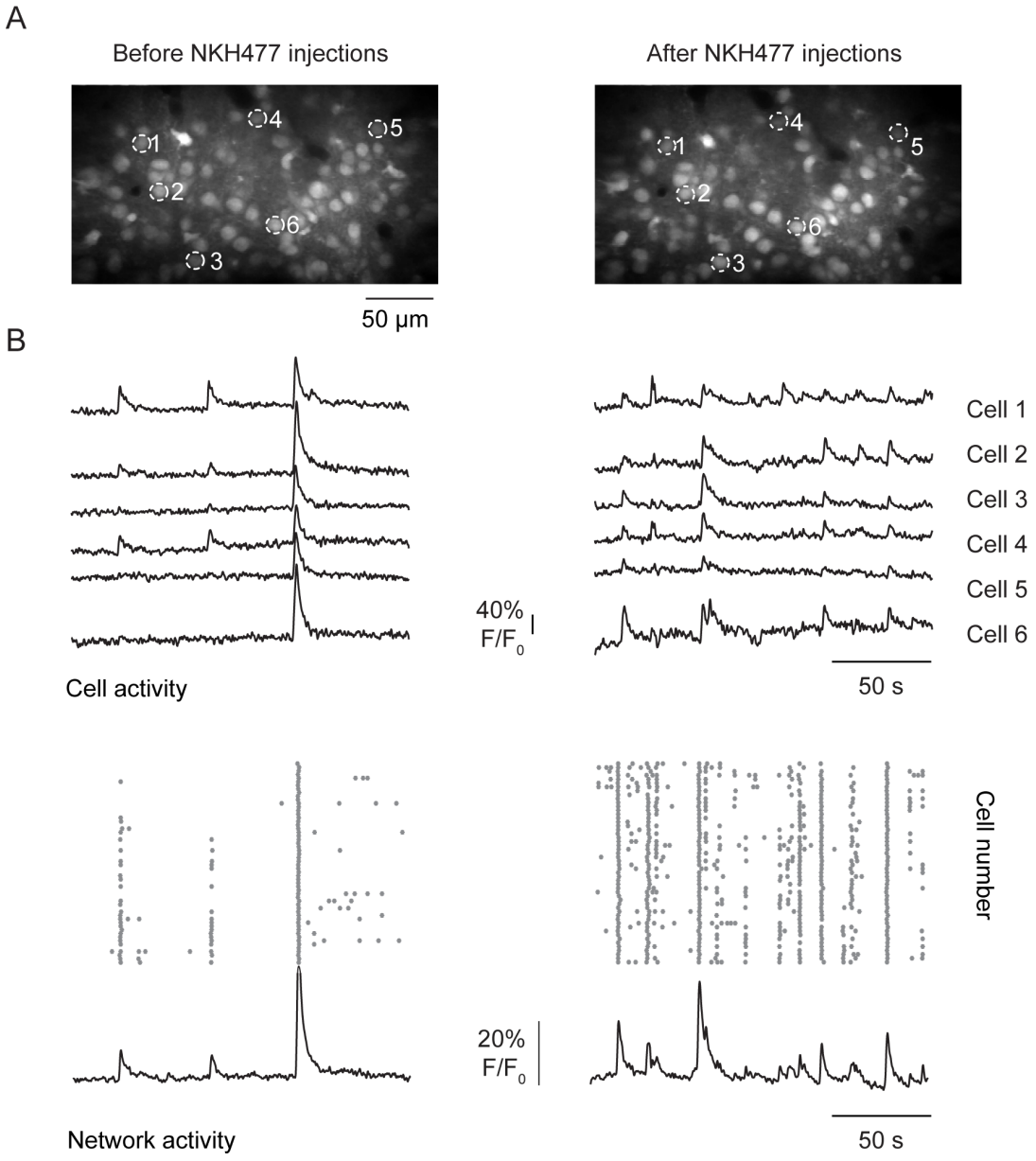


**Figure 4.10 The augmentation of spontaneous retinal activity increases the frequency of cortical network events.** (**A**) The same layer 2/3 cells in the visual cortex of a P9 animal were imaged before (left) and after (right) binocular injections of NKH477. (**B**) Top: Example traces of activity patterns of the cells depicted in (**A**). The frequency of cortical network events increases after retinal NKH477 application. Bottom: Gray bullets depict cellular activation during ongoing cortical network activity before and after injections of NKH477.

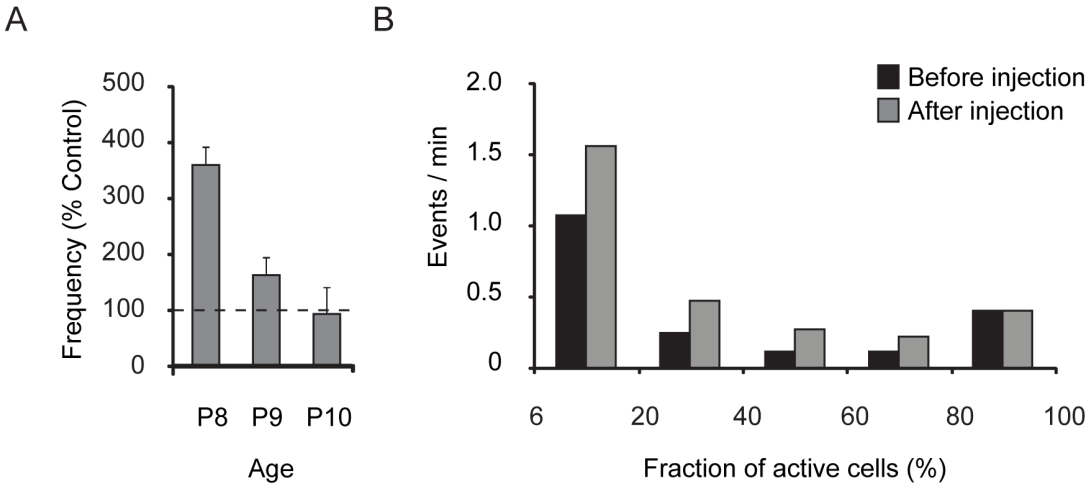


**Figure 4.11 The increase in frequency after binocular NKH477 injections is age-dependent and specifically affects cortical network events with low to medium participation rates.** (**A**) The effect of the augmentation of retinal activity is largest in P8 animals and less pronounced or absent in P9 and P10, respectively. (**B**) The cortical events with low to medium participation rates (6 – 80%) are specifically increased after binocular injections of NKH477 (gray bars) compared to baseline activity (black bars).

The increase was less pronounced at P9 (control: 0.9 / min ± 0.2; NKH477: 1.5 / min ± 0.4; n = 5 animals) and the drug had no effect at the age of P10 (control: 1.0 / min ± 0.07; NKH477: 0.9 / min ± 0.2; n = 4 animals). This finding can be explained by the characteristics of retinal waves at this stage of development. Retinal waves are purely cholinergic from the embryonic stage to P8 (Huberman et al., 2008) and are gradually replaced by glutamatergic stage III waves by approximately P10 – 12 (Figure 2.4). Forskolin and NKH477, however, act specifically on cholinergic activity patterns in the retina (Stellwagen et al., 1999) which might explain the smaller effect of NKH477 on event frequency at P10. For further analyses, I therefore included only animals from P8 – 9. While the frequency of network events increased after NKH477 injections (Figure 4.10 B and Figure 4.11 B), the average participation rate decreased (control: 65.1% ± 5.4; NKH477: 54.3% ± 4.8; n = 7 animals; $p < 0.05$; Figure 4.11 B). Primarily the frequency of events with low to medium participation rates were increased after binocular NKH477 injections while the frequency of events with a participation rate of more than 80% was not changed (Figure 4.11 B). It should be noted that the baseline

frequency of cortical network events can vary between experimental series (Figure 4.8 B and Figure 4.11 B). The reasons for this are currently unknown.

These findings nicely complemented the enucleation results showing that retinal inputs specifically trigger network events with low to medium participation rates. Moreover, increasing spontaneous retinal activity with NKH477 did not affect the frequency of cortical network events with a high participation rate. This is in line with our hypothesis that spontaneous activity from the retina specifically triggers cortical network events with low to medium participation rates.

## 4.5 Distinct mechanisms of spontaneous activity drive cortical network events

As pointed out in chapter 4.3 and 4.4, different retinal manipulations specifically affected cortical network events with low to medium participation rates (20 – 80% of the imaged cells). On the contrary, the frequency of cortical network events with high participation (> 80% of the imaged cells) was not changed. Network events in the visual cortex might thus arise from different origins and recruit varying proportions of cortical cells.

To characterize the impact of spontaneous retinal activity on cortical activity patterns in the developing cortex, the difference of cortical network events before and after a given treatment was calculated for each bin of participation rate. Hence, the frequency for each bin after binocular enucleation was subtracted from the activity before enucleation and vice versa for binocular NKH477 injections (Figure 4.12 A). This analysis revealed the fractions of different participation rate bins that were triggered by spontaneous retinal activity. Interestingly, the analysis of the proportions of network events that arise from the retina was very similar in both categories. In contrast, large network events remained unaffected by retinal manipulations (Figure 4.12 A). When plotting the frequencies of events of all animals with a low to medium participation rate (20 – 80%; Figure 4.12 B) and high participation rate separately (> 80%; Figure 4.12 C), the effects of retinal manipulations on the frequency of cortical network events were highlighted even more. First, the frequency of events with a low to medium participation rate was significantly changed and regulated in accordance with the manipulation of retinal inputs (enucleation: before: 1.1 / min ± 0.2; after: 0.5 / min ± 0.1; n = 6 animals; $p < 0.05$; NKH477: before: 0.5 / min ± 0.2; after: 1.2 / min ± 0.3; n = 11 animals; $p < 0.05$; Figure 4.12 B). Second, the events with a high participation rate were unaffected by either manipulation (enucleation: before: 0.5 / min ± 0.1; after: 0.5 / min ± 0.1; n = 6 animals; $p > 0.05$; NKH477: before: 0.3 / min ± 0.1; after: 0.4 / min ± 0.2; n = 11 animals; $p > 0.05$; Figure 4.12 C).

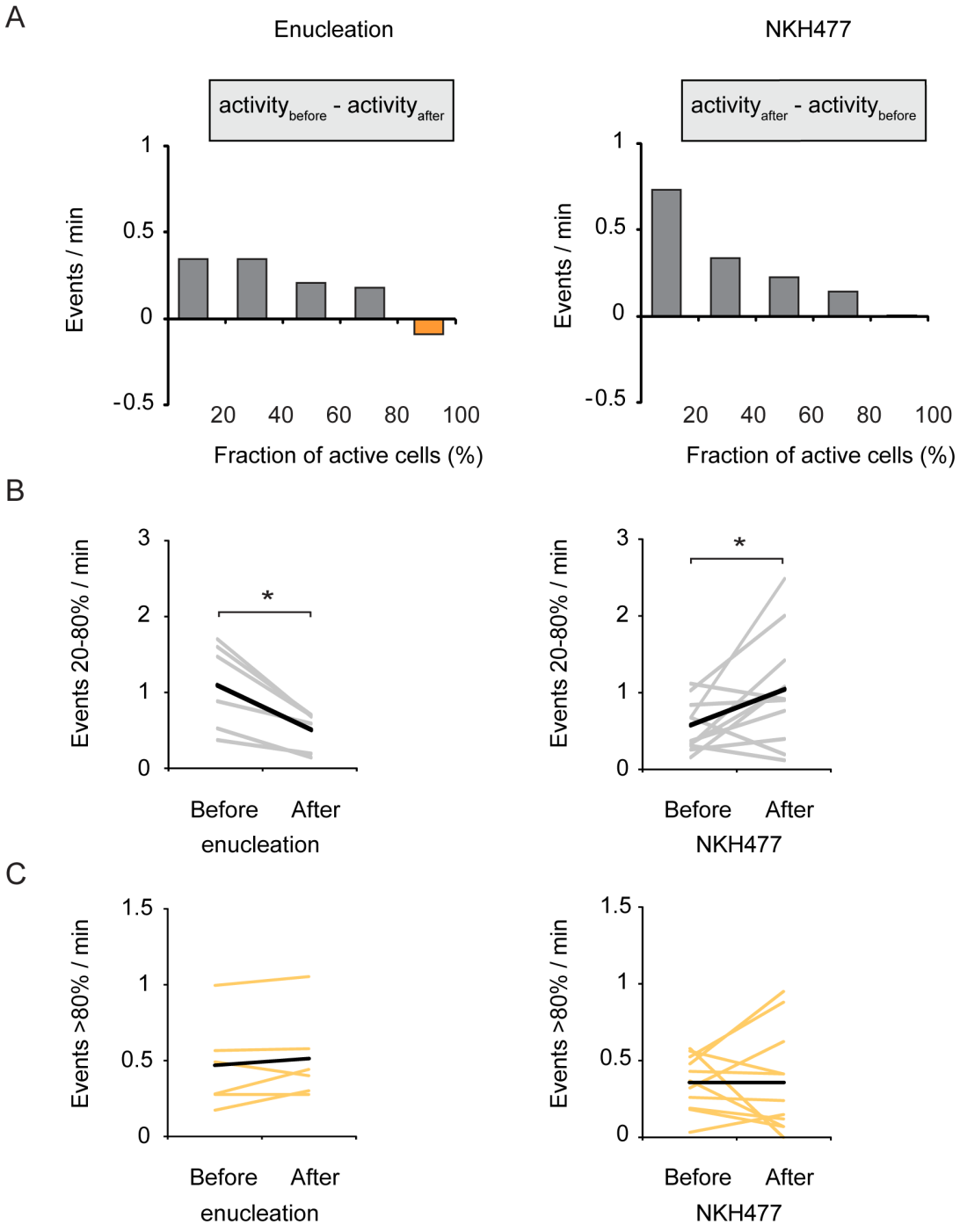


**Figure 4.12 The manipulation of retinal inputs reveals that events with a high participation rate are independent of spontaneous retinal activity.** (**A**) The subtraction of cortical activity before from that after binocular enucleation (left column) and vice versa for NKH477 injections (right column) reveals the fraction of cortical network events that are driven by retinal inputs. (**B**) The frequency of cortical network events with low to medium participation rates (20 – 80%) is changed after enucleation and NKH477 injections. (**C**) In contrast, the frequency of cortical events with a high participation (> 80%) is not affected by the respective retinal manipulation.

Taken together, these findings reveal that cortical network events with a high participation rate are not only unaffected by spontaneous retinal activity (Figure 4.12) but also differ in certain network parameters such as jitter and mean amplitude (Figure 4.5). Moreover, the frequency of these events is not changed during early development (Figure 4.4).

## 4.6 Binocular epibatidine injections have ambiguous effects on cortical network activity

The results presented here demonstrate that patterned retinal activity is relayed to the visual cortex before visually-driven inputs are present and that this activity affects specific patterns of cortical network events. Therefore, I sought to study how the disruption of patterned retinal activity affects the activity patterns in the visual cortex of neonatal mice. To de-correlate spontaneous waves in the retina, epibatidine was injected into both eyes of the animal. Epibatidine is a potent agonist of nicotinic acetylcholine receptors (nAChRs) and is known to disrupt the spatiotemporal properties of cholinergic retinal waves (Sun et al., 2008a), presumably by means of receptor desensitization (Marks et al., 1996). The application of epibatidine to retinal explants leads to the abolishment of patterned activity in the retina and to de-correlated firing of retinal ganglion cells (Cang et al., 2005b; Sun et al., 2008a). In addition, the application of epibatidine to retinal tissue results in increased firing in some retinal neurons while silencing others completely. Chronic epibatidine application causes severe wiring defects at all stages of the visual system (Penn et al., 1998; Chandrasekaran et al., 2005; Huberman et al., 2006).

To study how the acute disruption of the spatiotemporal properties of retinal waves affects network activity in the visual cortex, epibatidine (1 mM) was injected into both eyes of neonatal mice (~ 0.75µl, 1ml/min). Calcium imaging of cells in the visual cortex before and after binocular injections of epibatidine showed ambiguous effects on cortical activity patterns (Figure 4.13 A). Interestingly, no significant changes in frequency and participation rate of cortical network events after epibatidine injections were detected compared to baseline activity. The frequency of cortical network events was not significantly altered after epibatidine application (control: 1.8 / min ± 0.4; epibatidine: 2.0 / min ± 0.4; n = 6 animals; $p > 0.05$; Figure 4.13 B and C). As in the NKH477 study, the effect of epibatidine on spontaneous retinal activity appeared to be age-dependent (P8: control: 1.0 / min ± 0.1; epibatidine: 1.8 / min ± 1.0; n = 2 animals; P9: control: 2.2 / min ± 0.5; epibatidine: 2.1 / min ± 0.5; n = 4 animals; $p > 0.05$; Figure 4.13 D).

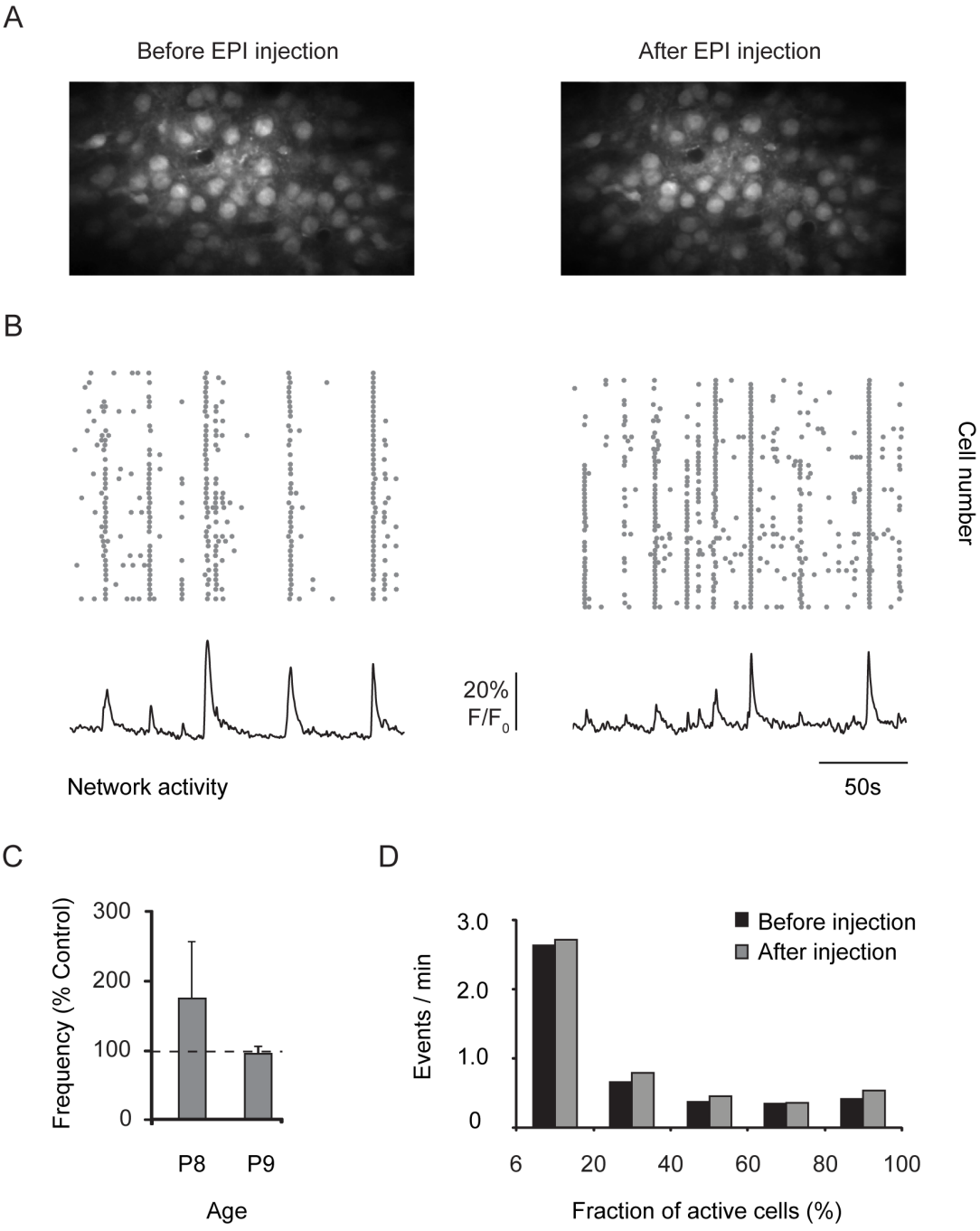


**Figure 4.13 The disruption of patterned spontaneous activity in the retina has ambiguous effects on cortical network dynamics.** (**A**) The same layer 2/3 cells in the visual cortex were imaged before and after binocular injections of epibatidine (EPI). (**B**) Top: Active cells are depicted as gray bullets over time before (left) and after (right) epibatidine injections. Bottom: Network activity plotted as the mean activity of all imaged cells. (**C**) The effect of epibatidine appears to be age-dependent. (**D**) Epibatidine does not significantly alter the frequency of network events with various participation rates.

Because injections of NKH477 showed no effect in P10 mice, epibatidine was not tested on P10 animals. The ambiguous effects of the epibatidine study are difficult to interpret and will need further experiments.

## 4.7 Comparison of the effects of different retinal manipulations on cortical network activity

The aim of this study was to dissect the different activity patterns that are present in the visual cortex during early development. I manipulated spontaneous activity patterns in the retina in different ways and recorded the effects on cortical network activity with two-photon calcium imaging (Table 4.1 and Figure 4.14). (i) The complete removal of all retinal inputs by means of binocular enucleation specifically abolished a large proportion of spontaneous network events with low to medium participation rates. Most of the remaining events recruited the majority of imaged cells which resulted in an overall increase in the average participation rate (Table 4.1 and Figure 4.14 A and B). (ii) The injections of the cAMP activator NKH477 into both eyes revealed a significant increase in the frequency of cortical network events, which specifically comprised events with a low to medium participation rate which in turn decreased the overall participation rate (Table 4.1 and Figure 4.14 A and B). (iii) Binocular injections of epibatidine, a nAChR agonist, had no significant effects on frequency and participation rate (Table 4.1 and Figure 4.14 A and B). To exclude the possibility that the process of injections and not the drug itself caused the effects, saline was injected into both eyes of the animals in control experiments. No significant effects in frequency and participation rate were observed after binocular saline injections (Table 4.1 and Figure 4.14 A and B). In contrast to the frequency and the participation rate, the jitter of cortical network events did not change significantly after enucleation as well as NKH477 and saline injections. After epibatidine injections, however, the jitter was significantly reduced (Table 4.1 and Figure 4.14 C). Although the trend of a reduction in jitter was observed after nearly all treatments, it is not clear what exactly caused the increase in synchronicity after epibatidine and saline application.

| | Frequency | | Participation rate | | Jitter | |
|---|---|---|---|---|---|---|
| | Before | After | Before | After | Before | After |
| **Enucleation** | 1.6 / min | 1.0/min(*) | 62.0% | 72.7% (*) | 0.5 s | 0.4 s |
| **NKH477** | 0.8 / min | 1.7/min(*) | 65.1% | 54.3% (*) | 0.4 s | 0.4 s |
| **Epibatidine** | 1.8 / min | 2.0 / min | 54.7% | 55.1% | 0.4 s | 0.3 s (*) |
| **Saline** | 1.4 / min | 1.2 / min | 61.9% | 65.1% | 0.4 s | 0.3 s |

**Table 4.1 Summary of basic parameters of cortical network events before and after retinal manipulations.** Significant differences after retinal manipulations are marked with an asterisk (*).

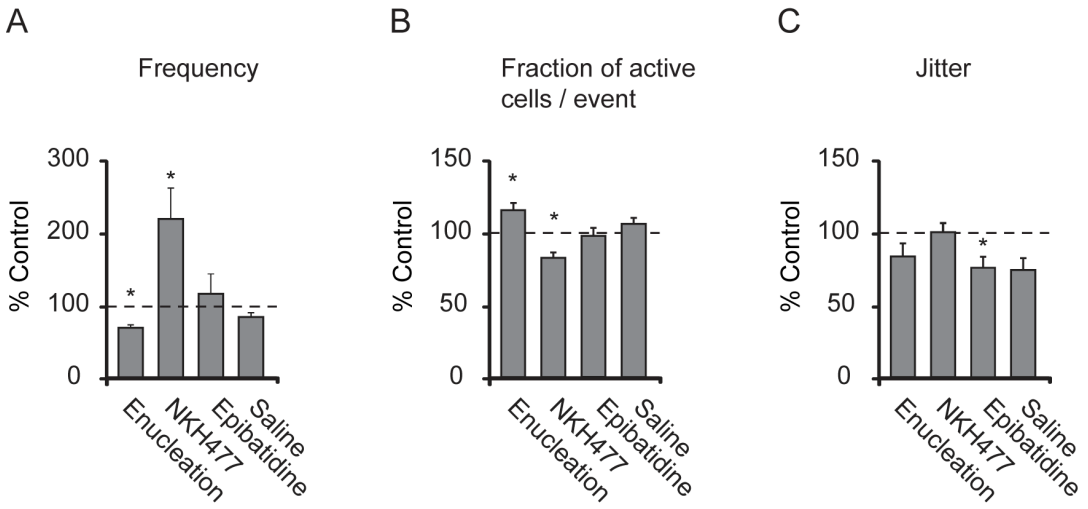


**Figure 4.14 Quantification of basic parameters of cortical network activity after different retinal manipulations.** (**A**) The frequency of cortical network events with a participation rate of > 20% is changed after enucleation and NKH477 application but unaffected by epibatidine and saline injections. (**B**) Similarly, the fraction of active cells per cortical network event is only significantly changed after enucleation and NKH477 injections. Epibatidine and saline injections have no effect on the fraction of co-active cells in the visual cortex. (**C**) In contrast, the jitter, a measure for synchronicity of network events, is significantly decreased after binocular injections of epibatidine. Enucleation as well as NKH477 and saline injections have no significant effect on the jitter of cortical network events.

In this study, I describe distinct cortical activity patterns present in the visual cortex before the onset of vision. One class of cortical events resembles large network oscillations that were described previously (Adelsberger et al., 2005). The majority of cells of the visual cortex are recruited to these events which are characterized by properties such as high synchronicity and high signal amplitude. Because this class of events was not altered after any retinal manipulation, I conclude that these events are not triggered by retinal inputs but arise instead centrally. On the contrary, spontaneous activity arising from the retina drives cortical network events that comprise a relatively low number of co-active cells. Retinally driven network events are less precisely aligned in time and have lower mean cellular amplitude. Moreover, the significance of this class of events increases with age as the connections from the periphery to the visual cortex mature.

# 5 Discussion

The developing nervous system is a highly dynamic structure that undergoes innumerous changes to sculpt the mature brain. These rearrangements are regulated by activity-dependent mechanisms that activate the neuronal network in a highly simultaneous manner. These mechanisms provide information about neighboring cells by means of Hebbian rules. Synapses between co-active neurons are strengthened whereas aberrant connections are eliminated. Good candidates that provide the simultaneous activation of neuronal ensembles are endogenously generated activity patterns. These spontaneous activity patterns are present early in the developing brain and have been described in a variety of neuronal structures (Khazipov and Luhmann, 2006; Blankenship and Feller, 2010). On the other hand, neuronal activity that is generated in the periphery is another important mediator to drive Hebbian-based network formation (Feller and Scanziani, 2005; Blankenship and Feller, 2010). Already present before the onset of sensation, these mechanisms become more prominent during later stages of development when an initial layout of the brain is already established. It is likely that there is a phase during development when both mechanisms overlap and work hand in hand to establish mature networks. However, the relative contributions of these two mechanisms to drive cortical network activity are unknown.

The findings of the present study provide strong evidence that spontaneous network activity in the developing visual cortex of neonatal mice comprises qualitatively distinct network patterns. In vivo two-photon imaging of spontaneous network dynamics reveals that the cortical network receives inputs from at least two different sources,

namely from the retina and central structures. Specific manipulations of spontaneous retinal activity affect only a particular fraction of cortical network events, including those with low to medium participation rates (20 – 80%). On the other hand, a specific fraction of cortical network events with a participation rate of more than 80% is unaffected by retinal manipulations and is thus likely to arise from other sources, presumably intrinsic to the cortex. These two differentially regulated patterns of network activity are likely to act in concert and reciprocally guide network maturation in the visual cortex before the onset of vision.

## 5.1 Cortical network dynamics under light anesthesia are comparable to activity during the awake state

Contrary to the assumption that anesthesia perturbs the overall characteristics of spontaneous activity patterns in the developing cortex (Adelsberger et al., 2005; Colonnese and Khazipov, 2010), I did not observe large differences in key parameters of network events during the anesthetized and the awake state of the animal. Apart from the expected increase in frequency, the overall quality of cortical network events was comparable (Figure 4.4). The experiments of previous reports were performed in animals that were either significantly younger (P3 – 4) or older (> P10) than the animals used in this study. Although unlikely, this age difference might explain the contradictory findings. It is more probable, however, that technicalities such as the isoflurane evaporator or the mode of anesthesia delivery account for the differences. Thus, although the same percentage of anesthetic was stated (approximately 1%), the amount of isoflurane that the animal inhaled could have been higher in these reports. As shown in figure 4.3, isoflurane concentrations of 1.5 % abolished all cortical network activity.

Light anesthesia did not significantly alter participation rate and jitter (Figure 4.4 C). However, the frequency of spontaneous network events increased in the awake state which is in line with other studies (Golshani et al., 2009; Colonnese et al., 2010). By comparing cortical network activity in the awake and anesthetized state, the results

obtained at low anesthesia concentrations can most likely be extrapolated to the awake state of the animal.

## 5.2 Binocular epibatidine application results in more synchronous cortical network events

The injection of epibatidine into both eyes of developing mice has ambiguous effects on cortical network events in the visual cortex (Figure 4.13). These findings might be explained by the differential effects of epibatidine on the behavior of individual RGCs (Sun et al., 2008a). While approximately half of the RGCs in the developing retina are completely silenced after epibatidine application, the other half exhibits increased firing rates (Sun et al., 2008a). This contradicts a previous study that report a complete loss of spontaneous activity upon epibatidine application in the ferret retina (Penn et al., 1998). It is likely that methodological differences contribute to these inconsistent results.

The differentially altered retinal firing patterns are likely to be responsible for the ambiguous effects in the visual cortex observed in the present study. The tonic firing of half of the RGCs in both eyes could be sufficient to drive network events in the visual cortex. Assuming that the probability of simultaneous firing of RGCs from both eyes increases after epibatidine application, this could explain that neither the frequency nor the participation rate of cortical network events are affected (Figure 4.13). Additionally, the altered spatiotemporal firing properties combined with the increased probability that individual RGCs from both eyes fire together could explain the smaller jitter of cortical network events. Similar to epibatidine application, RGCs of β2nAChR KO mice show disrupted firing (Rossi et al., 2001; Stafford et al., 2009). In contrast to previous reports (Bansal et al., 2000; Mclaughlin et al., 2003), it has been shown that β2nAChR KO retina exhibit stage II retinal waves under certain conditions (Stafford et al., 2009). The spatiotemporal properties of these waves, however, are altered compared to wild type waves. This includes a faster propagation speed while the frequency of retinal waves is not changed. It has not been shown whether these findings are

also true in retinas treated with epibatidine. It remains to be determined whether certain spatiotemporal properties are maintained after binocular epibatidine injections in vivo and how they affect cortical network dynamics.

To shed light on the mechanisms that underlie the ambiguous findings obtained with epibatidine injections, it would be valuable to simultaneously monitor spontaneous activity from the retina and the visual cortex in vivo. Combined electrophysiological recordings in the retina and calcium imaging in the visual cortex could reveal the retinal activity patterns that drive cortical activity after epibatidine application. In addition, since it has been shown that epibatidine is dose-dependent (Sun et al., 2008a), it would be useful to establish the effect of different epibatidine concentrations in the retina on cortical network dynamics.

## 5.3 Distinct patterns of cortical activity can be distinguished by synchronicity and amplitude

In addition to the participation rate, other distinctive features such as synchronicity and mean amplitude of cortical network events characterize the distinct classes of activity patterns. As a measure of synchronicity, I determined the degree of temporal alignment of active neurons during a network event. The jitter is significantly higher in cortical network events with low to medium participation rates than in events with high participation. Assuming a uniform increase in jitter with increasing participation rates, the jitter would be significantly higher for cortical network events with higher participation rates. However, the degree of synchronicity was similar in all network events with a participation rate of 20 – 80% (Figure 4.6 A). The jitter of network events with a participation of more than 80% was significantly lower and the activation of individual cells was precisely aligned in time (Figure 4.6 A). These findings are in agreement with previous reports that describe fast intrinsic cortical network oscillations that activate large parts of the developing brain (Chiu and Weliky, 2001; Colonnese and Khazipov, 2010).

How is this difference in the synchronicity of activation brought about? Several studies suggest that intrinsic network events are driven by gap junctions that interconnect a large number of neurons during early stages of development (Montoro and Yuste, 2004). In the neonatal visual cortex, gap junction-coupled mechanisms could drive the fast simultaneous activation of large populations of neurons required for intrinsically generated network events. On the other hand, synaptic mechanisms such as glutamatergic transmission arising from upstream areas initiate the slower cortical network events that are characterized by a higher temporal jitter and smaller participation rates. These differences in the synchronicity of cortical network events could be crucial to distinguish between events that arise from different origins. The temporal information implemented in different cortical network events could be an important mechanism for the correct wiring of neuronal networks.

In addition to differences in synchronicity, cortical network events of different origins also have various mean amplitudes in the cellular calcium signal (Figure 4.6 B). This implies that the distinct classes of network events are differentially activated by presynaptic inputs. Calcium imaging is a reliable reporter of neuronal activity (Svoboda et al., 1997; Rochefort et al., 2009). Although calcium-sensitive dyes do not directly provide information about the electrical signal such as electrophysiological recordings or voltage-sensitive dyes, they indirectly report neuronal activity upon intracellular calcium influx. In the developing visual cortex, the amplitude of the OGB-1 signal upon neuronal activation increases linearly with the number of action potentials (Rochefort et al., 2009). Thus, the amplitude of calcium signals provides a good measure of the neuron's synaptic inputs. In contrast to electrophysiological recording techniques, some variants of calcium indicators (e.g. OGB-1) allow monitoring the behavior of hundreds of individual neurons simultaneously. Although the findings of this study are restricted to layer 2/3 cells, it could be possible in the future to monitor network dynamics in more superficial layers or even in layer 4. 3D imaging techniques that record from large populations of cortical cells in different layers are available (Grewe and Helmchen, 2009) and could be implemented in the future to study network activity in different layers of the developing cortex.

Two-photon calcium imaging in large neuronal populations allows extrapolating the calcium signal of individual cells to the amount of action potentials. In the present

study, I show that the mean amplitude of cellular calcium signals is different depending on the type of cortical network event. Events that have low to medium participation rates have smaller mean amplitudes than cortical network events with a participation rate of more than 80% (Figure 4.6 B). This suggests that neurons receive more inputs during intrinsic network events than during retinally driven activation. Interestingly, not only the amplitude averaged over all co-active cells is generally higher in central network events than in retinally generated network events. But also individual cells show similar behavior depending on the type of cortical network event they participate in (Figure 4.9 B). This relationship is not altered after binocular enucleation and is thus not dependent on retinal inputs.

## 5.4 Spontaneous retinal activity triggers the activation of neuronal ensembles in the visual cortex

Different manipulations of retinal inputs reveal that the distinct classes of network events in the visual cortex are of different origin. This study shows for the first time the acute response of the developing visual cortex to retinal manipulations with high spatial resolution. Several studies have investigated the long term effects of chronic perturbations of spontaneous retinal activity on various aspects of visual cortex development. For instance, the chronic deprivation of retinal activity before the onset of vision leads to functional and structural aberrations in the visual cortex (Ruthazer and Stryker, 1996; Crair et al., 1998). Similarly, the analysis of β2nAChR KO mice that lack stage II retinal waves reveals structural and functional deficits in V1 (Cang et al., 2005b; Cang et al., 2008).

How do acute retinal manipulations influence activity patterns in the visual cortex before vision occurs? In newborn rats, the frequency of spindle bursts in the visual cortex decreases after binocular enucleation (Hanganu et al., 2006). Accordingly, pharmacologically increasing retinal waves results in an immediate increase in cortical network activity (Hanganu et al., 2006). However, the measurement of local field potential and multi-unit activity cannot provide information about individual cells. In

the present study, I describe the behavior of single cells upon different retinal manipulations. This includes the complete removal of all retinal inputs (binocular enucleation), the augmentation of stage II retinal waves (binocular injections of the cAMP activator NKH477) and the disruption of patterned retinal activity (binocular injections of the nAChR agonist epibatidine). Intriguingly, these different retinal manipulations reveal a group of cortical network events that were unaffected by any treatment. These results imply that this class of activity patterns is driven by central mechanisms. In contrast, specifically cortical network events with low to medium participation rates were altered after perturbations of retinal inputs. The removal of retinal inputs by binocular enucleation abolished a large portion of this class of cortical network events. In contrast, the augmentation of stage II retinal waves specifically increased the frequency of cortical network events with low to medium participation rates. These findings led me to conclude that cortical network events with low to medium participation rates are triggered by spontaneous retinal activity.

Intriguingly, the properties of retina-driven network events are very similar to the properties of stage II retinal waves. For instance, the frequency of stage II waves (Bansal et al., 2000; Sun et al., 2008a) is similar to the frequency of retina-driven network events observed in the visual cortex (approximately one wave per minute). This suggests that the majority of retinal waves that correspond to a specific retinotopic location in V1 are relayed to the visual cortex at this age. During the first postnatal week, however, the proportion of stage II retinal waves that triggers cortical activity is lower (Hanganu et al., 2006) and is probably due to immature connections from the dLGN to V1. In addition, the duration of stage II waves is in accordance with the average duration of retinally driven cortical network events in this study (Sun et al., 2008b; Stafford et al., 2009).

With the imaging parameters used in this study, it is currently difficult to make a statement about certain properties of retinally driven cortical network events. Because the field of view during the calcium imaging recordings was restricted, the extent of retinally driven network events in the visual cortex cannot be assessed. Increasing the field of view would provide more spatial information about the spread of cortical network activity. Accordingly, due to limitations in the temporal resolution with full frame scanning, characteristics such as speed and direction of cortical net-

work events cannot be described. Line scan recordings of the cortical network with high temporal resolution (approximately 500 Hz) will shed light on network event properties. For instance, it has been reported that individual RGCs have a directional bias during stage II retinal waves (Stafford et al., 2009). Line scan recordings will provide sufficient temporal information of whether network events travel across the visual cortex and whether these events have a directional bias. In addition, the spatial resolution of line scans is sufficient to provide information whether neurons preferably participate during network events of a certain type.

Compared to cortical network events with high participation, retinally driven events comprise fewer cells. The sparse activation during these events could be a prerequisite of activation patterns during adult vision and might be required for efficient information processing. Rochefort et al. report that a so called 'sparsification' process in the visual cortex sets in at the end of the second postnatal week with a gradual decrease in mean cellular participation per cortical network event at around eye-opening (Rochefort et al., 2009). However, the decrease in mean participation rate could also be due to the specific disappearance of intrinsically generated cortical events with a high participation rate. Thus, the sparsification process would not be the result of a lower participation rate in general but rather underlying specific adjustments in favor of retinally driven network events. Applying the analysis methods of the present study to recordings of cortical network activity in older animals might solve this question.

What is the role of retinally driven network events in the visual cortex before the onset of vision? The simultaneous activation of the same groups or patches of neurons during different network events that was reported in previous in vitro work (Yuste et al., 1992; Schwartz et al., 1998) could not be observed in the present study. Instead, the activity patterns closely resembled the salt-and-pepper organization of visual stimulus properties observed in the mature visual cortex of rodents (Ohki et al., 2005; Mrsic-Flogel et al., 2007). The spatiotemporal patterns of retinally induced network events described in this study could thus be a prerequisite of mature connectivity. For instance, ensembles of neurons are functionally grouped in a salt-and-pepper organization based on their receptive fields (Smith and Hausser, 2010) or their preference to stimulus orientation or direction (Ohki and Reid, 2007). Before eye-opening, sponta-

neous retinal activity could thus serve as 'training patterns' that trigger cortical network events with spatiotemporal characteristics, similar to adult patterns.

## 5.5 A fraction of cortical network events is generated independently of retinal inputs

The deprivation of retinal inputs by binocular enucleation largely eliminates cortical network events with low to medium participation rates (Figure 4.7 and 4.8 B), leaving mostly events with high participation. Since all activity arising from the retina is removed, it is likely that the residual activity is triggered by areas downstream of the retina. This group of remaining events resembles very closely synchronous network oscillations, such as ENOs that have been observed in organotypic cortical slice cultures (Garaschuk et al., 2000) as well as in the cortex of the mouse in vivo during the first postnatal week (Adelsberger et al., 2005). Although it has been postulated – based on in vitro findings – that ENOs disappear after the first postnatal days (Garaschuk et al., 2000; Corlew et al., 2004; Allene et al., 2008), this has not been tested in vivo yet. The cortical network events with a high participation rate described in this study might be remainders of ENOs that gradually disappear with cortical maturation. These events could serve as homeostatic mechanism to provide survival signals for the developing neurons before connections to peripheral inputs are established (Mennerick and Zorumski, 2000). It will be interesting to test whether the events resembling ENOs in this study are driven by glutamatergic transmission, as reported in in vitro studies (Allene and Cossart, 2010). Several other scenarios of network activation are also likely. Work in organotypic slice cultures of the developing cortex has shown that the activation of extrasynaptic NMDA receptors by ambient glutamate initiates ENOs in the immature cortex (Allene et al., 2008). This mechanism of extrasynaptic transmission has also been reported in other neuronal structures, suggesting that this might be a general mechanism to guide the formation of neuronal networks (Allene and Cossart, 2010; Blankenship and Feller, 2010). It will be interesting to test whether the intrinsic network events described here are triggered by extra-

synaptic glutamate. The application of an enzymatic glutamate scavenger to the cortical surface that enhances the clearance of extracellular glutamate could assess the role of extrasynaptic glutamate on the generation of centrally generated network events.

Other mechanisms that drive large neuronal populations to fire simultaneously are gap junction-mediated processes that support certain steps in the development of the cortical circuitry (Montoro and Yuste, 2004). Indeed, during the first two postnatal weeks, the neuronal network of the rodent neocortex is highly connected via gap junctions that are broken up shortly after (Peinado et al., 1993). These transient connections might provide a mechanism to synchronize the communication between large populations of neurons during early development. Several groups have shown that gap junction-mediated mechanisms play an important role in the generation of spontaneous network activity during cortical development (Kandler and Katz, 1998; Peinado, 2001; Dupont et al., 2006). In contrast, Minlebaev et al. have reported that gap junctions in the developing somatosensory cortex of live rats mediate inhibition of spindle bursts, a form of oscillatory network activity (Minlebaev et al., 2007; Minlebaev et al., 2009). It will be interesting to test what role gap junction-mediated mechanisms play in the generation of network activity in the visual cortex of neonatal mice. Applying gap junction blockers to the cortex could provide information of whether the generation of network events with a high participation rate is regulated by gap junction-mediated processes.

Other mechanisms, not intrinsic to the developing cortex, could play a role in the generation of network events with a high participation rate. For instance, spontaneous network activity is generated in the developing ferret dLGN before eye-opening both in vitro (McCormick et al., 1995) and in vivo (Weliky and Katz, 1999). These activity patterns in the dLGN show similar temporal properties as network activity in V1 during the same stages of development (Weliky and Katz, 1999; Chiu and Weliky, 2001). Binocular optic nerve transection leads to the temporary elimination of all dLGN activity (Weliky and Katz, 1999). Interestingly, unlike dLGN activity, V1 activity was present immediately after optic nerve transection, characterized by shorter durations of the cortical network events (Chiu and Weliky, 2001). DLGN activity resumed approximately 50 minutes after optic nerve transection and resembled the altered pat-

terns of cortical network activity (Weliky and Katz, 1999), suggesting that a thalamocortical feedback loop sustains spontaneous network activity in the dLGN. The adaptation of firing patterns of the visual cortex after the removal of retinal inputs demonstrates that dLGN activity does not drive cortical activity patterns but vice versa. Thus, assuming a similar circuitry in the developing mouse visual system, it is unlikely that the network events with a high participation rate described in the present study are driven by spontaneous dLGN inputs. Similar to reports that recorded activity from different stations of the visual system in the adult or developing animal (Kara et al., 2000; Hanganu et al., 2006), simultaneous recordings of activity patterns in the dLGN and V1 of developing mice could determine the impact of dLGN activity on cortical network events.

A key player in the development of the cortex is the subplate (Allendoerfer and Shatz, 1994; Kanold, 2009), a transient structure below the cortical plate. Subplate neurons are the first neurons to be generated in the cerebral cortex and show mature functional properties already early in development (Zhao et al., 2009; Kanold and Luhmann, 2010). In rodents, the majority of subplate neurons disappear with cortical maturation after the third postnatal week (Price et al., 1997). Subplate neurons form connections with both thalamic neurons (Friauf et al., 1990; Hanganu et al., 2002) and neurons in the cortical plate (Kanold and Luhmann, 2010). This puts the subplate in an ideal position to detect firing both in the thalamus and the cortex. The circuitry between the subplate, the thalamus and the cerebral cortex might thus provide a Hebbian-based mechanism for the strengthening of functional connections. Moreover, the subplate plays an important role in the generation of synchronous network activity in the cortex both in vitro (Sun and Luhmann, 2007) and in vivo (Yang et al., 2009). Taken together, the characteristics of subplate neurons provide an exciting mechanism to drive cortical network events with a high participation rate.

It has been proposed that the subplate serves as a hub to receive and distribute electrical information (Kanold and Luhmann, 2010). Due to their mature properties and their widespread axonal and dendritic arborizations early during development, subplate neurons could play an important role in the synchronization of network activity. Indeed, only a few GABAergic subplate neurons are sufficient to initiate cortical network activity in developing cortical neurons (Voigt et al., 2001), strongly resembling

pacemaker-like neurons such as described in the spinal cord (Yuste et al., 2005; Le Bon-Jego and Yuste, 2007). Moreover, gap junctions connect groups of subplate neurons with each other and with neurons in the cortical plate, forming electrically coupled columns of about 100 µm in diameter (Dupont et al., 2006). These so called hub cells could drive spontaneous network activity in the developing cortex (Voigt et al., 2001). To determine whether hub cells, either residing in the subplate or in cortical layers, play a role in the generation of cortical network events with a high participation rate will be an exciting question answered in the future.

The deprivation of retinal inputs in both eyes did not completely abolish network events with low to medium participation rates (Figure 4.8 and 4.12). What could be the source of these remaining events? As pointed out above, the dLGN is not capable in driving large-scale activity in V1. Thus, it is unlikely that the dLGN is responsible for triggering this class of network events. Another possibility could be that these events are generated by central neuronal circuits that are locally connected via gap junctions or chemical synapses. These sub-ensembles could be independent units that are neither driven by retinal inputs nor by large-scale cortical activation. Alternatively, the occurrence of these network events could be explained by the cross-activation of the visual cortex through activity from neighboring brain regions, such as the auditory or somatosensory cortex. Similarly, it has been reported in adult animals that cortical areas can be activated by stimuli of other modalities, suggesting that intracortical projections connect different sensory areas (Driver and Noesselt, 2008). Another possible scenario could be the spillover of network activity from other sensory areas due to the immature cortical circuitry. Since the boundaries of sensory areas are not fully defined yet, activity from other sensory areas could elicit network events with low participation in the developing visual cortex. A more detailed analysis of the cortical network events with low to medium participation rates after enucleation will uncover the specific spatiotemporal properties of these remaining events and shed light on their origin.

## 5.6 The contributions of retinal inputs that trigger cortical network activity increase with development

The properties of spontaneous activity patterns in the developing cortex change with development. Usually this involves the transition from a state with very little cellular participation that is mostly non-synaptic to the simultaneous activation of the entire network at older ages (chapter 2.2) (Allene and Cossart, 2010). Once sensory systems come into play, this synchronicity is broken up and highly synchronous network events are replaced by sparse activation patterns. This developmental transition is likely to be a prerequisite of signal processing in the adult animal (Rochefort et al., 2009; Golshani et al., 2009). Hence, the formation of neuronal networks seems to follow a sequence during the first weeks of life.

Despite the relatively small age differences used in this study, the changes in the quality of spontaneous network activity in the visual cortex of neonatal mice are substantial. While specifically network events with a participation rate of less than 80% are changed during this period of development, the frequency of network events with high participation is stable (Figure 4.5). These findings are consistent with the assumption that axonal projections from the dLGN to the cortex have arrived in layer 4 of V1 by the end of the first postnatal week (Lopez-Bendito and Molnar, 2003) and continue to mature until the end of the critical period. At this age, thalamocortical connections to the visual cortex are sufficiently strong to drive cortical network events that are generated in the retina (Hanganu et al., 2006; Colonnese and Khazipov, 2010). As these connections mature, it is likely that the proportions of peripheral spontaneous activity that drive cortical network events increase. This might explain the increase in frequency between P8 and P10 specifically in retinally driven network events with low to medium participation rates while the frequency of centrally generated network events does not change. Intriguingly, at these young ages individual neurons are fully embedded in the cortical network. The analysis of the participation probability of identified neurons revealed that nearly all neurons were active at least once during both retinally driven and centrally generated cortical network events (Figure 4.9 A).

Why does the developing visual cortex display different patterns of network activity? It seems reasonable that the various cortical network events provide a mechanism to guide different aspects of neuronal network formation. The different classes of network events could belong to distinct developmental processes that are differentially regulated during development with a period of overlap. This study demonstrates the increasing importance of retina-dependent cortical network events with age. On the contrary, cortical network events with high participation might be remainders of early network activity patterns (Adelsberger et al., 2005). These network events that are likely to be intrinsic to the cortex might eventually disappear to give way to more mature activity patterns that arise from the periphery. Such a transition could be crucial for the wiring of neuronal networks to meet the requirements of adult information processing.

# 6 Concluding Remarks and Outlook

It has long been assumed that activity-dependent mechanisms from different sources shape the development of neuronal networks in the developing cortex. The data presented here show for the first time on the cellular level that distinct patterns of network activity exist in the developing visual cortex. A fraction of the network activity is triggered by retinal activity whereas another fraction is generated independently of inputs from the retina and is thus likely to be generated centrally in the cortex. In this study, I used mice of the second postnatal week during which the retina is insensitive to light and stage II retinal waves are present. This is an interesting age since the projections from the dLGN to V1 are relatively mature at this age and spontaneous retinal activity is reliably relayed to V1. In light of these findings, it will be interesting to extend the study to other developmental stages. Studying the contributions of retinally driven network activity both before the dLGN projections are mature and shortly before eye-opening will give important insights into cortical activity patterns. These experiments will establish a developmental profile of the distinct activity patterns and will shed light on relative contributions of spontaneous retinal activity to network formation during different developmental stages.

The characterization of the mechanisms that drive network events in the visual cortex is crucial for understanding how they contribute to network formation. The data presented here show that network events with low to medium participation rates are retinally driven. Simultaneous recordings in the retina and the visual cortex will provide further insights into the relationship between retinal and cortical network activity. By specifically blocking different transmitter systems (e.g. glutamate, GABA) or gap junctions in the visual cortex, it will be possible to dissect the mechanisms that drive

both retina-driven and centrally generated network events. In addition, applying small lesions to specifically ablate connections to neighboring cortical areas could reveal whether network events with a high participation rate are triggered intrinsically or generated in other non-cortical regions.

The chronic perturbation of retinal activity before the onset of vision alters the layout of topographic maps, such as retinotopy or eye-specific territories (Huberman et al., 2008). Chronic deprivation of retinal inputs, such as binocular enucleation at very young ages, could provide information about compensatory mechanisms in the generation of network activity in the developing visual cortex. Likewise, the artificial augmentation of spontaneous retinal activity (chronic NKH477 injections) or the perturbation of the spatiotemporal properties of retinal activity (chronic epibatidine injections, β2nAChR KO) could be helpful to study the significance of retinal activity patterns on cortical network activity. These studies might provide insights into the interactions of distinct activity patterns in the visual cortex. The selective disruption of one class of cortical network events (i.e. retinally driven events) could lead to an upregulation of centrally driven cortical network events to maintain a homeostatic balance of network activity in the developing cortex. In addition to functional studies, it would also be revealing to study the effects of chronic changes of retinal activity on the structural level. The use of GFP reporter mice could provide information about the dependence of network formation on retinal activity on the level of subcellular processes.

The findings of this study provide a basis for the future study of the concerted interplay of molecular guidance cues and activity-dependent mechanisms. By choosing the appropriate mouse models (e.g. axon guidance: ephrin-A2/3/5 KO, synaptogenesis: SynCAM1 KO), it will be possible to study the interdependence of specific molecules and distinct classes of cortical network events. It will be interesting to reveal whether different patterns of cortical network activity are differently affected by the absence of specific molecules. In addition, crossing either of these knockout animals with mice that lack stage II retinal waves (β2nAChR KO) will reveal important aspects of the interplay of network activity and molecular factors during cortical network formation. Ultimately, the study of cortical network activity in animal models for neurodevelopmental diseases could shed light on the origin of these disorders. It will be important

to determine whether cortical activity patterns are disrupted or even absent in these animal models to understand the significance of cortical network activity during important stages of cortical development in the healthy brain.

# Bibliography

Adelsberger H, Garaschuk O, Konnerth A (2005) Cortical calcium waves in resting newborn mice. Nat Neurosci 8:988-990.

Akerman CJ, Smyth D, Thompson ID (2002) Visual experience before eye-opening and the development of the retinogeniculate pathway. Neuron 36:869-879.

Allendoerfer KL, Shatz CJ (1994) The subplate, a transient neocortical structure: its role in the development of connections between thalamus and cortex. Annu Rev Neurosci 17:185-218.

Allene C, Cossart R (2010) Early NMDA receptor-driven waves of activity in the developing neocortex: physiological or pathological network oscillations? J Physiol 588:83-91.

Allene C, Cattani A, Ackman JB, Bonifazi P, Aniksztejn L, Ben-Ari Y, Cossart R (2008) Sequential Generation of Two Distinct Synapse-Driven Network Patterns in Developing Neocortex. J Neurosci 28:12851-12863.

Antonini A, Fagiolini M, Stryker MP (1999) Anatomical correlates of functional plasticity in mouse visual cortex. J Neurosci 19:4388-4406.

Bansal A, Singer JH, Hwang BJ, Xu W, Beaudet A, Feller MB (2000) Mice lacking specific nicotinic acetylcholine receptor subunits exhibit dramatically altered spontaneous activity patterns and reveal a limited role for retinal waves in forming ON and OFF circuits in the inner retina. J Neurosci 20:7672-7681.

Ben Ari Y (2001) Developing networks play a similar melody. Trends Neurosci 24:353-360.

Ben-Ari Y, Cherubini E, Corradetti R, Galarsa J-L (1989) Giant synaptic potentials in immature rat CA3 hippocampal neurones. J Physiol (Lond) 416:303-325.

Bence M, Levelt CN (2005) Structural plasticity in the developing visual system. Prog Brain Res 147:125-139.

Biederer T, Sara Y, Mozhayeva M, Atasoy D, Liu X, Kavalali ET, Sudhof TC (2002) SynCAM, a synaptic adhesion molecule that drives synapse assembly. Science 297:1525-1531.

Bjartmar L, Huberman AD, Ullian EM, Renteria RC, Liu X, Xu W, Prezioso J, Susman MW, Stellwagen D, Stokes CC, Cho R, Worley P, Malenka RC, Ball S, Peachey NS, Copenhagen D, Chapman B, Nakamoto M, Barres BA, Perin MS (2006) Neuronal pentraxins mediate synaptic refinement in the developing visual system. J Neurosci 26:6269-6281.

Blankenship AG, Feller MB (2010) Mechanisms underlying spontaneous patterned activity in developing neural circuits. Nat Rev Neurosci 11:18-29.

Blanks JC, Adinolfi AM, Lolley RN (1974) Synaptogenesis in the photoreceptor terminal of the mouse retina. J Comp Neurol 156:81-93.

Bollmann JH, Engert F (2009) Subcellular topography of visually driven dendritic activity in the vertebrate visual system. Neuron 61:895-905.

Bonhoeffer T, Grinvald A (1991) Iso-orientation domains in cat visual cortex are arranged in pinwheel-like patterns. Nature 353:429-431.

Bonifazi P, Goldin M, Picardo MA, Jorquera I, Cattani A, Bianconi G, Represa A, Ben-Ari Y, Cossart R (2009) GABAergic Hub Neurons Orchestrate Synchrony in Developing Hippocampal Networks. Science 326:1419-1424.

Boulanger LM, Shatz CJ (2004) Immune signalling in neural development, synaptic plasticity and disease. Nat Rev Neurosci 5:521-531.

Cang J, Kaneko M, Yamada J, Woods G, Stryker MP, Feldheim DA (2005a) Ephrin-as guide the formation of functional maps in the visual cortex. Neuron 48:577-589.

Cang J, Niell CM, Liu X, Pfeiffenberger C, Feldheim DA, Stryker MP (2008) Selective Disruption of One Cartesian Axis of Cortical Maps and Receptive Fields by Deficiency in Ephrin-As and Structured Activity. Neuron 57:511-523.

Cang J, Renteria RC, Kaneko M, Liu X, Copenhagen DR, Stryker MP (2005b) Development of Precise Maps in Visual Cortex Requires Patterned Spontaneous Activity in the Retina. Neuron 48:797-809.

Chandrasekaran AR, Plas DT, Gonzalez E, Crair MC (2005) Evidence for an instructive role of retinal activity in retinotopic map refinement in the superior colliculus of the mouse. J Neurosci %20;25:6929-6938.

Chapman B (2000) Necessity for afferent activity to maintain eye-specific segregation in ferret lateral geniculate nucleus. Science 287:2479-2482.

Chen C, Regehr WG (2000) Developmental Remodeling of the Retinogeniculate Synapse. Neuron 28:955-966.

Cheng HJ, Nakamoto M, Bergemann AD, Flanagan JG (1995) Complementary gradients in expression and binding of ELF-1 and Mek4 in development of the topographic retinotectal projection map. Cell 82:371-381.

Chiu C, Weliky M (2001) Spontaneous activity in developing ferret visual cortex in vivo. J Neurosci 21:8906-8914.

Cline H (2003) Sperry and Hebb: oil and vinegar? Trends Neurosci 26:655-661.

Colonnese MT, Kaminska A, Minlebaev M, Milh M, Bloem B, Lescure S, Moriette G, Chiron C, Ben-Ari Y, Khazipov R (2010) A conserved switch in sensory processing prepares developing neocortex for vision. Neuron 67:480-498.

Colonnese MT, Khazipov R (2010) "Slow activity transients" in infant rat visual cortex: a spreading synchronous oscillation patterned by retinal waves. J Neurosci 30:4325-4337.

Corlew R, Bosma MM, Moody WJ (2004) Spontaneous, synchronous electrical activity in neonatal mouse cortical neurones. J Physiol 560:377-390.

Corriveau RA, Huh GS, Shatz CJ (1998) Regulation of class I MHC gene expression in the developing and mature CNS by neural activity. Neuron 21:505-520.

Crair MC, Gillespie DC, Stryker MP (1998) The role of visual experience in the development of columns in cat visual cortex. Science 279:566-570.

Crair MC, Ruthazer ES, Gillespie DC, Stryker MP (1997) Relationship between the ocular dominance and orientation maps in visual cortex of monocularly deprived cats. Neuron 19:307-318.

Crowley JC, Katz LC (1999) Development of ocular dominance columns in the absence of retinal input. Nat Neurosci 2:1125-1130.

Crowley JC, Katz LC (2000) Early development of ocular dominance columns. Science 290:1321-1324.

Crowley JC, Katz LC (2002) Ocular dominance development revisited. Curr Opin Neurobiol 12:104-109.

Dalva MB, McClelland AC, Kayser MS (2007) Cell adhesion molecules: signalling functions at the synapse. Nat Rev Neurosci ..

Demas J, Sagdullaev BT, Green E, Jaubert-Miazza L, McCall MA, Gregg RG, Wong RO, Guido W (2006) Failure to maintain eye-specific segregation in nob, a mutant with abnormally patterned retinal activity. Neuron 50:247-259.

Demas J, Eglen SJ, Wong ROL (2003) Developmental Loss of Synchronous Spontaneous Activity in the Mouse Retina Is Independent of Visual Experience. J Neurosci 23:2851.

Drager UC (1975) Receptive fields of single cells and topography in mouse visual cortex. J Comp Neurol 160:269-290.

Drescher U, Kremoser C, Handwerker C, Löschinger J, Noda M, Bonhoeffer F (1995) In vitro guidance of retinal ganglion cell axons by RAGS, a 25 kDa tectal protein related to ligands for Eph receptor tyrosine kinases. Cell 82:359-370.

Driver J, Noesselt T (2008) Multisensory interplay reveals crossmodal influences on 'sensory-specific' brain regions, neural responses, and judgments. Neuron 57:11-23.

Dupont E, Hanganu IL, Kilb W, Hirsch S, Luhmann HJ (2006) Rapid developmental switch in the mechanisms driving early cortical columnar networks. Nature 439:79-83.

Feldheim DA, Vanderhaeghen P, Hansen MJ, Frisen J, Lu Q, Barbacid M, Flanagan JG (1998) Topographic guidance labels in a sensory projection to the forebrain. Neuron 21:1303-1313.

Feller MB, Scanziani M (2005) A precritical period for plasticity in visual cortex. Curr Opin Neurobiol 15:94-100.

Firth SI, Wang CT, Feller MB (2005) Retinal waves: mechanisms and function in visual system development. Cell Calcium 37:425-432.

Flanagan JG (2006) Neural map specification by gradients. Curr Opin Neurobiol 16:59-66.

Fogel AI, Akins MR, Krupp AJ, Stagi M, Stein V, Biederer T (2007) SynCAMs Organize Synapses through Heterophilic Adhesion. J Neurosci 27:12516-12530.

Friauf E, McConnell SK, Shatz CJ (1990) Functional synaptic circuits in the subplate during fetal and early postnatal development of cat visual cortex. J Neurosci 10:2601-2613.

Fuerst PG, Bruce F, Tian M, Wei W, Elstrott J, Feller MB, Erskine L, Singer JH, Burgess RW (2009) DSCAM and DSCAML1 function in self-avoidance in multiple cell types in the developing mouse retina. Neuron 64:484-497.

Fuerst PG, Burgess RW (2009) Adhesion molecules in establishing retinal circuitry. Curr Opin Neurobiol 19:389-394.

Galli L, Maffei L (1988) Spontaneous impulse activity of rat retinal ganglion cells in prenatal life. Science 242:90-91.

Garaschuk O, Hanse E, Konnerth A (1998) Developmental profile and synaptic origin of early network oscillations in the CA1 region of rat neonatal hippocampus. J Physiol London 507:219-236.

Garaschuk O, Linn J, Eilers J, Konnerth A (2000) Large-scale oscillatory calcium waves in the immature cortex. Nat Neurosci 3:452-459.

Garaschuk O, Milos RI, Grienberger C, Marandi N, Adelsberger H, Konnerth A (2006a) Optical monitoring of brain function in vivo: from neurons to networks. Pflugers Arch 453:385-396.

Garaschuk O, Milos RI, Konnerth A (2006b) Targeted bulk-loading of fluorescent indicators for two-photon brain imaging in vivo. Nat Protoc 1:380-386.

Golshani P, Goncalves JT, Khoshkhoo S, Mostany R, Smirnakis S, Portera-Cailliau C (2009) Internally Mediated Developmental Desynchronization of Neocortical Network Activity. J Neurosci 29:10890-10899.

Gordon JA, Stryker MP (1996) Experience-dependent plasticity of binocular responses in the primary visual cortex of the mouse. J Neurosci 16:3274-3286.

Greenberg DS, Houweling AR, Kerr JND (2008) Population imaging of ongoing neuronal activity in the visual cortex of awake rats. Nat Neurosci 11:749-751.

Grewe BF, Helmchen F (2009) Optical probing of neuronal ensemble activity. Curr Opin Neurobiol In Press, Corrected Proof.

Grubb MS, Rossi FM, Changeux JP, Thompson ID (2003) Abnormal functional organization in the dorsal lateral geniculate nucleus of mice lacking the beta 2 subunit of the nicotinic acetylcholine receptor. Neuron 40:1161-1172.

Hanganu IL, Ben Ari Y, Khazipov R (2006) Retinal waves trigger spindle bursts in the neonatal rat visual cortex. J Neurosci 26:6728-6736.

Hanganu IL, Kilb W, Luhmann HJ (2002) Functional synaptic projections onto subplate neurons in neonatal rat somatosensory cortex. J Neurosci 22:7165-7176.

Hanson MG, Landmesser LT (2004) Normal patterns of spontaneous activity are required for correct motor axon guidance and the expression of specific guidance molecules. Neuron 43:687-701.

Hebb DO (1949) The Organization of Behavior: A Neuropsychological Theory. New York: Wiley.

Hensch TK (2005) Critical period mechanisms in developing visual cortex. Curr Top Dev Biol 69:215-37.:215-237.

Hindges R, Mclaughlin T, Genoud N, Henkemeyer M, O'Leary DD (2002) EphB forward signaling controls directional branch extension and arborization required for dorsal-ventral retinotopic mapping. Neuron 35:475-487.

Hofer SB, Mrsic-Flogel TD, Bonhoeffer T, Hubener M (2009) Experience leaves a lasting structural trace in cortical circuits. Nature 457:313-317.

Hofer SB, Mrsic-Flogel TD, Bonhoeffer T, Hubener M (2006) Lifelong learning: ocular dominance plasticity in mouse visual cortex. Curr Opin Neurobiol 16:451-459.

Hooks BM, Chen C (2007) Critical periods in the visual system: changing views for a model of experience-dependent plasticity. Neuron 56:312-326.

Hubel DH, Wiesel TN (1959) Receptive fields of single neurones in the cat's striate cortex. J Physiol 148:574-591.

Hubel DH, Wiesel TN (1970) The period of susceptibility to the physiological effects of unilateral eye closure in kittens. J Physiol 206:419-436.

Hubel DH, Wiesel TN, LeVay S (1977) Plasticity of ocular dominance columns in monkey striate cortex. Philos Trans R Soc Lond [Biol ] 278:377-409.

Huberman AD, Clandinin TR, Baier H (2010) Molecular and cellular mechanisms of lamina-specific axon targeting. Cold Spring Harb Perspect Biol 2:a001743.

Huberman AD, Feller MB, Chapman B (2008) Mechanisms Underlying Development of Visual Maps and Receptive Fields. Annu Rev Neurosci 31:479-509.

Huberman AD, Murray KD, Warland DK, Feldheim DA, Chapman B (2005) Ephrin-As mediate targeting of eye-specific projections to the lateral geniculate nucleus. Nat Neurosci 8:1013-1021.

Huberman AD, Speer CM, Chapman B (2006) Spontaneous retinal activity mediates development of ocular dominance columns and binocular receptive fields in v1. Neuron 52:247-254.

Huh GS, Boulanger LM, Du H, Riquelme PA, Brotz TM, Shatz CJ (2000) Functional requirement for class I MHC in CNS development and plasticity. Science 290:2155-2159.

Kandler K, Katz LC (1998) Relationship between dye coupling and spontaneous activity in developing ferret visual cortex. Dev Neurosci 20:59-64.

Kanold PO (2009) Subplate neurons: crucial regulators of cortical development and plasticity. Front Neuroanat 3:16.

Kanold PO, Luhmann HJ (2010) The subplate and early cortical circuits. Annu Rev Neurosci 33:23-48.

Kara P, Reinagel P, Reid RC (2000) Low response variability in simultaneously recorded retinal, thalamic, and cortical neurons. Neuron 27:635-646.

Kerr JND, de Kock CPJ, Greenberg DS, Bruno RM, Sakmann B, Helmchen F (2007) Spatial Organization of Neuronal Population Responses in Layer 2/3 of Rat Barrel Cortex. J Neurosci 27:13316-13328.

Khazipov R, Sirota A, Leinekugel X, Holmes GL, Ben-Ari Y, Buzsaki G (2004) Early motor activity drives spindle bursts in the developing somatosensory cortex. Nature 432:758-761.

Khazipov R, Luhmann HJ (2006) Early patterns of electrical activity in the developing cerebral cortex of humans and rodents. Trends in Neurosciences 29:414-418.

Kobayashi T, Nakamura H, Yasuda M (1990) Disturbance of refinement of retinotectal projection in chick embryos by tetrodotoxin and grayanotoxin. Brain Res Dev Brain Res 57:29-35.

Koch SM, Ullian EM (2010) Neuronal pentraxins mediate silent synapse conversion in the developing visual system. J Neurosci 30:5404-5414.

Krug K, Akerman CJ, Thompson ID (2001) Responses of neurons in neonatal cortex and thalamus to patterned visual stimulation through the naturally closed lids. J Neurophysiol 85:1436-1443.

Kuhn B, Denk W, Bruno RM (2008) In vivo two-photon voltage-sensitive dye imaging reveals top-down control of cortical layers 1 and 2 during wakefulness. Proc Natl Acad Sci U S A 105:7588-7593.

Lang SB, Stein V, Bonhoeffer T, Lohmann C (2007) Endogenous brain-derived neurotrophic factor triggers fast calcium transients at synapses in developing dendrites. J Neurosci 27:1097-1105.

Le Bon-Jego M, Yuste R (2007) Persistently active, pacemaker-like neurons in neocortex. Front Neurosci 1:123-129.

Leinekugel X, Khazipov R, Cannon R, Hirase H, Ben-Ari Y, Buzsaki G (2002) Correlated bursts of activity in the neonatal hippocampus in vivo. Science 296:2049-2052.

LeVay S, Stryker MP, Shatz CJ (1978) Ocular dominance columns and their development in layer IV of the cat's visual cortex. J Comp Neurol 179:223-244.

LeVay S, Wiesel TN, Hubel DH (1980) The development of ocular dominance columns in normal and visually deprived monkeys. J Comp Neurol 191:1-51.

Levitt P, Eagleson KL, Powell EM (2004) Regulation of neocortical interneuron development and the implications for neurodevelopmental disorders. Trends Neurosci 27:400-406.

Lidman O, Olsson T, Piehl F (1999) Expression of nonclassical MHC class I (RT1-U) in certain neuronal populations of the central nervous system. Eur J Neurosci 11:4468-4472.

Linden DC, Guillery RW, Cucchiaro J (1981) The dorsal lateral geniculate nucleus of the normal ferret and its postnatal development. J Comp Neurol 203:189-211.

Lohmann C (2009) Calcium signaling and the development of specific neuronal connections. Prog Brain Res 175:443-452.

Lopez-Bendito G, Molnar Z (2003) Thalamocortical development: how are we going to get there? Nat Rev Neurosci 4:276-289.

Lyckman AW, Horng S, Leamey CA, Tropea D, Watakabe A, Van Wart A, McCurry C, Yamamori T, Sur M (2008) Gene expression patterns in visual cortex during the critical period: Synaptic stabilization and reversal by visual deprivation. Proc Natl Acad Sci U S A.

Mann F, Ray S, Harris W, Holt C (2002) Topographic mapping in dorsoventral axis of the Xenopus retinotectal system depends on signaling through ephrin-B ligands. Neuron 35:461-473.

Marks MJ, Robinson SF, Collins AC (1996) Nicotinic agonists differ in activation and desensitization of 86Rb+ efflux from mouse thalamic synaptosomes. J Pharmacol Exp Ther 277:1383-1396.

Matthews BJ, Kim ME, Flanagan JJ, Hattori D, Clemens JC, Zipursky SL, Grueber WB (2007) Dendrite self-avoidance is controlled by Dscam. Cell 129:593-604.

McCormick DA, Trent F, Ramoa AS (1995) Postnatal development of synchronized network oscillations in the ferret dorsal lateral geniculate and perigeniculate nuclei. J Neurosci 15:5739-5752.

Mclaughlin T, O'Leary DD (2005) Molecular gradients and development of retinotopic maps. Annu Rev Neurosci 28:327-355.

Mclaughlin T, Torborg CL, Feller MB, O'Leary DD (2003) Retinotopic map refinement requires spontaneous retinal waves during a brief critical period of development. Neuron 40:1147-1160.

Meister M, Wong ROL, Baylor DA, Shatz CJ (1991) Synchronous bursts of action potentials in ganglion cells of the developing mammalian retina. Science 252:939-943.

Mennerick S, Zorumski CF (2000) Neural activity and survival in the developing nervous system. Mol Neurobiol 22:41-54.

Metin C, Godement P, Imbert M (1988) The primary visual cortex in the mouse: receptive field properties and functional organization. Exp Brain Res 69:594-612.

Millard SS, Flanagan JJ, Pappu KS, Wu W, Zipursky SL (2007) Dscam2 mediates axonal tiling in the Drosophila visual system. Nature 447:720-724.

Minlebaev M, Ben-Ari Y, Khazipov R (2007) Network mechanisms of spindle-burst oscillations in the neonatal rat barrel cortex in vivo. J Neurophysiol 97:692-700.

Minlebaev M, Ben-Ari Y, Khazipov R (2009) NMDA receptors pattern early activity in the developing barrel cortex in vivo. Cereb Cortex 19:688-696.

Montoro RJ, Yuste R (2004) Gap junctions in developing neocortex: a review. Brain Res Brain Res Rev 47:216-226.

Mooney R, Penn AA, Gallego R, Shatz CJ (1996) Thalamic Relay of Spontaneous Retinal Activity Prior to Vision. Neuron 17:863-874.

Morgan J, Thompson ID (1993) The segregation of ON- and OFF-center responses in the lateral geniculate nucleus of normal and monocularly enucleated ferrets. Vis Neurosci 10:303-311.

Mrsic-Flogel TD, Hofer SB, Ohki K, Reid RC, Bonhoeffer T, Hubener M (2007) Homeostatic Regulation of Eye-Specific Responses in Visual Cortex during Ocular Dominance Plasticity. Neuron 54:961-972.

Muir-Robinson G, Hwang BJ, Feller MB (2002) Retinogeniculate axons undergo eye-specific segregation in the absence of eye-specific layers. J Neurosci 22:5259-5264.

Murayama M, Perez-Garci E, Nevian T, Bock T, Senn W, Larkum ME (2009) Dendritic encoding of sensory stimuli controlled by deep cortical interneurons. Nature.

Myhr KL, Lukasiewicz PD, Wong RO (2001) Mechanisms Underlying Developmental Changes in the Firing Patterns of ON and OFF Retinal Ganglion Cells during Refinement of their Central Projections. J Neurosci 21:8664-8671.

Neumann H, Cavalie A, Jenne DE, Wekerle H (1995) Induction of MHC class I genes in neurons. Science 269:549-552.

Niell CM, Stryker MP (2010) Modulation of Visual Responses by Behavioral State in Mouse Visual Cortex. Neuron 65:472-479.

ODonovan MJ, Chub N, Wenner P (1998) Mechanisms of spontaneous activity in developing spinal networks. J Neurobiol 37:131-145.

Ohki K, Chung S, Ch'ng YH, Kara P, Reid RC (2005) Functional imaging with cellular resolution reveals precise micro-architecture in visual cortex. Nature 433:597-603.

Ohki K, Reid RC (2007) Specificity and randomness in the visual cortex. Curr Opin Neurobiol 17:401-407.

Olshausen BA, Field DJ (2004) Sparse coding of sensory inputs. Curr Opin Neurobiol 14:481-487.

Owens DF, Kriegstein AR (1998) Patterns of intracellular calcium fluctuation in precursor cells of the neocortical ventricular zone. J Neurosci 18:5374-5388.

Peinado A (2001) Immature neocortical neurons exist as extensive syncitial networks linked by dendrodendritic electrical connections. J Neurophysiol 85:620-629.

Peinado A, Yuste R, Katz LC (1993) Extensive dye coupling between rat neocortical neurons during the period of circuit formation. Neuron 10:103-114.

Penn AA, Riquelme PA, Feller MB, Shatz CJ (1998) Competition in retinogeniculate patterning driven by spontaneous activity. Science 279:2108-2112.

Pfeiffenberger C, Cutforth T, Woods G, Yamada J, Renteria RC, Copenhagen DR, Flanagan JG, Feldheim DA (2005) Ephrin-As and neural activity are required for eye-specific patterning during retinogeniculate mapping. Nat Neurosci 8:1022-1027.

Potez S, Larkum ME (2008) Effect of common anesthetics on dendritic properties in layer 5 neocortical pyramidal neurons. J Neurophysiol 99:1394-1407.

Price DJ, Aslam S, Tasker L, Gillies K (1997) Fates of the earliest generated cells in the developing murine neocortex. J Comp Neurol 377:414-422.

Prusky GT, Douglas RM (2004) Characterization of mouse cortical spatial vision. Vision Res 44:3411-3418.

Rheims S, Minlebaev M, Ivanov A, Represa A, Khazipov R, Holmes GL, Ben Ari Y, Zilberter Y (2008) Excitatory GABA in Rodent Developing Neocortex in vitro. J Neurophysiol.

Rochefort NL, Garaschuk O, Milos RI, Narushima M, Marandi N, Pichler B, Kovalchuk Y, Konnerth A (2009) Sparsification of neuronal activity in the visual cortex at eye-opening. Proceedings of the National Academy of Sciences 106:15049-15054.

Rossi FM, Pizzorusso T, Porciatti V, Marubio LM, Maffei L, Changeux JP (2001) Requirement of the nicotinic acetylcholine receptor beta 2 subunit for the anatomical and functional development of the visual system. Proc Natl Acad Sci U S A 98:6453-6458.

Ruthazer ES, Stryker MP (1996) The role of activity in the development of long-range horizontal connections in area 17 of the ferret. J Neurosci 16:7253-7269.

Ruthazer ES, Akerman CJ, Cline HT (2003) Control of Axon Branch Dynamics by Correlated Activity in Vivo. Science 301:66.

Sato M, Stryker MP (2008) Distinctive Features of Adult Ocular Dominance Plasticity. J Neurosci 28:10278-10286.

Sawtell NB, Frenkel MY, Philpot BD, Nakazawa K, Tonegawa S, Bear MF (2003) NMDA receptor-dependent ocular dominance plasticity in adult visual cortex. Neuron 38:977-985.

Schuett S, Bonhoeffer T, Hubener M (2002) Mapping Retinotopic Structure in Mouse Visual Cortex with Optical Imaging. J Neurosci 22:6549-6559.

Schwartz TH, Rabinowitz D, Unni V, Kumar VS, Smetters DK, Tsiola A, Yuste R (1998) Networks of coactive neurons in developing layer 1. Neuron 20:541-552.

Seamon KB, Daly JW (1986) Forskolin: its biological and chemical properties. Adv Cyclic Nucleotide Protein Phosphorylation Res 20:1-150.

Shatz CJ, Stryker MP (1978) Ocular dominance in layer IV of the cat's visual cortex and the effects of monocular deprivation. J Physiol 281:267-283.

Simon DK, Prusky GT, O'Leary DDM, Constantine-Paton M (1992) N-methyl-D-aspartate receptor antagonists disrupt the formation of a mammalian neural map. Proc Natl Acad Sci USA 89:10593-10597.

Simon W, Hapfelmeier G, Kochs E, Zieglgansberger W, Rammes G (2001) Isoflurane blocks synaptic plasticity in the mouse hippocampus. Anesthesiology 94:1058-1065.

Smith SL, Hausser M (2010) Parallel processing of visual space by neighboring neurons in mouse visual cortex. Nat Neurosci 13:1144-1149.

Song S, Abbott LF (2001) Cortical development and remapping through spike timing-dependent plasticity. Neuron 32:339-350.

Sperry RW (1963) Chemoaffinity in the orderly growth of nerve fiber patterns and connections. Proc Natl Acad Sci USA 50:703-710.

Sretavan DW, Shatz CJ (1986) Prenatal development of retinal ganglion cell axons: segregation into eye-specific layers. J Neurosci 6:234-251.

Stafford BK, Sher A, Litke AM, Feldheim DA (2009) Spatial-temporal patterns of retinal waves underlying activity-dependent refinement of retinofugal projections. Neuron 64:200-212.

Stellwagen D, Shatz CJ (2002) An instructive role for retinal waves in the development of retinogeniculate connectivity. Neuron 33:357-367.

Stellwagen D, Shatz CJ, Feller MB (1999) Dynamics of retinal waves are controlled by cyclic AMP. Neuron 24:673-685.

Stevens B, Allen NJ, Vazquez LE, Howell GR, Christopherson KS, Nouri N, Micheva KD, Mehalow AK, Huberman AD, Stafford B, Sher A, Litke AM, Lambris JD, Smith SJ, John SW, Barres BA (2007) The classical complement cascade mediates CNS synapse elimination. Cell 131:1164-1178.

Stoppini L, Buchs P-A, Muller D (1991) A simple method for organotypic cultures of nervous tissue. J Neurosci Meth 37:173-182.

Stosiek C, Garaschuk O, Holthoff K, Konnerth A (2003) In vivo two-photon calcium imaging of neuronal networks. Proceedings of the National Academy of Sciences 100:7319.

Stryker MP, Harris WA (1986) Binocular impulse blockade prevents the formation of ocular dominance columns in cat visual cortex. J Neurosci 6:2117-2133.

Sun C, Speer CM, Wang GY, Chapman B, Chalupa LM (2008a) Epibatidine application in vitro blocks retinal waves without silencing all retinal ganglion cell action potentials in developing retina of the mouse and ferret. J Neurophysiol 100:3253-3263.

Sun C, Warland DK, Ballesteros JM, van der List D, Chalupa LM (2008b) Retinal waves in mice lacking the +¦2 subunit of the nicotinic acetylcholine receptor. Proceedings of the National Academy of Sciences 105:13638-13643.

Sun JJ, Luhmann HJ (2007) Spatio-temporal dynamics of oscillatory network activity in the neonatal mouse cerebral cortex. Eur J Neurosci 26:1995-2004.

Svoboda K, Denk W, Kleinfeld D, Tank DW (1997) In vivo dendritic calcium dynamics in neocortical pyramidal neurons. Nature 385:161-165.

Syed MM, Lee S, Zheng J, Zhou ZJ (2004) Stage-dependent dynamics and modulation of spontaneous waves in the developing rabbit retina. J Physiol ..

Tavazoie SF, Reid RC (2000) Diverse receptive fields in the lateral geniculate nucleus during thalamocortical development. Nat Neurosci 3:608-616.

Tian N, Copenhagen DR (2003) Visual stimulation is required for refinement of ON and OFF pathways in postnatal retina. Neuron 39:85-96.

Van Quyen M, Khalilov I, Ben Ari Y (2006) The dark side of high-frequency oscillations in the developing brain. Trends in Neurosciences 29:419-427.

Voigt T, Opitz T, de Lima AD (2001) Synchronous oscillatory activity in immature cortical network is driven by GABAergic preplate neurons. J Neurosci 21:8895-8905.

Waites CL, Craig AM, Garner CC (2005) Mechanisms of vertebrate synaptogenesis. Annu Rev Neurosci 28:251-274.

Wallace W, Bear MF (2004) A Morphological Correlate of Synaptic Scaling in Visual Cortex. J Neurosci 24:6928-6938.

Weissman TA, Riquelme PA, Ivic L, Flint AC, Kriegstein AR (2004) Calcium waves propagate through radial glial cells and modulate proliferation in the developing neocortex. Neuron 43:647-661.

Weliky M, Bosking WH, Fitzpatrick D (1996) A systematic map of direction preference in primary visual cortex. Nature 379:725-728.

Weliky M, Katz LC (1999) Correlational structure of spontaneous neuronal activity in the developing lateral geniculate nucleus in vivo [see comments]. Science 285:599-604.

Wiesel TN, Hubel DH (1963) SINGLE-CELL RESPONSES IN STRIATE CORTEX OF KITTENS DEPRIVED OF VISION IN ONE EYE. J Neurophysiol 26:1003-1017.

Wiesel TN, Hubel DH (1974) Ordered arrangement of orientation columns in monkeys lacking visual experience. J Comp Neurol 158:307-318.

Wong ROL (1999) Retinal waves and visual system development. Annu Rev Neurosci 22:29-47.

Wong ROL, Chernjavsky A, Smith SJ, Shatz CJ (1995) Early functional neural networks in the developing retina. Nature 374:716-718.

Wong ROL, Meister M, Shatz CJ (1993) Transient Period of Correlated Bursting Activity During Development of the Mammalian Retina. Neuron 11:923-938.

Yamagata M, Weiner JA, Sanes JR (2002) Sidekicks: Synaptic adhesion molecules that promote lamina-specific connectivity in the retina. Cell 110:649-660.

Yamagata M, Sanes JR (2008) Dscam and Sidekick proteins direct lamina-specific synaptic connections in vertebrate retina. Nature 451:465-469.

Yang JW, Hanganu-Opatz IL, Sun JJ, Luhmann HJ (2009) Three Patterns of Oscillatory Activity Differentially Synchronize Developing Neocortical Networks In Vivo. J Neurosci 29:9011-9025.

Yuste R, MacLean JN, Smith J, Lansner A (2005) The cortex as a central pattern generator. Nat Rev Neurosci 6:477-483.

Yuste R, Peinado A, Katz LC (1992) Neuronal domains in developing neocortex. Science 257:666-669.

Zhao C, Kao JP, Kanold PO (2009) Functional excitatory microcircuits in neonatal cortex connect thalamus and layer 4. J Neurosci 29:15479-15488.

# Acknowledgements – Danksagung

First and foremost, I wish to thank Dr. Christian Lohmann for being a great and very supportive supervisor. His encouraging and open-minded attitude enabled me to pursue my own ideas. He always took his time for discussions and help if needed. I am very grateful for his belief in me and the project.

I am very thankful to Prof. Dr. Tobias Bonhoeffer for giving me the opportunity to work in his department in the first years of my PhD training. I always felt assured of his support and encouragement.

Many thanks go to the members of my thesis advisory committee, Prof. Dr. Mark Hübener, Dr. Thomas Misgeld and Dr. Volker Scheuss. I really appreciate your support and interest in my work.

I would also like to express my thanks to Prof. Dr. Rainer Uhl for reviewing this thesis.

Some years ago, Prof. Dr. Rachel Wong sparked my interest in neuroscience. Her passion for neuroscience and her warm personality confirmed my wish to become a neuroscientist for which I am very grateful.

Thanks to the Lohmann group for being around all year long! Thank you, Thomas and Johan for helping me with data analysis and for innumerous lunch and coffee breaks. I would especially like to thank my flat and office mate Kristin. In the short time she became a very good friend of mine. I will never forget the evenings on my couch! Many thanks go to all present and former members of the Bonhoeffer and Stein group for a great work atmosphere that I often thought of during my time in Amsterdam.

Mein ganz besonderer Dank gilt meinen Eltern sowie meiner Schwester und ihrer großartigen Familie. Vielen Dank für Eure bedingungslose Liebe und dafür, dass Ihr da seid! Ebenso möchte ich Alexander danken, der mein Leben in den letzten Jahren so bereichert hat. Die unzähligen Flüge quer durch Europa waren nur möglich, weil ich fest an uns glaube!

# Curriculum Vitae

## Personal Data

| | |
|---|---|
| Name: | Friederike Siegel |
| Date of birth: | 16th August, 1979 |
| Place of birth: | Freising/Germany |
| Citizenship: | German |

## Education

| | |
|---|---|
| 07 / 2006 – present | PhD under the supervision of Dr. Christian Lohmann and Prof. Dr. Tobias Bonhoeffer at the department of Synapse and Network Development at the Netherlands Institute of Neuroscience, Amsterdam/Netherlands and the department of Cellular and Systems Neurobiology, Max-Planck-Institute of Neurobiology, Martinsried /Germany. |
| 02 / 2005 – 05 / 2006 | Diploma thesis and research project under the supervision of Prof. Dr. Rachel Wong at the department of Anatomy and Neurobiology at Washington University, School of Medicine, Saint Louis/USA.<br>„In vivo $Ca^{2+}$ imaging in the developing zebrafish retina" |
| 10 / 2000 – 11 / 2005 | Undergraduate studies in biology at the Ludwig-Maximilians-Universität, Munich/Germany.<br>Major subject: neurobiology, minor subjects: immunology, microbiology and zoology. |
| 11 / 2000 – 04 / 1999 | Undergraduate studies in history, Romance studies and musicology at the University of Regensburg, Regensburg/Germany. |
| 09 / 2000 – 06 / 1999 | Abitur (High School Diploma) at Camerloher-Gymnasium, Freising/Germany. |