All human beings by nature desire to know.

A sign of this is our liking for the senses; for even apart from their usefulness we like them for themselves – especially the sense of sight, since we choose seeing above practically all the others, not only as an aid to action, but also when we have no intention of acting.

The reason is that sight, more than any of the other senses, gives us knowledge of things and clarifies many differences among them.

-Aristotle, Metaphysics, Book 1
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Abstract

The visually evoked potential (VEP) is a local field potential (LFP) evoked in visual cortex in response to visual stimuli. Unlike extracellular single unit recordings, which allow us to probe the function of single spiking cells acutely, the chronic VEP technique gives us insight into ensemble synaptic activity. However, while action potentials are easily interpreted as the output of the recorded neuron, LFPs are difficult to interpret because they may reflect the sum of activity occurring at or beyond the site of recording. The goal of this study was to use the current source density (CSD) method to derive information about synaptic activity occurring at the site of recording and to determine how this activity relates to the concurrent LFP.

The mouse has recently become a widely-used experimental model for studying the mechanisms of plasticity and there has been an increase in the use of VEP recordings to study experience-dependent changes in mouse primary visual cortex (V1). These studies typically focus on changes occurring in the layer 4 VEP after a variable period of visual deprivation. Layer 4 of mouse V1 receives heavy direct input from the lateral geniculate nucleus. This initial input is followed by strict hierarchical connectivity from cortical layer 4 to superficial layers 2/3 and from 2/3 to deep layers 5/6. Using a method for silencing cortical activity without affecting geniculate input activity in conjunction with CSD analyses, we found that the laminar flow of activity in mouse V1 in response to various grating stimuli was consistent with the anatomical connectivity going from layer 4 → 2/3 → 5/6.

To determine if the layer 4 VEP is indeed reflecting synaptic activity occurring in layer 4, we applied the CSD method to field potentials recorded from mouse V1. Our results indicate that changes in the layer 4 VEP strongly and significantly covaries with changes in layer 4 current sink activity suggesting that the layer 4 VEP is indeed reflecting local layer 4 synaptic activity. This layer 4 activity is likely due to direct geniculate input since it persisted after intracortical activity was blocked.

If the layer 4 VEP reflects synaptic activity due to direct geniculo-cortical input and if this input is carrying information about the visual world then we would expect the VEP to change as the parameters of the stimuli vary. Indeed the binocular-driven VEP broadened in shape as we increased the spatial frequency (SF) of grating stimuli. Using CSD analyses, we were able to trace the transformations of the layer 4 VEP waveform to changes happening in layer 4 current sinks and layer 4 current sinks were in turn affected by events in deep layers. Specifically, increasing SF of the grating stimuli led to a reduction of current sink activity in deep layers and this unmasked prolonged current sink activity in layer 4. This prolonged layer 4 current sink activity persisted after cortical silencing suggesting that it is likely due to late-onset direct
geniculate input. We suggest that late-onset activity from the ipsilateral-eye may be unmasked with increasing SF.

VEPs have been used extensively in the clinical and laboratory setting to determine visual acuity in humans as well as anaesthetized animals. If the layer 4 VEP is to be a useful measure of visual function in awake head-fixed mice, VEP-assessed visual acuity and contrast sensitivity should be consistent with behaviorally-assessed measures. We found that VEP-assessed visual acuity agreed with previous behaviorally-assessed acuity; however, VEP-assessed contrast-sensitivity values were slightly higher.

One of the reasons why inbred laboratory mice are becoming increasingly useful in Neuroscience is because individual mice are genetically identical and any behavioral variability should be experience-driven. While this is true for mice within a given strain, it is not true between strains since strains are genetically different. Therefore, it is crucial to understand how strain differences in genes affects neural activity before comparing results from different strains. To this end, we compared the VEP response of two commonly used laboratory mouse strains: C57BL/6 and 129/Sv and found important differences in the VEP waveform which may translate into differences in visual function. Specifically, our data suggest that 129/Sv mice may have better acuity than C57BL/6 mice.

The advent of molecular engineering tools is another reason why the mouse has become the preferred model system for studying the cellular and molecular mechanisms underlying behavioral and physiological phenomena. Genetically modified mice are routinely screened for behavioral deficits using tasks such as the Morris water-maze – a test for spatial navigation which assumes that the mice have functional vision. In order to remove the experimental confound of vision, the layer 4 VEP can be used to assay the visual function of mice prior to behavioral experimentation. Using the VEP technique, we determined the visual function of Shank1⁻/⁻ mice to be normal in response to low SF gratings but impaired in response to high SF gratings. Shank1⁻/⁻ mice were not impaired in the eight-arm radial maze task - another test of spatial navigation - suggesting that low SF vision may be sufficient for performing this task.

Taken together, this study demonstrates that the VEP is an interpretable and useful recording technique which can be combined with CSD analysis to determine the laminar activity patterns which underlie visual function in the awake mouse.
METHODS
Subjects

Juvenile, male C57/BL6 (Charles River Laboratories), 129/SvJae (gift from Rudolf Jaenisch; bred inhouse), and Shank$^{-}$ mice in the 129/SvJae background (bred inhouse) were group housed, on a 12 hr/12 hr light/dark cycle, with food and water available ad libitum. All animals were treated according to NIH and MIT guidelines for animal use.

VEP Recording Procedure

VEP recordings (Figure 1) were performed as previously described (Sawtell et al., 2003). Male C57Bl/6 and 129SvJae mice were anesthetized with 50 mg/kg ketamine and 10 mg/kg xylazine i.p. For purposes of head fixation, a post was glued to the skull just anterior to bregma using cyanoacrylate. Two small burr holes (<0.5 mm) were made in the skull overlaying the binocular visual cortex (3mm lateral of lambda), and tungsten microelectrodes (FHC) were inserted 450 µm below the cortical surface. Reference electrodes were placed bilaterally in frontal cortex. Electrodes were secured in place using cyanoacrylate, and the entire exposure was covered with dental cement. VEP recordings were conducted in awake mice. Mice were habituated to the restraint apparatus prior to the first recording session. The animals were alert and still during recording. Various components of the VEP amplitude were measured; negative-going component (Figure 1c, black arrow), positive-going component (red arrow), and trough-to-peak (blue arrow).
Stagger Dagger Recording Procedure

For current source density (CSD) analysis and single-unit recordings, a custom-made multichannel linear array (Figure 2) consisting of eight tungsten microwires (outer diameter: 22.86 µm; California Fine Wire) was constructed. One end of the wires was wound around the pins of an omnetics connector (Omnetics #A8177-001) and the recording end was fixed together with cyanoacrylate glue. This recording end was cut with fine scissors so that the ends of the wires were staggered relative to one another. The distance between each wire’s tip can vary depending on the angle of the cut. A steep angle results in distances that can span all layers of the mouse cortex. The electrode was implanted normal to the cortical surface of V1 and fixed in place with glue and dental cement. Field potentials and spike activity evoked by visual stimuli in awake, head-restrained mice were collected using commercially available hardware and software. Output from each recording electrode was split, directed to preamplifiers, bandpassed filtered for spikes (300–3000 Hz) and for local field potentials (1–300 Hz), and sent to a PC running the data acquisition software.

Cannula surgery and muscimol+SCH50911 infusion

Following electrode implantation, a second small craniotomy was made 0.5 mm lateral and 0.5 mm posterior to the electrode placement. A guide cannula (Plastics One, Roanoke, VA) was inserted 150 µm below the cortical surface at ~45º angle to the plane of electrode placement, thereby minimizing the distance between the tip of the electrode and the tip of the cannula. Electrodes and guide cannulae were secured in place by
cyanoacrylate glue and dental cement. On the day of infusion, the dummy cannula was removed and replaced with a 33 GA infusion cannula, attached with tubing to a 100 µL Hamilton syringe (VWR, West Chester, PA). A cocktail of muscimol (4 mM) (Sigma, St. Louis, MO) and SCH50911 (6 mM) (Sigma, St. Louis, MO) was infused with an infusion pump (VWR, West Chester, PA) over a 5 min period at a rate of 6 µL/hr. VEPs were recorded throughout the infusion and after the infusion for an additional 1 hr or until the effect of the drug was observed.

Current source density and single unit analysis

Current source density (CSD) analysis was performed to determine the spatiotemporal pattern of current sinks and sources evoked in mouse V1 by the various visual stimuli. From the field potentials collected, the corresponding CSD profiles were approximated by the second spatial derivative of field potentials (Mitzdorf, 1985) collected on channels immediately adjacent to each other (differentiation grid = 1) by the equation 

\[
\frac{d(n) \cdot (V_{n+1} - V_n) - d(n') \cdot (V_n - V_{n-1})}{0.5 \cdot d(n) \cdot d(n') \cdot [d(n) + d(n')]} ,
\]

where \( V_{n-1}, V_n, \) and \( V_{n+1} \) stand for the field potentials recorded at electrode \( n-1, \) \( n, \) and \( n+1, \) and \( d(n) \) is the distance between electrode \( n-1 \) and \( n, \) and \( d(n') \) is the distance between electrode \( n \) and \( n+1. \) The variable distance between the electrodes was taken into account. This analysis was performed assuming that the tissue conductivity through V1 was homogenous and that current flows normal to the cortical surface (Mitzdorf, 1985). Although these assumptions may not be completely valid, the CSD analysis has proved to be a useful first approximation of the spatiotemporal patterns of cortical activation (Freeman and Nicholson, 1975; Mitzdorf, 1985; Mitzdorf and Singer, 1978; Schroeder et
al., 1991). A full account of the theoretical basis of CSD analysis has previously been presented (Freeman and Nicholson, 1975; Mitzdorf, 1985).

For recorded spikes, offline discrimination of single unit activity was based on principal component analysis (PCA) as described by Csicsvari et al (1998). Typically, the first three principal components (PCs) were calculated for each channel. Graphically, units were identified in either two or three dimensions using the first three principle components, and hand-drawn polygons were used to define the cluster borders. To determine peak firing, spike trains were first smoothed by convolution with a Gaussian kernel (sigma = 20 ms). Neurons with average firing rate across the entire trial length (1 second, 300 presentations) in response to a grating stimulus that was greater than 2 standard deviations above the average firing rate in response to a gray screen of equal luminance were used for further analysis. Baseline peak firing during the presentation of a gray screen was subtracted from peak firing during the presentation of an isoluminant grating stimulus.
Visual Stimuli

Visual stimuli (Figure 3) consisted of full-field phase-reversing (1Hz) sine wave horizontal or vertical gratings of varying spatial frequencies (0.05 - 0.6 cycles/deg) and varying contrast levels (0% - 100%). The stimuli were generated by a VSG2/2 card (Cambridge Research System, Cheshire, UK) and presented on a computer monitor suitably linearized by correction. The display was positioned 20 cm in front of the mouse and centered on the midline, thereby occupying 92° x 66° of the visual field. For acuity experiments, mice received 20 presentations of the visual stimuli binocularly. All other experiments consisted of 200 presentations of the visual stimuli to either or both eyes.

Monocular Deprivation

Monocular deprivation was performed by eyelid suture. Mice were anesthetized by inhalation of isoflurane (IsoFlo 2%-3%) and placed under a surgical microscope. Lid margins were trimmed and the antibiotic (Vetropolycin, Pharmaderm) was applied to the eye. Three to five mattress stitches were placed using 6-0 silk, opposing the full extent of the trimmed lids. Animals were fully recovered by breathing room air (20-30mins). Animals whose eyelids did not fully remain shut during the deprivation period were excluded from further experiments. At the end of the deprivation period, mice were reanesthetized, stitches were removed, and lid margins were separated. Eyes were then flushed with sterile saline and checked for clarity under a microscope. Mice with corneal opacities or signs of infection were excluded from further study.


Figure 1. Recording VEPs from the binocular cortex of awake head-fixed mice. **A**, Schematic of the mouse visual system. Input from the contralateral eye arrives via the LGN to the monocular and binocular zones of V1, whereas input from the ipsilateral eye projects only to the binocular zone. **B**, Representation of the VEP recording setup. A mouse previously implanted with recording electrodes in the binocular zone is placed in a restraint apparatus 20 cm away from a computer monitor displaying visual stimuli. The visual stimulus consists of a horizontal or vertical phase reversing (1 Hz) sinusoidal grating. Mice are fully awake during all recording sessions. Image courtesy of M. Frenkel. **C**, A typical VEP in response to one phase of a 0.05 cyc/deg grating stimulus. Arrowhead indicates cue onset. The trace is an average of 100 presentations of the stimulus. The negative-going component is indicated by the black arrow, the positive-going component by the red arrow. The VEP amplitude can be determined by taking the trough-to-peak measure (blue arrow). **D**, After the final recording session a lesion at the recording site is made, the mouse is euthanized and the brain is removed and fixed in formalin. 50 µm slices of the visual cortex are made and stained with cresyl violet to confirm the site of recording.
Figure 2. Steps in the construction of the stagger dagger for use in simultaneously recording field potentials and single unit activity across multiple layers. A, 9-pin Omnetics connector. B, Bare silver wire (0.10"; AM systems #782500) is attached to the leftmost pin to act as recording ground. The connector is glued to a small plastic board made from western blotting film and the assembly is then held in place with double sided tape. 8 tungsten wires (22.86 mm outer diameter) are fixed closely together with cyanoacrylate glue to form a ribbon. The tip of the ribbon is cut in a staggered fashion and then attached to the film with glue. The free end of the ribbon is wound around the remaining pins of the connector. A close up of the tip of the electrode is shown in (C). D, A completed electrode is shown next to a dime for comparison. E, A juvenile mouse implanted with a stagger dagger.
Figure 3. Mice were exposed to full-field isoluminant horizontal or vertical phase-reversing (1 Hz) gratings that were varied in spatial frequency and contrast levels. For acuity experiments, each stimulus of the 48 stimuli shown was randomly presented. In all other experiments, gratings at 100% contrast at three spatial frequencies (0.05, 0.15, and 0.3 cyc/deg) were used.
Chapter 1:

Laminar Pattern of Visually Evoked Activity in Mouse V1: A Current Source Density Analysis of Visually Evoked Potentials
Introduction

Local Field Potentials

It is generally accepted that one form of neural communication consists of single neurons firing spikes and releasing neurotransmitters. Because spiking activity indicates that a neuron has received enough neurotransmitter from presynaptic contacts to push it over action potential threshold, it is a direct measure of that neuron’s output. Measuring single unit activity is therefore the gold standard electrophysiological technique for measuring the brain’s response to external stimuli. When a single neuron spikes, this activity can be detected (using high-pass filters) through a recording electrode placed within ~50 – 100 µm of its location (Bullock, 1997). Action potentials, as well as postsynaptic potentials (PSPs) in non-spiking neurons, consist of the inward and outward movement of current across the neural membrane and these fluctuating currents contribute to local field potentials (LFPs). LFPs are “vector sums of the intercellular currents of many cells,” and may be a second form of neural communication (Bullock, 1997). In an excitatory network, synaptic potentials that result in a net movement of current into cells generate current sinks extracellularly (Figure 4). A net current sink is reflected as a negative-going field potential. Positive ions entering cells must leave in order for the membrane to recharge, and when they do, this generates a loop-closing capacitative current source (Mitzdorf, 1985) and a net current source extracellularly is reflected as a positive-going field potential.

LFPs are easy to record and this tool was extensively used in the early pioneering works of neurophysiology and is still being used in the clinical and laboratory setting (Figure 5). To study the electrical activity of the various elements of the optic
tract, Bartley and Bishop recorded from the surface of the rabbit visual cortex while stimulating the optic nerve (Bartley and Bishop, 1932). They found that a single stimulus to the optic tract elicits a succession of electrical potentials (Figure 5a) which likely “signalizes the activity of successive neurones along the visual pathway, probably successive layers of cortical cells” (Bartley and Bishop, 1932). Visually evoked potentials (VEPs) are LFPs evoked in response to visual stimuli. VEPs have been used in clinical applications (Figure 5b) for many years to assess the functional integrity of the developing visual system in infants and young children (Brecelj, 2003). The VEP technique has been adapted for use in laboratory animals such as rats (Dyer et al., 1987) and mice (Porciatti et al., 1999; Sawtell et al., 2003). Porciatti et al (1999) determined the spatial visual acuity of anaesthetized C57BL/6 mice (Figure 5c) using VEPs recorded from layer 4 of the primary visual cortex (V1). More recently, the VEP recording technique has been used in awake head-fixed mice to study experience-dependent changes (Figure 5d) in V1 (Frenkel and Bear, 2004; Sawtell et al., 2003).

Current Source Density Analysis in the Hippocampus

LFPs, though easy to record, are hard to interpret because they only indirectly reflect the underlying neuronal activities (Mitzdorf, 1985). Since an LFP recorded at one site is a sum of current sinks and current sources occurring at many different sites, the site of recording does not necessarily correspond to the location of major current generators. In order to localize the current sinks and sources which contribute to the LFP, Nicholson and Freeman (1975) applied a method of analyzing field potentials,
known as current source density analysis (CSD). The CSD method localizes the current sinks and sources that generate the local field potential. In contrast to the single-unit method, this analysis “reveals the site of excitatory synaptic interactions rather than locations of cell somata, and like intracellular recordings it discloses even subthreshold synaptic events” (Mitzdorf and Singer, 1978). It has been used extensively to trace the flow of activity within and between cortical layers of various laminated neural structures including the spinal cord, hippocampus, cerebellum, and neocortex (Mitzdorf, 1985).

In an ideal excitatory network, active ionic current flows into the cells at the site of the synapse (e.g. dendrites or cell body) to generate a current sink at that location, and a loop-closing capacitative current of equal amplitude flows out at proximal and more distant membrane sites (Mitzdorf, 1985). In order to generate detectable current sinks and sources, the dendrites or cell bodies of multiple neurons would have to be packed together and have a laminar organization such that all the cell bodies or dendrites are in the same plane. If this requirement is satisfied, then synchronous activation of cells in this structure will result in current flow that is restricted to the laminar plane of the tissue (Richardson et al., 1987). The CA1 region of the hippocampus is this ideal excitatory network (Figure 6). The cells bodies of CA1 pyramidal cells are densely packed and restricted to the stratum pyramidale (SP); the basal dendritic tree of these cells branch extensively in the stratum oriens (SO), while the apical dendrites extend through stratum radiatum (Richardson et al., 1987). Richardson et al (1987) performed CSD analysis on field potentials evoked in SO and stratum radiatum (SR) using subthreshold stimulation (1987). Subthreshold stimulation of either SO or SR evoked graded
negative going potentials and corresponding current sinks restricted to the site of stimulation (Figure 7). A current source at SP and beyond indicates the passive conduction of the EPSPs toward and past the cell body layer. Suprathreshold stimulation of the inputs in SR evoked a population spike response upon synaptic potentials associated with the depolarization of the dendritic arbors. The largest-amplitude and earliest-onset population spike response was found in SP and proximal SO (Figure 8). The negativity of the population spike continued through SR as a biphasic negative/positive potential with an increase in peak latency. The largest-amplitude and shortest peak-latency current sink was found in the region of SP. Like the population spike response, the current sink continued through SR gradually increasing in peak latency to becoming the sink component of a biphasic dendritic source and sink. Using CSD analysis in the CA1 region of the rat hippocampus, Robertson et al (1987) gained insight into the conductance of dendritic EPSPs and the site of action potential generation.

Current Source Density Analysis in Cat Primary Visual Cortex

The CSD profile of field potentials in hippocampus CA1 is easily interpreted because of the laminar structure of the region. While regions of the neocortex are anatomically and functionally organized into columns, there is not a clustering of cell bodies and dendrites into distinct laminae, as in the hippocampus CA1 (Figure 9a). The considerable inter- and intralaminar connectivity in cortex means that CSD analysis carried out in the cortex will be harder to interpret. However, data from anatomical and
single-unit studies help to identify CSD components according to various arguments, including the argument of temporal coincidence and the succession of events as determined by the circuit (Mitzdorf, 1985). The anatomical circuitry in the cat visual cortex has been well studied (Figure 9b). Most of direct lateral geniculate (LGN) input is to layer 4 with fewer afferents going to layer 6 (Reid, 2003). LGN afferents have been grouped into three main classes: X, Y, and W according to various criteria including receptive field size and conductance velocity (Stone, 1983). Y-cells have broad receptive fields, are fast conducting and project more superficially in layer 4 than X cells, which make up the smaller receptive field, slow conducting group. The circuit after the LGN input to layer 4 continues as follows: 4 \rightarrow 2/3 \rightarrow 5 \rightarrow 6. There is considerable interlaminar connectivity as well as feedback connections from 5 \rightarrow 2/3 and 6 \rightarrow 4 (Reid, 2003; Stone, 1983). To determine if evidence of this flow of activity can be detected in the CSD profile, Mitzdorf and Singer performed a CSD analysis in cat primary visual cortex (Mitzdorf and Singer, 1978).

Field potentials evoked by electrical and visual stimulation were recorded in primary visual area 17 of the cat cortex and were subsequently subjected to CSD analysis. Using different stimuli and comparison with anatomical and single-unit data helped them to interpret the CSDs (Mitzdorf, 1985). Field potentials at various cortical depths (pia to white matter) were evoked by electrical stimulation of the optic radiation (Figure 10a). The LFP increased in amplitude across the depth and had large amplitudes in the white matter below the cortex. The corresponding CSD profile (Figure 10b) does not reveal current sinks in white matter indicating that the LFP peaks in white matter reflect current activity in other regions of area 17. The earliest onset current
sinks were monosynaptic and occurred at depths corresponding to layer 4 (Figure 10b, sink a) as well as deep layer 6 (Figure 10b, sink e) consistent with anatomical data that LGN afferent fibers terminate in laminae 4 and 6 (Mitzdorf and Singer, 1978). Late onset sinks reflect polysynaptic intracortical activity and demonstrate the main pathways along which afferent activity is processed within area 17. One to two milliseconds following the peak of the initial layer 4 current sinks are sink-peaks in layers 2 and 3 (Mitzdorf and Singer, 1978). The latencies of the polysynaptic current sinks in supragranular layers (Figure 10b, sinks b,c) suggest that fast-conducting Y-type activity is projected to these layers and processed along this pathway (Mitzdorf, 1985). Polysynaptic current sinks in layer 5 peaked about three milliseconds after the sink peaks in layer 4 (Figure 10b, sink f) and likely reflect slow-conducting X-type afferent activity, which is relayed along this pathway (Mitzdorf, 1985). Electrical stimulation evoked a CSD profile that revealed the two different processing pathways of X-type and Y-type cells in cat area 17. To determine if the same profile can be evoked with visual stimuli, Mitzdorf performed CSD analysis on VEPs recorded in response to strobe flashes and phase-reversing gratings (Mitzdorf, 1985).

Single unit studies have revealed laminar preferences for the receptive fields of single units in cat area 17 (Berman et al., 1982; Gilbert, 1977). Therefore, it is expected that these lamina-specific differences are manifested in the CSDs. The CSD profiles evoked by visual stimuli are qualitatively similar to the profile evoked by electrical stimulation of the optic radiation (Figure 11 b-c cf. 11a). Quantitatively the visually evoked CSDs differ from the electrically evoked CSD in latencies, durations, and amplitudes (Mitzdorf, 1985). For both visual stimuli, earliest onset monosynaptic current
sinks are evoked in layer 4 (Figure 11 b-c, sinks a,d) and layer 6 (Figure 11 b-c, sink e). However, the layer 4 current sinks in response to strobe flash stimuli have shorter onset latencies, are compressed in time, and have higher amplitudes (Figure 11b cf. 11c). These differences may be attributed to higher coherence of the afferent input following flash stimulation (Mitzdorf, 1985). Using a phase-reversing grating stimulus (Figure 11b cf. 11c) leads to an increase in the onset latencies of the sinks in layers 4 and 6, an increase in the amplitude of sinks in layer 6, and a decrease in the amplitude of supragranular sinks. The modulations observed in response to these two different stimuli may be attributed to the differences in the proportion of X- and Y-type afferent input. A contour-rich stimulus evokes more X-type activity and corresponding activity along the second pathway involving infragranular layers which is pronounced, while activity along the first pathway involving supragranular layers is attenuated (Mitzdorf, 1985).

Using data from anatomical and single-unit studies to guide their interpretation of the cat area 17 CSD profile, Mitzdorf and Singer gained insight into the laminar flow of activity involved in processing various stimuli in the visual cortex (Mitzdorf and Singer, 1978). The goal of the present study was to apply the same analysis to the mouse primary visual cortex. Mouse V1, like cat V1, is a laminar structure with anatomical segregation of afferents and functional segregation of receptive field types (Drager, 1974, 1975; Mangini and Pearlman, 1980; Niell and Stryker, 2008). In the mouse, fibers direct from the LGN terminate mainly in layer 4 (Figure 12a), with fewer fibers terminating in layers 2/3, 5, and 6 (Burkhalter and Wang, 2008). The circuit after the LGN input continues as follows (Figure 12b): 4 → 2/3 → 5 → 6. As in the cat, there is
considerable interlaminar connectivity as well as feedback connections from 5 → 2/3 and 6 → 4 (Figure 12b) (Burkhalter and Wang, 2008; Maffei et al., 2006; Maffei and Turrigiano, 2008). While different functional cell types have yet to be clearly identified in the mouse LGN (Grubb, 2008; Grubb and Thompson, 2003), studies in mouse visual cortex have reported variation in receptive field properties across V1 laminae. Of interest to our study are laminar-specific differences in spatial frequency tuning. A recent study by Niell and Stryker (2008) reported that in C57BL/6 mice, cells in layers 2/3, 4, and 5 are preferentially tuned to higher spatial frequency grating stimuli (0.04 cyc/deg) compared with layer 6 cells which are tuned to lower spatial frequencies (0.02 cyc/deg; Figure 13). Taking these anatomical and single unit data into consideration, we set out to interpret mouse area 17 visually evoked CSD profiles.

**Results**

The bases for the present CSD-analysis are sets of visually evoked potentials measured through mouse visual cortical laminae in response to phase-reversing grating stimuli of various spatial frequencies at 100% contrast. Example field potential and CSD profiles from two animals are shown in Figure 14.

*Laminar Pattern of Evoked Activity in Response to 0.05 cyc/deg Gratings*

In general, the laminar flow of current sink activity in mouse V1 was consistent with what we know about the anatomy: a current sink-peak in layer 4 occurred at 62 +/- 1 msec post-stimulus onset (Figure 14a, c, 15a₁) and was followed 8 milliseconds later by a current sink-peak in superficial layers (70 +/- 2 msecs post-stimulus onset; Figure 14a, 15a₁); current sinks in deep layers peaked last at 81 +/- 3 msecs post-stimulus
Layer 4 current sinks in response to 0.05 cyc/deg gratings had, on average, larger peak amplitudes than sink-peaks in superficial and deep layers (Figure 15b; one-way anova, p = 0.1037). The difference in latency to peak (8 msecs between layer 4 and layers 2/3 and 11 msec between layers 2/3 and layers 5/6) most likely reflects polysynaptic intracortical activity following the first synaptic input into layer 4. If this is the case, silencing cortical activity should eliminate the late peaking current sinks in superficial and deep layers. Indeed, infusing a cocktail of muscimol (GABA$_A$ agonist) and SCH50911 (GABA$_B$ antagonist) at the site of recording led to elimination of sinks in superficial and deep layers (Figure 16a, b). However, the amplitude of the early current sink in layer 4 was reduced in response to the drug (Figure 16b). Muscimol is a GABA$_A$ agonist, which acts primarily on postsynaptic receptors, but at high concentrations it also acts on presynaptic GABA$_B$ receptors and inhibits presynaptic activity (Liu et al., 2007). SCH50911 is a GABA$_B$ antagonist which counteracts the presynaptic side effect of muscimol. It is possible that the concentration of SCH50911 used for these experiments did not fully counteract the effect of muscimol on presynaptic GABA$_B$ receptors and the presynaptic response was reduced as a result. Muscimol may also reduce the magnitude of layer 4 current sinks via shunting inhibition. The opening of postsynaptic inhibitory synaptic channels increases membrane resting conductance, thereby decreasing the size of depolarization during the EPSP (Kandel et al., 2000).

*Neural Correlates of the 0.05 cyc/deg Evoked Layer 4 VEP*

In superficial layers, the onset of the field potential waveform is mostly positive-going and becomes more negative-going in deeper layers (Figure 14a, c, left). As in cat
area 17, large-amplitude negative-going VEPs were also detected in white matter of mouse area 17 (Figure 14a, c cf. Figure 10a). Since these VEPs did not coincide with current sinks in white matter (Figure 14c), large far-field components likely contribute to these potentials. The earliest onset, earliest to peak, and largest amplitude negative-going VEP occurred in layer 4 (Figure 14a, c). In the mouse, this peak latency coincided with the peak of current sinks in layer 4 (62 +/- 1 msec; Figure 14a, c, 15a1). Since the negative-going component of the layer VEP peaked at the same time as the layer 4 current sink, this activity likely contributes to the layer 4 VEP. Indeed, there is a significant correlation between the amplitude of the negative-going component of the layer 4 VEP and amplitude of the layer 4 current sink (Figure 17a, r² = 0.4171, p = 0.0437). There is no significant correlation between the negative-going component of the layer 4 VEP and the current sink-peak amplitudes in superficial or deep layers (Supplemental figure 1a, b; superficial: r² = -0.0199, p = 0.7898; deep: r² = 0.1648, p = 0.1187) suggesting that sink activity in these layers may not contribute significantly to the layer 4 VEP. While the standard measure of the LFP is amplitude, we measured the area under the negative-going VEP component (Figure 15d) to determine if it yields a stronger correlation with the area of the corresponding layer 4 CSD sink (Figure 15e). The correlation between the area of the negative-going VEP component and the area of the corresponding layer 4 sink was weak (Supplemental figure 1c, r² = 0.2024, p = 0.1920) confirming that the amplitude measure of the negative-going VEP component better reflects the layer 4 CSD sink. The excitation generated by thalamic input onto layer 4 cells is strong enough to cause these cells to spike and the amplitude of the
spikes correlates with the amplitude of the negative-going VEP component (Figure 17e, $r^2 = 0.3910$, $p = 0.0532$).

The second most prominent component of the layer 4 VEP in response to 0.05 cyc/deg is a positive-going component that peaks at 122 +/- 7 msecs post-stimulus onset (Figure 15a1, red shaded region). This peak coincided in time with a source occurring in layer 4 (126 +/- 6 msecs post-stimulus onset, Figure 15a1) and there was a significant correlation between the amplitude of the positive-going component of the VEP and the amplitude of layer 4 current source peak (Figure 17b, $r^2 = 0.4288$, $p = 0.0399$). The source in layer 4 may be due to an active inhibitory network, or may reflect a passive current source due to recently peaking current sink activity. Prior to the layer 4 source-peak, current sink-peaks occurred in deep layers at 81 +/- 3 millisecond post-stimulus onset (Figure 15a1). Furthermore, the layer 4 current sinks reversed polarity at 88 +/- 6 msecs post-stimulus onset, shortly after sinks in deep layers peaked (82 +/- 3 msecs; Figure 15a1) suggesting that in response to 0.05 cyc/deg, layer 4 may be a current source for deep layer sinks. Therefore, we would expect to see a correlation between the amplitudes of current sinks in deep layers with current sources in layer 4. Indeed, there is a significant correlation between current sinks in deep layers and the layer 4 current source (Figure 17c, $r^2 = 0.2817$, $p = 0.0418$). However, this does not discount the possibility of active inhibitory connections or current sinks from other layers contributing to the current source in layer 4. Since the positive-going component of the layer 4 VEP correlates with layer 4 current sources, and they in turn correlate with current sink activity in deep layers, it is not surprising that the positive-going VEP component correlates with current sink activity in deep layers (Figure 17d, $r^2 = 0.3669$, p
The positive-going VEP component does not correlate with layer 4 current sink amplitudes (Supplemental figure 1d, $r^2 = 0.0342, p = 0.6088$). Silencing the polysynaptic activity, which likely results in deep layer sinks, should eliminate the positive-going VEP component. Indeed after infusing muscimol+SCH50911 into the site of recording, only 6% of the positive-going VEP component remained (Figure 16c). As expected, more of the negative-going component remained (40%) since it likely reflects layer 4 current sinks, which are generated as a result of direct geniculate input. However, the amplitude of the negative-going VEP component was reduced and as mentioned above, this could be due to the side-effect of muscimol on presynaptic GABA$_B$ channels or shunting inhibition in the cortex.

Laminar Pattern of Evoked Activity in Response to 0.15 cyc/deg Gratings

The CSD pattern obtained in response to the 0.15 cyc/deg grating stimulus (Figure 14b, d) was, in some ways, similar to the CSD pattern in response to 0.05 cyc/deg (Figure 14a, c). The higher spatial frequency gratings evoked a current sink in layer 4 that peaked at 64 +/- 1 msecs post-stimulus onset (Figure 15a$_2$). This layer 4 sink-peak was followed 12 msecs later by a current sink in superficial layers, which peaked at 76 +/- 4 msecs post-stimulus onset (Figure 15a$_2$). These latencies were delayed by 1-3 msecs relative to their 0.05 cyc/deg counterparts. However, current sinks in deep layers peaked at 100 +/- 3 msecs post-stimulus onset (Figure 15a$_2$), 18 msecs later than their 0.05 cyc/deg counterparts (Figure 15a$_1$). These late peaking current sinks in superficial and deep layers are likely due to polysynaptic intracortical activity as they were virtually eliminated when muscimol+SCH50911 was infused at the site of recoding (Figure 16d, e).
More differences between 0.05 and 0.15 cyc/deg evoked CSDs were revealed in the current sink amplitudes (Figure 15b). In general, the sinks in response to 0.15 cyc/deg peaked at lower amplitudes, particularly in layer 4 and deep layers, compared to sinks in response to 0.05 cyc/deg (Figure 15b). The lower layer 4 current sink-peak response to 0.15 cyc/deg (paired t-test; \( p = 0.1213 \)) may be attributed to weaker coherence of the afferent input (Mitzdorf, 1985). The current sink amplitudes in superficial layers were only slightly lower in response to 0.15 cyc/deg (-3.2 +/- 0.7 mV/mm²) compared to 0.05 cyc/deg (-3.5 +/- 0.9 mV/mm²; \( p = 0.5968 \)). By contrast, the current sink response to 0.15 cyc/deg in deep layers was noticeably lower (-2.8 +/- 0.4 mV/mm²) than its 0.05 cyc/deg evoked counterpart (-3.8 +/- 0.8 mV/mm²; \( p = 0.1151 \)). The combination of a significantly longer latency to sink-peak (\( p = 0.0008 \)) and a lower amplitude sink-peak suggest that higher spatial frequency stimuli do not drive deep layer responses as strongly as low spatial frequency stimuli. This is consistent with the recent single-unit finding (Niell and Stryker, 2008) that cells in layer 6 prefer lower spatial frequency stimuli compared to cells in all other laminae (Figure 13). If less activity is evoked in deep layers by high spatial frequency gratings, then deep layer sinks should gradually disappear as spatial frequency is gradually increased. Indeed, we observed this in response to spatial frequencies ranging from 0.05 cyc/deg up to 0.6 cyc/deg (Supplemental figure 2).

**Neural Correlates of the 0.15 cyc/deg Evoked Layer 4 VEP**

Similar to the 0.05 cyc/deg evoked VEP profile, the initial VEP response to 0.15 cyc/deg progressed from being mostly positive going at the cortical surface, to negative going from layer 4 and into white matter. However, the field potential profile evoked in
response to the 0.15 cyc/deg grating stimulus (Figure 14b, d) was morphologically different from the VEP in response to 0.05 cyc/deg (Figure 14a, c). In contrast to the 0.05 cyc/deg evoked VEP profile, the 0.15 cyc/deg evoked VEPs had more polarity transitions that made them appear more rippled in shape. The earliest onset, earliest to peak, and largest negative-going response still occurred in the layer 4 region (Figure 14b, d). Similar to the 0.05 cyc/deg evoked VEP, the layer 4 negative-going VEP component in response to 0.15 cyc/deg gratings peaked at 62 +/- 2 msecs post-stimulus onset (Figure 15a2, gray shaded region). The negative-going VEP component in response to 0.05 cyc/deg was followed by one positive-going peak at about 120 msecs post-stimulus onset. In contrast, the 0.15 cyc/deg negative-going VEP component was followed by two positive going components (Figure 14b, d). The polarity inversion after the first negative-going component peaked at 82 +/- 3 msecs post-stimulus onset and was followed by a second negative-going deflection that peaked at 101 +/- 2 msecs post-stimulus onset. The final positive-going deflection peaked at 144 +/- 7 msecs post-stimulus onset (Figure 15a2, red shaded region). A summary table of these peak latencies can be found in Supplemental table 1.

The peak of the first negative-going VEP component in response to 0.15 cyc/deg coincided in time with the layer 4 current sink-peak (Figure 15a2, gray shaded region) suggesting that current sink activity in layer 4 could contribute to this component. There is a correlation between negative-going VEP amplitudes and layer 4 current sink-peaks though it is not significant (Figure 17f, $r^2 = 0.2692, p = 0.1244$). Interestingly, the area under the negative-going VEP component is significantly correlated with the area under the layer 4 current sink-peaks (Supplemental figure 1g, $r^2 = 0.3870, p = 0.0548$). This
suggests that area may be the better measure of the layer 4 VEP in response to 0.15 cyc/deg. The area of the negative-going component did not correlate with amplitudes of single-unit spikes in layer 4 (r² = 0.0130, p = 0.7705, figure not shown); however there was a better correlation between the amplitude of the negative-going component and layer 4 spikes (Figure 17j, r² = 0.2952, p = 0.1306) which again suggests a relationship between strength of the geniculate input and the layer 4 output spiking response. While the negative-going layer 4 VEP amplitudes significantly correlated with current sinks in deep layers (Supplemental figure 1f, r² = 0.5345, p = 0.0013), the latency to peak of the VEP negativity occurred significantly earlier (62 msecs post-stimulus onset) than the latency to peak of the deep layer sinks (100 msecs, p < 0.0001). Therefore, it is unlikely that sinks in deep layers contribute to the negative-going VEP component. Across all spatial frequencies tested (0.05 – 0.6 cyc/deg) the negative-going VEP component temporally overlapped with the layer 4 current sink-peak (Supplemental Figure 2). Collapsed across spatial frequencies, there was a significant correlation between the amplitude of layer 4 currents sinks and the amplitude of the layer 4 negative-going component (Supplemental Figure 3a, r² = 0.5036, p < 0.0001). When normalized to the 0.05 cyc/deg responses, the change in the VEP amplitude strongly correlated with the change in layer 4 sinks (Supplemental Figure 3b, r² = 0.8749, p < 0.0001) suggesting that the layer 4 VEP is a measure of layer 4 synaptic strength.

The peak of the first positive-going VEP component occurred about 6 milliseconds after the peak of current sinks in superficial layers indicating that layer 4 may be a current source to superficial layer sinks (Supplemental table 1). In response to 0.05 cyc/deg, current sink-peaks in superficial layers were followed closely (~ 11
msecs) by large amplitude current sink-peaks in deep layers. However, in response to 0.15 cyc/deg, relatively smaller amplitude deep layer current sinks (Figure 15b) followed ~26 milliseconds after superficial layers sink-peaks (Supplemental table 1). As a result, in response to 0.15 cyc/deg, the layer 4 current sink may be more apparent as it is not being obscured due to competing sinks in deep layers as it did in response to 0.05 cyc/deg. Since layer 4 maintained its current sink for a longer period of time (Figure 14b,d, 15a2), the net effect of this on the layer 4 VEP is that its negative-going component was also extended. This extended VEP negativity reversed and maintained a positive-going polarity at 101 +/- 2 msecs post-stimulus onset, right at the time that current sinks in deep layers peaked (100 +/- 3 msecs, Supplemental table 1). This VEP positivity reached its peak at 144 +/- 7 msecs post-stimulus onset (Figure 15a2, red shaded region) overlapping with the latency to source-peaks occurring in layer 4 (149 +/- 4 msecs post-stimulus onset). Unlike the 0.05 cyc/deg evoked positive-going component, the layer 4 current source-peak amplitudes did not significantly correlate with the amplitude of the positive-going VEP component in response to 0.15 cyc/deg (Figure 17g, r² = 0.0497, p = 0.5360). Furthermore, the layer 4 source-peak in response to 0.15 cyc/deg did not significantly correlate with deep layer sink-peak amplitudes (Figure 17h, r² = 0.2940, p = 0.1054). Consequently, the amplitudes of the positive-going VEP component in response to 0.15 cyc/deg did not significantly correlate with deep layer sink-peak amplitudes (Figure 17i, r² = 0.0004, p = 0.9383). The positive-going component also did not correlate with current-sink activity in layer 4 (Supplemental figure 1h, r² = 0.0294, p = 0.6355). The positive-going VEP component in response to 0.15 cyc/deg is fundamentally different from the positive-going VEP
component in response to 0.05 cyc/deg in that the latter reflects current sink activity in
deep layers while the former appears not to. Nevertheless, the 0.15 cyc/deg evoked
positive-going component is likely due to intracortical activity since only 6% of its
amplitude remains after cortical activity was silenced with muscimol+SCH50911 (Figure
16f). It is worth mentioning that the layer 4 VEP waveform in response to 0.15 cyc/deg
after muscimol+SCH50911 appears narrower in shape, but about 10% of the 2nd
negative-going peak (N100) remained (Figure 16f). The broad shape of the 0.15
cyc/deg evoked VEP under baseline conditions is likely reflecting the ongoing current
sink activity in layer 4 (Figure 14b, d, 15a2). This late sink activity in layer 4 may reflect
intracortical activity or it may be due to slow-peaking activity from direct LGN afferents.
Evidence suggests that both may contribute to the late layer 4 current sink activity.

Silencing cortical activity reduced but failed to completely eliminate the late
ongoing current sink in layer 4 (Figure 16e) suggesting that intracortical activity may be
a major, but not the only, contributor. Evidence for contributions from LGN afferents
comes indirectly from the observation that the layer 4 VEP and current sink have a
longer latency to peak in response to stimulation of the ipsilateral eye than in response
to contralateral eye stimulation (Supplemental figure 4). Layer 4 of mouse V1 receives
contralateral (97%) and ipsilateral (3%) LGN input (Drager, 1974). Contralateral-eye
VEPs in response to 0.15 cyc/deg gratings reached maximum negativity at the same
time (61 +/- 4 msec post-stimulus onset, supplemental figure 4b) as the first negative-
going peak of the binocular evoked VEP (62 +/- 2 msecs). Latency to peak negativity of
Ipsilateral-eye VEPs was longer (88 +/- 8 msecs) and almost overlaped with the second
negative-going peak of the binocular evoked VEP (102 +/- 2 msecs). Latency to layer 4
current sinks evoked in response to 0.15 cyc/deg was also longer for the ipsilateral eye (supplemental figure 4a, 4c). These data suggest that the prolonged layer 4 current sink activity giving rise to the second negative-going peak in the layer 4 VEP may be due to late peaking input from the ipsilateral eye. This delay in peak VEP responses for the ipsilateral eye was also seen in response to low spatial frequency gratings (supplemental figure 4d). However, in response to 0.05 cyc/deg, this late ipsilateral eye input was not resolved into two peaks in the binocular VEP (e.g. figure 14). Layer 4 current sinks evoked by the ipsilateral-eye response to 0.05 cyc/deg may be masked by large amplitude current-sinks in deep layers. Since these deep layer current sinks were smaller in amplitude and peaked later in response to 0.15 cyc/deg, it is possible that current sinks evoked by ipsilateral-eye input to layer 4 became unmasked under this condition.

Discussion

Laminar Pattern of Visually Evoked Activity in Mouse V1

In general, the laminar pattern of visually evoked activity in mouse primary visual cortex was consistent with mouse anatomical and single-unit data. In mouse V1, LGN afferents terminate heavily in layer 4, with fewer fibers terminating in superficial and deep layers (Burkhalter and Wang, 2008). Consistent with this, the earliest onset, earliest peaking, and largest amplitude current sink was observed in layer 4. We did not detect early onset current sink activity in superficial or deep layers indicating that any direct LGN afferents to these layers are not sufficiently activated by the stimuli used in our experiments. We observed prolonged current sink activity in layer 4 in response to high spatial frequency gratings which could be attributed to intracortical activity and/or
activity from late-peaking ipsilateral-eye input. This later portion of the layer 4 current sinks were reduced, but not completely eliminated when cortical activity was silenced. Layer 4 of mouse V1 receives both contra- and ipsilateral LGN input, although 97% of that input is from the contralateral eye (Drager, 1974). As a result, input from the ipsilateral-eye into layer 4 is likely to be weaker and peak later than contralateral eye input. Following the layer 4 sink-peak was a sink-peak in superficial layers, which likely results from layer 4 afferent activity because superficial layers receive direct input from layer 4. It is not likely that the later peaking superficial sinks are due to a second class of late-onset LGN afferents for two reasons. First, when we silenced cortical activity leaving LGN afferent inputs intact, we were able to eliminate current sinks in superficial layers. Second, different functional cell classes have yet to be identified in the mouse LGN (Grubb, 2008). Current sink-peaks in deep layers follow the superficial layer sinks at different latencies depending on the spatial frequency of the grating stimulus. A high spatial frequency stimulus results in a longer latency to peak of deep layer current sinks. For the reasons just mentioned, it is not likely that these sinks are the result of late-onset LGN afferents. More likely is that they are generated as a result of input from layers 2/3. The short latency, large amplitude current sinks in deep layers in response to 0.05 cyc/deg suggest a high coherence of afferent input from layer 2/3 (Mitzdorf, 1985) to these layers. By contrast, the longer latency and smaller amplitude of the deep layer current sink response to 0.15 cyc/deg suggests that in response to higher spatial frequencies, the layers 2/3 input to deep layers is weaker. This would mean that layers 4 and 2/3 are likely acting as low-pass spatial frequency filters. Consistent with this are recent single-unit data from Niell and Stryker showing that cells in layer 5 of
anesthetized mice have a preference for lower spatial frequency gratings than cells in layer 4 and superficial layers (2008). Taken together, the data suggest that the laminar pattern of activity in response to gratings is consistent with what we know about the anatomy of mouse V1. Input into layer 4 is followed by activity in superficial 2/3, which is followed by activity in deep layers. However, the magnitude of the activity in deep layers may depend on the spatial frequency of the gratings. Low spatial frequency gratings are more likely than high spatial frequency gratings to evoke activity in deep layers.

*Neural Correlates of the Layer 4 VEP*

A second major finding of this study involves the neural correlates of the various layer 4 VEP components. The shape of the layer 4 VEP varied in response to low and high spatial frequency gratings. In response to 0.05 cyc/deg gratings, the layer 4 VEP waveform had one negative-going peak followed by a positive-going peak. The earliest peaking current sink in layer 4 likely contributes to the negative-going VEP component because this sink was temporally coincident with the negative-going peak. Furthermore, the positive correlation between the layer 4 current sink amplitudes and the negative-going VEP amplitudes supports the contribution of layer 4 sinks to the layer 4 negative-going VEP component. Concurrent ongoing sinks in superficial or deep layers may also contribute to this negativity but the correlation with these layers was weaker. That the layer 4 negative-going component reflects the initial input activity in layer 4 is no surprise since this layer receives the heaviest geniculate input. The positive-going layer 4 VEP component evoked by 0.05 cyc/deg is correlated with current sink activity in deep layers and is temporally coincident with the latency to peak of
current sinks in these layers. This component appears positive going in layer 4 because layer 4 is likely a current source for current sink activity in deep layers. In response to 0.15 cyc/deg gratings, the layer 4 waveform had two negative- and positive-going components and was broader in shape compared to the 0.05 cyc/deg evoked waveform. The long duration of the concurrent layer 4 current sink likely contributed to the longer duration of the layer 4 VEP. In response to 0.05 cyc/deg gratings, the VEP may have been cut short due to large and early peaking sinks in deep layers that were competing for current with layer 4. However, in response to 0.15 cyc/deg gratings, the layer 4 current sink was in competition with smaller and later peaking sinks in deep layers that may not be of large enough magnitude to cause a polarity reversal. The late negative-going portions of the layer 4 current sink and the layer 4 VEP in response to 0.15 cyc/deg gratings may reflect intracortical activity and/or late peaking activity of ipsilateral-eye input. The peak positive-going VEP component in response to 0.15 cyc/deg, though it likely reflects intracortical activity, did not correlate with current sinks in deep layers. Taken together, the data suggest that measurements of various components of the layer 4 VEP can give insight into current sink activity occurring in layer 4 and outside of layer 4.

Interpretation of CSD Profiles in Neocortex

The one-dimensional CSD method has proven useful for analyzing the laminar flow of activations in visual cortex in response to electrical and visual stimulation (Aizenman et al., 1996; Heynen and Bear, 2001; Mitzdorf, 1985; Mitzdorf and Singer, 1978; Schroeder et al., 1991). However, this method contains some assumptions that may limit our ability to fully interpret the data. For example, the ideal network to apply
the CSD method would be a laminar structure in which the cell bodies and dendritic elements are aligned to create anatomical homogeneity along the x- and y-plane (Freeman and Nicholson, 1975; Mitzdorf, 1985). When applied to such a network, the CSD method reveals current activity along a line running normal to the cortical surface (Aizenman et al., 1996). The CA1 network of the hippocampus is the closest approximation to this ideal network (Richardson et al., 1987). The neocortex on the other hand, is not a homogeneous structure. As a result, in the neocortex a CSD analysis in the z-dimension will reveal the activation of pyramidal cell apical dendrites that are aligned normal to the cortical surface. The activation of cells with short dendrites or cells with dendrites not aligned along a line normal to the cortical surface may not be easily detected (Aizenman et al., 1996). For example, the activity of stellate cells in layer 4 may remain unresolved or underestimated. Therefore, as a general caveat, the absence of current sinks does not indicate the absence of synaptic activity (Aizenman et al., 1996).

There can also be ambiguity in interpreting the CSD with respect to the neuronal activities that cause them. Activation of axons, synaptic activation, and somatic and dendritic action potentials can cause current sinks and sources in a CSD distribution (Mitzdorf, 1985). The activation of fibers results in a source/sink/source distribution that passes along the fibers extending 1-2 mm. This dissipated CSD distribution is not resolvable in the cortex, where events that extend over ~50 – 300 μm are resolved (Mitzdorf, 1985). In a structure where a large number of cell bodies and dendrites are aligned, synchronous activation of action potentials can generate sink and source patterns. However, where the dendrites and somata are scattered in depth, as they are
in the neocortex, the contribution of synchronous cell firing to sinks is considered to be negligible (Aizenman et al., 1996; Mitzdorf, 1985). Therefore, it is likely that the major current sinks that we detected result from excitatory synaptic currents.

Synaptic activations can be excitatory or inhibitory. However in the visual cortex, inhibitory post-synaptic potentials (IPSPs) do not contribute significantly to the CSDs (Mitzdorf, 1985; Mitzdorf and Singer, 1978). Although inhibitory synapses are located on the cell somata, while excitatory are on proximal and distal dendrites, the rising phase of most cortical IPSPs is 5 to 20 times slower than that of excitatory post-synaptic potentials (EPSPs). Therefore, the currents generated by IPSPs are “at least one order of magnitude smaller in amplitude than the EPSP-currents” (Mitzdorf and Singer, 1978). If IPSPs are major contributors to CSD sinks and sources, decreasing IPSPs should decrease the CSD components. In contrast, the opposite has been demonstrated. Decreasing the strength of inhibitory synaptic activation increased the amplitude of all the cortical sinks and sources evoked by electrical stimulation to the optic radiation (Aizenman et al., 1996; Mitzdorf, 1985). Conversely, none of the sinks or sources were increased by increasing the strength of IPSPs (Mitzdorf, 1985). Therefore, it is unlikely that the current sinks and sources detected in our study received significant contributions from IPSPs.


Figure 4. Schematic of current sink and sources that generate the local field potential (LFP) in an excitatory network. The flow of positive ions (red arrows) inward results in an active current sink (blue shaded region) extracellularly. The net activity is reflected as a negative-going field potential. To recharge the membrane, positive ions must exit the intracellular space and when this happens, a passive current source (red shaded region) is generated extracellularly. A net current source is manifested as a positive-going field potential. The LFP reflects the net sum of the current sinks and sources generated at various locations.
Figure 5. The local field potential (LFP) has been extensively used in early pioneering works of neurophysiology and is still used in the clinical and laboratory setting. A, Sample surface LFPs recorded from the optic cortex of rabbits during electrical stimulation of the optic tract. S, S1, and S2 show location of stimulus. Each stimulus evoked a series of LFPs the components of which are numbered. Records A and B contain the complete series of five components, while record C contains only three. Adapted from Bartley and Bishop, 1932. Visually evoked potentials (VEPs) are LFPs in response to visual stimuli. VEPs have been adapted for use in the clinical setting. B, Maturation of the surface recorded VEP waveform to pattern-reversal stimulus in human infants. Arrow indicates the N70 component. Brecelj, 2003. C, Left: VEPs recorded from the input layer (layer 4) of the primary visual cortex (V1) of anaesthetized C57BL/6 mice in response to grating stimuli of varying spatial frequencies at a constant contrast. Right: The highest amplitude VEP response was elicited by low spatial frequency gratings. The highest spatial frequency to elicit a measurable VEP response was 0.6 cyc/deg. Porciatti et al, 1999. VEPs have also been used to assess experience-dependent changes in the binocular zone of mouse V1. D, In juvenile C57BL/6 mice, three days of monocular deprivation causes a significant reduction in the deprived (contralateral) eye VEP response while the VEP response to the non-deprived (ipsilateral) eye remains unchanged. Adapted from Sawtell et al, 2003.
Figure 6. Schematic diagram of the rat pyramidal cell in the CA1 region of the hippocampus. Pyramidal cell bodies are densely packed and restricted to the stratum pyramidale. Basal dendrites of the pyramidal cells extend to stratum oriens, while the apical dendrites project through stratum radiatum. Due to this laminar organization, the CA1 is ideal for current source density analysis. Adapted from Richardson et al. 1987.
Figure 7. Laminar profiles of subthreshold stratum oriens or stratum radiatum-evoked field potentials and current source density (CSD). **A** and **C**: evoked potentials at 25 µm steps parallel to the dendrosomatic axis of the pyramidal cell. **B** and **D**: CSD calculations for each 25-µm interval. CSD measurements are displayed with a zero line to facilitate comparison of sink-source relationships. Sinks are upward-going and shaded, sources are down-ward going. A scaled schematic diagram of the pyramidal cell is interposed between profiles with the borders of stratum pyramidale denoted by dotted lines. Distance along the cell axis is taken from the ventral border of stratum radiatum (0 µm) and voltage polarity is negative down. Modified from Richardson et al, 1987.
Figure 8. Laminar profiles of suprathreshold stratum radiatum-evoked field potentials and current source density (CSD). A, evoked potentials at 25-µm steps parallel to the dendrosomatic axis of the pyramidal cell. B, CSD calculations for each 25-µm interval. CSD measurements are displayed with a zero line to facilitate comparison of sink-source relationship. Current sinks are upward-going and shaded; current sources are downward-going. A scaled schematic diagram of the rat pyramidal cell is interposed between profiles with the borders of stratum pyramidale denoted by dotted lines. Comparison of field potentials (C) and CSD measurements (D) at the level of pyramidal cell somata (-50 µm) and apical dendrites (200 µm; waveforms were taken from the position of asterisks on laminar profiles in A and B). The onset of the dendritic spike response in stratum radiatum is shown by an arrow in C. Modified from Richardson et al, 1987.
Figure 9. Cell bodies and dendrites are not clustered into distinct laminae in cortex. **A**, Morphology of individual cells in cat cortical area 17. Thick lines: dendrites, thin lines: axons. **B**, Schematic diagram of connections in visual cortex of the cat. Most thalamic (LGN) input is concentrated in layer 4 and, to a lesser degree, in layer 6. Y cells tend to project more superficially in layer 4 than X cells. There is a fairly strict hierarchical pathway from layers 4 → 2+3 → 5 → 6 with feedback connections from 5 → 2+3 and 6 → 4. There are also many lateral connections between neurons within the same layer. From C. Reid, Fundamentals Neuroscience, 2nd edition.
Figure 10. **A**, field potentials in primary visual cortex (area 17) of cat evoked by electrical stimulation of the optic radiation (arrow). Distance between adjacent recordings is 50 µm. Profile was obtained by successive recordings with a single micropipette. **B**, current source-density (CSD) distribution obtained from potential profile in A, with a differentiation grid of 2. As a result, the highest and lowest regions of recording range are missing in CSD profile. Sinks are upward-going and shaded; sources are downward-going. At left, depth regions of cortical laminae are indicated. Sinks a and e reflect Y-type and X-type monosynaptic activity; sinks b and c reflect polysynaptic Y-type activity; sinks d and f reflect polysynaptic X-type activity. Adapted from Mitzdorf, 1985.
Figure 11. Electrically and visually evoked current source density (CSD) distributions in area 17. Stimuli were: A, electrical stimulation of the optic radiation; B, strobe flash; C, reversal of 0.2 cycle/deg grating (square wave, 40% contrast). Time of stimuli onset are indicated by vertical bars and arrow. Sinks are upward-going and shaded; sources are downward-going. Time scales (in msec) and gain (relative units) are given below CSDs. Borders of cortical laminae are indicated at left. All CSDs are qualitatively similar (monosynaptic components a, d, and e; polysynaptic components b, c, and f), although their time courses and amplitudes differ by 1 or 2 orders of magnitude. Adapted from Mitzdorf, 1985.
Figure 12. Circuitry of mouse cortical area 17. A, Thalamocortical axons project to both layer 4 and layer 3 of the mouse visual cortex. Axons were labeled with biotin-conjugated dextran injected into binocular dLGN of a P28 mouse. Fixed coronal sections containing visual cortex were imaged four days later. Laminar borders were determined based on Nissl staining. Image courtesy of J. Coleman. B, Schematic diagram of connections based on anatomical data from mouse V1 and S1. Most thalamic (LGN) input is concentrated in layer 4, and to a lesser extent in layers 2/3, 5, and 6. As in the cat, there is a hierarchical pathway from layers 4 $\rightarrow$ 2/3 $\rightarrow$ 5 $\rightarrow$ 6 with feedback connections from 5 $\rightarrow$ 2/3 and 6 $\rightarrow$ 4. There are also many lateral connections between neurons within the same layer. See text for references.
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Figure 17. Correlations among various components of the layer 4 VEP and current sink/source activity in the different cortical layers of C57BL/6 mice (n = 10). Responses to 0.05 (A – E) and 0.15 (F – J) cyc/deg grating stimuli. See text for details.
Supplemental figure 1. Correlations among various components of the layer 4 VEP and current sink/source activity in the different cortical layers of C57BL/6 mice (n = 10). Responses to 0.05 (A – D) and 0.15 (E – H) cyc/deg grating stimuli. See text for details.
Supplemental figure 2.  A, Field potential and CSD profiles in response to grating stimuli of increasing spatial frequency from the primary visual cortex of a C57BL/6 mouse implanted with a stagger dagger (see Methods). Currents sinks are downward going and shaded black; current sources are upward going. Concurrent layer 4 field potentials are shown on top of each CSD profile. Note that current sinks in deep layers are gradually reduced in amplitude in response to gratings of increasing spatial frequency. See figure 14 for conventions.  B, Current sink-peak amplitudes, mean +/- SEM, from superficial layers (left, n = 5 animals), layer 4 (center, n = 6 animals), and deep layers (right, n = 6 animals). The gray shaded region indicates the baseline response, mean +/- SEM, to a gray screen of equal luminance.
Supplemental Figure 3. Relationship between the layer 4 VEP and the layer 4 current sink. **A**, Across all spatial frequencies tested (0.05 – 0.6 cyc/deg), the amplitude of the layer 4 negative-going component (y-axis) is significantly correlated with the amplitude of the layer 4 current sink-peak. **B**, The layer 4 VEP (y-axis) and current sink-peak (x-axis) amplitudes for all spatial frequencies tested normalized to 0.05 cyc/deg responses.
Supplemental figure 4. CSD profiles of the binocular (A, left, green), contralateral-eye (A, center, cyan), and ipsilateral-eye (A, right, yellow) response to 0.15 cyc/deg gratings. The two negative-going VEP peaks in response to binocular visual stimulation may be due to early peaking contra-eye input and late peaking ipsi-eye input. See figure 14 for conventions. B, The latency to the peak of the layer 4 VEP negativity shows the ipsi-eye VEP response peaks significantly later than the contra-eye VEP response (n = 5 animals, post-hoc paired t-test, p = 0.0152). C, In the same set of animals, current sinks responses of the ipsi-eye to 0.15 cyc/deg gratings also peaked significantly later than contra-eye responses (p = 0.0178). This latency shift was also observed in VEP responses to 0.05 cyc/deg gratings (D).
Supplemental Table 1. Latency to VEP and current sink/source peaks in response to (A) 0.05 and (B) 0.15 cyc/deg gratings. C57BL/6 mice.

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<td>Negative Component</td>
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Chapter 2:

Measuring Mouse Visual Acuity and Contrast Sensitivity Using Visually Evoked Potentials
Introduction

Visual Acuity and Contrast Sensitivity Testing in Human Subjects

Visual acuity (VA) is a common quantitative measure of how well the visual system can see and resolve fine details. It reflects clearness of vision which is an important factor for performing a variety of tasks such as reading text. In humans, VA is assessed with a Snellen chart during the routine ophthalmic examination (Trick, 2003). Contrast sensitivity (CS) is a second less common measure of visual function that describes the ability to distinguish differences in luminance. While visual acuity tests use objects with high contrast, tests of contrast sensitivity use low-contrast objects varying in orientation as well as spatial frequency (Haegerstrom-Portnoy, 1993). As a result, the CS test may better reflect real-world conditions. Both tests typically rely on verbal confirmation that the object was detected by the subject. However, for non-verbal infants and adults, an objective assessment is required. The visually evoked potential (VEP) technique provides this objective measure of visual function at the level of the visual cortex. In human subjects, VEP activity is monitored via scalp electrodes (Figure 18a) placed over the occipital cortex while the subject views stimuli such as flash or pattern gratings (Almoqbel et al., 2008; Norcia et al., 1986; Tyler et al., 1979). An acuity estimation that is comparable to the psychophysical acuity can then be made (Figure 18b).

Visual Acuity Testing in Rodents

An early attempt at developing an operant visual discrimination task to test visual function in rodents was made by Lashley (1930). He introduced the jumping stand test that required the rat to jump from an elevated stand to one of two windows containing
the patterns to be discriminated; failure to choose the correct window resulted in a short fall. Because the task required “…much manipulation and time to interchange stimulus-cards, to lock appropriate windows, to return animals to the jumping-platform and to record data,” it never quite caught on (Feldman, 1948). Nevertheless, these initial experiments stimulated future studies that improved behavioral tests of visual function in rodents.

One such study by Prusky and Douglas (2004) used a computer-based, two-alternative, forced-choice visual discrimination swim task to characterize mouse cortical spatial vision. In this task, mice were placed in a pool of water (Figure 19a) and were required to swim towards the screen displaying a grating stimulus (Figure 19b) in order to climb atop a submerged platform and escape from the water. Using this task, Prusky and Douglas (2004) determined that adult (P89, P125, P235) C56BL/6 mice have a visual acuity of 0.55 cyc/deg (2004; Prusky et al., 2000), which is lower than that of other species (Petry et al., 1984) including humans, macaques, cats, and hooded rats (Figure 19c,d). Despite having relative low visual acuity, the contrast sensitivity function (CSF) of these mice had the typical inverted “U” shape that peaked at intermediate spatial frequencies (0.1 – 0.2 cyc/deg). If the layer 4 VEP is an accurate measure of visual function, then the VEP measured visual acuity should match previously reported behavioral measures of visual acuity. To determine if this is true, we measured the layer 4 VEP to approximate the visual acuity in juvenile (P27 – P30) C57BL/6 mice. Prusky et al (2004) previously reported that visual acuity in C57BL/6 mice reaches a maximum value of 0.4 cyc/deg by P28. In addition to C57BL/6 mice, we assessed the visual function of the 129/Sv strain using VEPs and current source density (CSD)
analysis. This strain, although commonly used to generate genetically modified mice, is not widely used for behavioral testing. In general, C57BL/6 mice are preferred because they tend to outperform 129/Sv mice, as well as other strains, in behavioral tasks such as the Morris water maze test for spatial learning and memory (Crawley et al., 1997). However, few studies have directly tested the visual function of 129/Sv mice.

We previously used current source density (CSD) analysis to determine the laminar pattern of activity in mouse primary visual cortex (V1) in response to two spatial frequency gratings (0.05 and 0.15 cyc/deg) at 100% contrast. We suggested that in mouse V1 processing proceeds from the input layer 4 up to 2/3 and then down to 5/6. However, layers 4 and 2/3 may act as low-pass spatial frequency filters so that the activity in 5/6 is reduced in amplitude in response to high spatial frequency gratings. In addition, we described differences in the layer 4 VEP waveform in response to low and high spatial frequency gratings. In the first 150 milliseconds (msec) post-stimulus onset, the 0.05 cyc/deg evoked VEP had single negative and positive peaks while 0.15 cyc/deg stimuli evoked a VEP with two negative and positive peaks that resulted in a broader waveform. We suggested that this broad VEP waveform resulted from layer 4 current sinks of longer duration. In this study, we used the CSD method to look at the laminar pattern of activity in response to stimuli of fixed spatial frequency and varying contrast levels.
Results

Contrast Sensitivity and Visual Acuity of C57BL/6 Mice

We recorded layer 4 VEPs in the binocular zone of V1 in response to 48 grating stimuli of various spatial frequencies and contrast levels (see Methods). The VEP waveform responses averaged across all animals (n = 20) are shown in Figure 20a. The waveforms were consistent across all animals so averaging did not produce an artifact in the shape of the waveform (supplemental figure 5). The negative-going VEP component, which reflects initial geniculate input, is clearly visible in response to gratings of low spatial frequencies (0.05 = 0.1 cyc/deg) at about 6% contrast (Figure 20a). For a fixed spatial frequency, this negative-going component increased in amplitude as contrast levels increased (Figure 20a). This modulation of the negative-going component in response to contrast changes may reflect an increase in current sink amplitude. For a fixed contrast, the amplitude also decreased with increasing spatial frequency but the most striking difference is in the shape of the VEP waveform which goes from narrow in response to low spatial frequencies to broad in response to higher spatial frequencies.

Since we previously determined that the negative-going VEP component correlates best with layer 4 current sink activity (Chapter 1), all the analysis was done with this component. There was a significant effect of contrast on the VEP amplitude (one-way anova, p < 0.0001) such that holding each spatial frequency constant, the VEP amplitude decreased with decreasing contrast levels (Figure 20b). This likely reflects a decrease in layer 4 current sink activity, which was confirmed by CSD
analysis (Figure 21). In response to gratings of 0.05 cyc/deg spatial frequency at 25% contrast, the VEP negative-going component was significantly smaller in amplitude (Figure 21c) and the peak appeared later (Figure 21e) compared with the VEP evoked by 100% contrast. The laminar pattern of current sink activity was the same in response to gratings of both contrast levels (Figure 21a, b): layer 4 current sinks peaked first, followed by sinks in superficial layers 2/3, and finally current sinks in deep layers 5/6 peaked last (Figure 21f1-f3). However, the relative amplitudes and latencies to current sink-peak varied. Current sinks in layer 4 and layers 5/6 were significantly smaller in response to gratings at 25% contrast (Figure 21d1,d3); current sinks in layers 2/3 were also smaller (Figure 21d2) although this difference was not significant (p = 0.1704). In all laminae, the peak latencies were significantly longer in response to the reduced contrast (Figure 21f1 - f3). The smaller amplitudes and longer latencies to peak may reflect lower coherence of the afferent input (Mitzdorf, 1985).

There was also a significant effect of spatial frequency on the amplitude of the VEP response (one-way anova, p <0.0001). On average, 0.05 cyc/deg gratings evoked the largest layer 4 VEP amplitudes that were detected with as little as 6% contrast (Figure 20a, b) and as a result, the contrast sensitivity at this spatial frequency is high (Figure 20c). Although the amplitudes varied, this pattern was consistent across individual animals (supplemental figure 6). As spatial frequency of the grating stimuli increased, the amplitude of the negative-going component decreased and higher contrast was required to evoke a VEP response above baseline. As a result, contrast sensitivity dropped with increasing spatial frequencies (Figure 20c). Interpolating between the recorded values, we found 0.55 cyc/deg to be the highest spatial frequency
able to evoke a VEP response above baseline. This acuity value is consistent with the behaviorally assessed acuity value determined using the visual water task (Prusky and Douglas, 2004; Prusky et al., 2000). However, the VEP assessed contrast sensitivity function did not have the typical inverted “U” shape observed with the visual water task ((Prusky and Douglas, 2004). Instead of peaking at intermediate spatial frequencies, the VEP measured CSF peaked at low spatial frequencies (0.05 – 0.1 cyc/deg).

Curiously, the contrast sensitivity function determined by measuring the area under the negative-going component, rather than amplitude, had the typical inverted “U” shape that peaked at 0.1 cyc/deg (supplemental figure 7).

**Contrast Sensitivity and Visual Acuity of 129/Sv Mice**

We found similarities and differences in the visual function of age-matched 129/Sv mice compared to that of C57BL/6 mice when we applied the same analysis to VEPs recorded in V1 of 129/Sv mice. Like the C57BL/6 response, the amplitude of the layer 4 negative-going VEP component in 129/Sv mice increased with increasing contrast (Figure 22a, b) and the waveform shape became broader with increasing spatial frequency (Figure 22a). However, the average amplitude response of the negative-going component was smaller overall in 129/Sv mice (one-way anova, p <0.0001, Figure 22b, note the difference in scale compared to Figure 20b). Whereas the greatest amplitude response in C57BL/6 was skewed towards 0.05 cyc/deg, large amplitude responses in 129/Sv mice were spread out over several spatial frequencies (Figure 22b) with a concentration around 0.3 cyc/deg (one-way anova, p < 0.0001). This pattern was consistently seen in individual animals (n = 18, supplemental figure 8). The increase in the amplitude of the negative-going component in response to higher
spatial frequencies effectively increased contrast sensitivity at those spatial frequencies (Figure 22c). As a result, the VEP assessed acuity of 129/Sv mice was higher (0.6 cyc/deg) than that of C57BL/6 mice.

One of the most striking differences between the 129/Sv and C57BL/6 VEP waveform is in the positive going component (Figure 23). In C57BL/6 mice, the largest amplitudes of the positive-going component were in response to 0.05 cyc/deg gratings (Figure 23a-b, p < 0.0001). The amplitudes evoked by 0.05 cyc/deg were modulated by contrast levels and were fairly large even at low contrasts (Figure 23b, p < 0.0001). The positive-going amplitudes decreased with increasing spatial frequency (p < 0.0001) and were less affected by contrast at high spatial frequencies. In 129/Sv mice, the largest amplitudes of the positive-going component were spread out over intermediate spatial frequencies and contrast levels (Figure 23c). Another feature of the positive-going VEP component is that, collapsed across all conditions, it peaked significantly later in 129/Sv mice compared to C57BL/6 (p = 0.01) and there is a significant interaction between contrast and genotype (three-way anova; p = 0.0036), as well as spatial frequency and genotype (p = 0.0006, Figure 23d) on the latency to peak positivity. Therefore, in order to investigate the current sink activity coincident with this positive-going component in 129/Sv mice, we extended the CSD analysis time window from 150 to 300 msecs post-stimulus onset.

Laminar pattern of visually evoked activity in V1 of 129/Sv mice

Using stagger dagger implants, we recorded field potentials from multiple layers of V1 simultaneously and calculated the CSD profile using the formula outlined in the
methods section. Example CSD distributions from a 129/Sv animal in response to 0.05 (Figure 24a) and 0.15 cyc/deg (Figure 24b) indicate that latency to the peak current sink occurred first in layer 4 consistent with layer 4 being the recipient of heavy geniculate input. There was a significantly longer latency to the layer 4 current sink-peak in response to 0.3 cyc/deg (mean +/- SEM: 99 +/- 6 msecs post-stimulus onset, Figure 24c1) compared with the peak current-sink response to 0.05 cyc/deg (71 +/- 5 msecs, paired t-test, p = 0.0293), which may be due to weaker coherence of the afferent input. However, this is not likely for two reasons. First, if the longer latency to peak were due to a weaker input, the peak amplitude of the layer 4 current sink should be lower in response to 0.3 cyc/deg (-2.4 +/- 0.9 mV/mm²) compared to 0.05 cyc/deg (-2.3 +/- 0.7 mV/mm²), but it was not (Figure 24f1, paired t-test, p = 0.9137). Second, there was a significant increase in the onset latency of the layer 4 current-sink response to 0.3 cyc/deg (67 +/- 6 msecs post-stimulus onset, Figure 24d) compared with the response to 0.05 cyc/deg (44 +/- 3 msecs, paired t-test, p = 0.0177) and this could have caused the delay in the time to sink-peak. The slow-onset layer 4 current sink response to 0.3 cyc/deg compared with the fast-onset response to 0.05 cyc/deg is characteristic of the response of cat X- and Y-geniculate afferents, respectively (Mitzdorf, 1985; Stone, 1983). X-cells in the LGN are slow-conducting, have smaller receptive fields, and respond to high spatial frequency grating stimuli with a sustained response. In contrast, fast-conducting Y-cells in the LGN have larger receptive fields and respond to low spatial frequency gratings with a transient response. To determine if the layer current sink responses to 0.05 and 0.3 cyc/deg gratings fall into these two categories of “transient” and “sustained” activity, the time from onset of the current sink to the time it
reversed polarity was measured (Figure 24e). Indeed, there was a more sustained
layer 4 current sink response to 0.3 cyc/deg (116 +/- 26 msecs) compared with the
response to 0.05 cyc/deg (79 +/-8 msecs, paired t-test, p = 0.1409) although it was not
statistically significant.

Following the layer 4 current sink-peak were current sink-peaks in deep and
superficial layers (Figure 24a,b). As with the layer 4 current sink-peak, current sinks in
superficial layers peaked later in response to 0.3 cyc/deg (182 +/- 18 msecs post-
stimulus onset, Figure 24c3) compared with the response to 0.05 cyc/deg (151 +/- 12
msecs, paired t-test, p = 0.0141). In contrast, latency to peak of current sinks in deep
layers did not vary between 0.05 cyc/deg (137 +/- 15 msecs post stimulus onset, Figure
24c2) and 0.3 cyc/deg (142 +/- 7 msecs, paired t-test, p = 0.6927). In response to both
spatial frequencies, deep layer current sinks peaked earlier than superficial layer current
sinks, but this could be due to the position of the electrodes in superficial layers or
perhaps early peaking current sinks in superficial layers were masked by the layer 4
sinks.

Neural Correlates of the Layer 4 VEP in 129/Sv Mice

The difference in the latency to layer 4 current sink-peak was manifested in the
latency to peak of the negative-going VEP component (Figure 24h). The negative-going
VEP component peaked later in response to 0.30 cyc/deg (95 +/- 8 msecs post-stimulus
onset) compared with the VEP response to 0.05 cyc/deg (78 +/- 8 msec, paired t-test, p
= 0.2027). Nevertheless, both negative-going peaks temporally overlapped with current
sink-peaks in layer 4 (supplemental table 2) and there was a significant correlation
between layer 4 sink amplitudes and amplitudes of the VEP negative component \((r^2 = 0.5513, p = 0.0057;\) supplemental figure 9a). Current sink-peaks in superficial and deep layers did not temporally overlap with the peak of the VEP negative-going component and the correlations were weaker.

The latency to peak of current sinks in superficial layers, but not deep layers, temporally overlapped with the latency to peak of the layer 4 positive-going VEP component. In response to 0.05 cyc/deg, the positive going-component was smaller in amplitude (Figure 24i) and peaked at 159 +/- 17 msecs post-stimulus onset, shortly after current sinks in superficial layers peaked (151 +/- 12 msecs) but well after current sinks in deep layers peaked (137 +/- 15 msecs). In response to 0.3 cyc/deg, the latency to peak of the positive-going component (208 +/- 21 msecs post-stimulus onset) was closer to the peak latency of current sinks in superficial layers (182 +/- 18 msecs) than the peak latency of deep layers (142 +/- 7 msecs). The temporal proximity between current sink-peaks in superficial layers and the positive-going layer 4 VEP response suggest that the positive-going layer 4 VEP component may reflect sinks occurring in superficial layers. Indeed, the amplitudes of the positive-going VEP component best correlated with the amplitudes of current sink-peaks in superficial layers (supplemental figure 9b, \(r^2 = 0.3730, p = 0.0459\)). The amplitude of current sink-peaks in superficial and deep layers varied with spatial frequency and is further evidence that the layer 4 positive-going VEP component is probably not influenced by current sink activity in deep layers. Peak current sink amplitudes in deep layers were significantly smaller in response to 0.3 cyc/deg (Figure 24f2, paired t-test, \(p = 0.0124\)), the frequency that evoked the larger layer 4 positive-going VEP component (Figure 24i). In contrast,
current sink-peak amplitudes in superficial layers were significantly larger in response to 0.3 cyc/deg (-1.28 +/- 0.26 mV/mm^2, paired t-test, p = 0.0494, Figure 24f3) compared with the response to 0.05 cyc/deg (-0.87 +/- 0.15 mV/mm^2). These data also suggest that deep layers are more active in response to the low spatial frequency grating stimulus while processing of the high spatial frequency gratings evokes more activity in superficial layers. Indeed, gradually increasing the spatial frequency of the gratings evoked increasing current sinks in layers 4 and 2/3 that peaked at 0.3 cyc/deg, while current sinks in deep layers 5/6 decreased (Figure 25).

Discussion

Contrast Sensitivity in C57BL/6 Mice

In response to a grating stimulus of fixed spatial frequency, increasing contrast levels is concomitant with an increase in the amplitude of the negative-going VEP component in V1 of C57BL/6 mice. In cats, VEPs recorded from the surface of visual cortex were also higher in amplitude in response to gratings at 100% contrast relative to gratings at 25% contrast (Freeman et al., 1983). This increase in the VEP amplitude reflects an increase in layer 4 current sink activity suggesting higher coherence of the afferent input in response to higher contrast levels (Mitzdorf, 1985). However, single unit studies have shown that in the cat, LGN cells have contrast invariant responses and that contrast modulation of single unit responses arise in the striate cortex (Ohzawa et al., 1985; Rossi and Paradiso, 1999). Therefore it is likely that in the mouse, LGN afferent input is the same in response to 25% and 100% contrast, but the cortical excitatory postsynaptic potential response is modulated in the cortex.
Regardless of the site of origin, contrast sensitivity was found to be reflected in the amplitude of the negative-going VEP component. In the mouse, VEP-assessed contrast sensitivity peaked at low spatial frequencies (0.05 – 0.1 cyc/deg), which is contrary to what has been previously reported in other species including humans, monkeys, cats, rats and tree shrews (Petry et al., 1984). In addition, using a behavioral task to measure contrast sensitivity, Prusky et al reported peak contrast sensitivity at intermediate spatial frequencies (0.1 – 0.2 cyc/deg) in adult C57BL/6 mice (2004). Since we used juvenile mice in our study, the difference in our measure could be age-dependent. However, VEP amplitudes in anaesthetized adult C57BL/6 mice (Porciatti et al., 1999) also peaked at low spatial frequencies (0.05 – 0.1 cyc/deg). Therefore, the more likely explanation of the difference between VEP measured and behaviorally-assessed contrast sensitivity is in the experimental paradigm. In the VEP studies, mice were never exposed to the grating stimuli prior to the recording session. In the behavioral study, there were pretraining and training phases in which mice learned to locate a platform hidden below a computer screen displaying a vertically oriented low spatial frequency grating (~0.12 cyc/deg) and to distinguish that stimulus from the gray screen of a second monitor (Prusky et al., 2000). As a result, the mice were exposed to gratings at the 0.12 cyc/deg spatial frequency for more than 1 week prior to testing their response to gratings of other spatial frequencies (Prusky et al., 2004). Therefore, it is likely that the high contrast sensitivity to spatial frequencies in this range was a potentiated response due to repeated exposure over multiple days (Hager and Dringenberg, 2008). This phenomenon is called stimulus specific response potentiation (SRP) and has been observed in the visual cortex of adult C57BL/6 using VEPs.
(Frenkel et al., 2006). Using the awake VEP recording setup described in the Methods section, Frenkel et al (2006) exposed mice to repeated presentations of a grating of the same orientation over a period of 5 days. Mice were previously implanted with recording electrodes in layer 4 of binocular V1 and their VEP amplitudes increased gradually over the course of stimulus exposure. This potentiation was specific to the trained orientation because exposing animals to the orthogonal orientation during the last session resulted in a smaller VEP amplitude response. The Frenkel et al study (2006) showed that SRP can be specific to the orientation of the stimulus, and it is likely that SRP can extend to other features of the grating stimulus such as spatial frequency. This suggests testing C57BL/6 mice in a behavioral paradigm that does not require pretraining may eliminate the experimental confound of SRP. The optomotor response reflex task is one such task that is already used to probe the visual function of birds, rats, and human infants, (Tauber and Koffler, 1966; Wallman, 1975) and was adapted for use in mice by Prusky and colleagues (2004). Because the response is a reflex that both juvenile and adult mice have, no behavioral training is required. Testing the visual function of juvenile and adult mice using this task, Prusky et al found that indeed, contrast sensitivity peaks at low spatial frequencies (0.06 cyc/deg), consistent with our VEP assessed contrast sensitivity. It is also possible that high spatial frequency vision in the visual water behavioral task was subserved by cortical mechanisms outside of layer 4. Both the optomotor response reflex and our VEP recordings are biased towards detecting the layer 4 input response (Prusky et al., 2004). However, this response is likely filtered by higher cortical mechanisms before it is translated into a behavioral output.
Visual Acuity in 129/Sv Mice

While we were able to check the VEP assessed visual acuity and contrast sensitivity of C57BL/6 mice against already existing behavioral measures, comparable reports have not been published for the 129/Sv strain used in this study. Therefore, what we report here using VEPs will have to be confirmed with a carefully designed behavioral study. Our results show that 129/Sv mice have slightly better visual acuity (0.6 cyc/deg) than C57BL/6 mice (0.55 cyc/deg). While the amplitude of the negative-going VEP component was smaller in 129/Sv mice relative to C57BL/6, this component increased in response to increasing spatial frequencies in 129/Sv mice, contrary to the decrease seen in C57BL/6. This effectively led to better contrast sensitivity at higher spatial frequencies in 129/Sv mice relative to C57BL/6 mice. As a result, we predict that psychophysical measures of visual acuity comparing the two mice should find that 129/Sv mice see high spatial frequency gratings better than C57BL/6 mice. Spatial frequency detection depends on receptive field size (Stone, 1983) so we further predict that single unit studies of cells in cortex should find that visual receptive fields are smaller in 129/Sv mice compared to C57LB/6 mice. Single unit studies have revealed laminar preferences for the receptive fields of single units in cat area 17. In the cat, small to intermediate receptive field lengths (1 – 3º) are found in layers 2,3, and 4; layer 5 cells have intermediate sized receptive fields (3º) and the largest receptive fields (8º) are found in layer 6 (Berman et al., 1982; Gilbert, 1977). A recent study looking at receptive field size in V1 of C57BL/6 mice found a similar distribution of receptive field radius: cells in layers 2/3 and 4 have the smallest receptive fields (∼5 – 7º) followed by somewhat larger receptive fields (∼8 – 12º) in deep layers 5 and 6 (Niell and Stryker,
2008). Consistent with this, cells in superficial layers and layer 4 have a preference for high spatial frequency (0.04 cyc/deg) gratings and cells in deep layers respond best to low (0.02 cyc/deg) spatial frequencies. We found evidence of this laminar specific preference to the spatial frequency of the stimulus in our CSD analysis of VEPs elicited by low (0.05 cyc/deg) and high (0.3 cyc/deg) spatial frequency gratings. High spatial frequency gratings evoked increasing amplitude current sinks in superficial layers, which peaked at 0.15 – 0.3 cyc/deg. This is different from the spatial frequency modulation of current sinks in C57BL/6 mice wherein the response to increasing spatial frequencies was attenuated in superficial layers. In both strains, deep layers were most responsive to low-spatial frequency stimuli.


Figure 18. Visually evoked potential (VEP) recording in humans. **A**, Schematic of the stimulus and recording apparatus. **B**, VEP amplitude versus spatial frequency. Three separate traces are superimposed to show the repeatability of the data. The solid line is drawn by eye, and extrapolated through the noise (dashed line) to the 0 voltage level to determine the VEP acuity. The psychophysical acuity is shown by the arrow (Observer D.L.). Adapted from Tyler et al, 1979.
Figure 19. Mouse visual acuity assessed behaviorally using the visual water task. Schematic diagram and components of the visual water box. A, View from above showing the major components including pool, midline divider, platform, starting chute and two monitors. The pool is filled with clean water (gray), and the braces are needed to resist its weight. Following release from the chute, animals choose to swim on the side of the pool displaying the grating in order to find the hidden platform and escape from the water. B, Front view showing monitor screens, submerged platform and midline divider. From Prusky and Douglas, 2000. C, Contrast sensitivity function (CSF) of the mouse. Spatial frequency (x-axis) is plotted as a function of contrast sensitivity (y-axis) on log scales. The mice exhibited an inverted “U”-shaped function, typical of a mammal, which had maximum sensitivity at 0.208 c/d. The solid line is a best-fit curve of the data. The arrow points to the spatial frequency expected to be the threshold at 100% contrast (acuity). +/- SEMs are plotted on each symbol. From Prusky et al, 2004. D, Contrast sensitivity of other species. Adapted from Petry et al, 1984. The mouse CSF from (C) was added for comparison.
Figure 20. VEP assessed visual function of C57BL/6 mice.  

A, Mice were exposed to 40 presentations of horizontal or vertical phase reversing (1 Hz) gratings that varied in contrast levels and spatial frequency (see Methods). The VEP response to one phase of each stimulus was averaged across all animals (n = 20) and is shown beneath the stimulus. Grayed out are the stimuli that did not produce a response significantly different (paired t-test, p < 0.001) from the baseline response to a gray screen of equal luminance. Note that for a given spatial frequency, as contrast levels increase, the amplitude of the VEP increases. For a given contrast, the VEP amplitude decreases as the width of the waveform increases.  

B, Colorplot of the group averaged VEP amplitude response (µV) to gratings of various spatial frequencies and contrast levels. Values in between the tick marks were interpolated.  

C, Contrast sensitivity function based on the interpolated VEP amplitudes in (B) indicates high contrast sensitivity at low spatial frequencies and acuity of 0.55 cyc/deg.
Figure 21. Current sink amplitudes in response to gratings of 100% and 25% contrast levels. Field potential and CSD profiles from V1 of one mouse in response to 0.05 cyc/deg gratings at 100% (A) and 25% (B) contrast. See figure 14 for conventions. The amplitude of the negative-going VEP component is significantly reduced (C, n = 5 animals) and peaks later (E) in response to gratings of 25% contrast. Current sink amplitudes across all layers (D1 – D3) were reduced in response to 25% contrast gratings and the latency to current sink-peaks were significantly longer (F1 – F3).
Figure 22. VEP assessed visual function of 129/Sv mice. 

A, Mice were exposed to 40 presentations of horizontal or vertical phase reversing (1 Hz) gratings that varied in contrast levels and spatial frequency (see Methods). The VEP response to one phase of each stimulus was averaged across all animals (n = 18) and is shown beneath the stimulus. Grayed out are the stimuli that did not produce a response significantly different (paired t-test, p < 0.001) from the baseline response to a gray screen of equal luminance. 

B, Colorplot of the group averaged VEP amplitude response (µV) to gratings of various spatial frequencies and contrast levels. Values in between the tick marks were interpolated. On average, VEP amplitudes were lower in 129/Sv mice compared with C57BL/6 mice (note the difference in scale compared to Fig. 20b).

C, Contrast sensitivity function based on the interpolated VEP amplitudes in (B) indicates high contrast sensitivity at low spatial frequencies and acuity of 0.6 cyc/deg.
Figure 23. High spatial frequency stimuli evoke a large positive-going component in 129/Sv mice. **A**, Group averaged VEP waveforms from figures 21 and 23 are overlaid to highlight the differences between the C57BL/6 (black) and 129/Sv (red) VEP response to gratings of various spatial frequencies and contrasts. Note the larger positive-going VEP component evoked in 129/Sv mice in response to increasing spatial frequency. **B** and **C**, Colorplots of the positive-going VEP amplitudes (measured from baseline) in C57BL/6 (B) and 129/Sv (C) mice. Values in between the tick marks were interpolated. **D**, In response to 0.3 cyc/deg gratings at 100% contrast, the positive-going component peaked significantly later in 129/Sv mice (red) than in C57BL/6 mice (black, unpaired t-test, p <0.0001).
Figure 24. Current sink activity in response to 0.05 (A) and 0.3 cyc/deg (B) grating stimuli at 100% contrast in primary visual cortex of 129/Sv mice. CSD profiles determined from field potentials recorded at various cortical depths in a mouse implanted with stagger dagger electrodes (see Methods). See figure 14 for conventions. Note the shift in latency to peak of current sinks in response to 0.3 cyc/deg. A significant increase in current sink-peak latency was observed in response to 0.3 cyc/deg in layer 4 (C₁, n = 6 animals) and superficial layers (C₃, n = 5 animals) but not in deep layers (C₂, n = 6 animals). This increase in latency to sink-peak may be due to a significant increase in latency to current sink onset in response to 0.3 cyc/deg (D). High spatial frequency gratings evoked a more “sustained” layer 4 current sink response (E). Layer 4 current sink-peak amplitudes were not modulated by spatial frequency (F₁). However, current sinks in deep layers were larger in amplitude in response to low spatial frequency gratings (F₂) while high spatial frequency gratings evoked larger current sinks in superficial layers (F₃). The amplitude of the corresponding layer 4 negative-going VEP component was not affected by spatial frequency (G) although the latency to peak was slightly longer in response to 0.3 cyc/deg (H). The amplitude of the positive-going VEP component was larger (I) and peaked later (J) in response to 0.3 cyc/deg.
Figure 25. **A**, Field potential and CSD profiles in response to grating stimuli of increasing spatial frequency from the primary visual cortex of a 129/Sv mouse implanted with a staggered dagger (see Methods). Current sinks are downward going and shaded black; current sources are upward going. Concurrent layer 4 field potentials are shown on top of each CSD profile. See figure 14 for conventions. **B**, Current sink-peak amplitudes, mean +/- SEM, from superficial layers (*left*, *n* = 5 animals), layer 4 (*center*, *n* = 6 animals), and deep layers (*right*, *n* = 6 animals). The gray shaded region indicates the baseline response, mean +/- SEM, to a gray screen of equal luminance.
Supplemental figure 5. VEP waveforms are consistent in shape between animals. Each waveform was collected from one animal and is an average response to 40 presentations of phase-reversing (1Hz) gratings of various spatial frequencies at fixed contrast (100%) and luminance.
Supplemental Figure 6. Colorplot of VEP amplitude response (µV) to 40 presentations of phase-reversing gratings at various spatial frequencies and contrast levels. Each panel is the response from one C57BL/6 mouse. See figure 21 for the group averaged response. Values in between the tick marks were interpolated. Same scale for all plots.
Supplemental Figure 7. VEP-assessed acuity using amplitude and area measures of the negative-going component in C57BL/6 mice. A, Mice were exposed to 40 presentations of horizontal or vertical phase reversing (1 Hz) gratings that varied in contrast levels and spatial frequency (see Methods). The VEP response to one phase of each stimulus was averaged across all animals (n = 20) and is shown beneath the stimulus. Colorplots of the amplitude (B) or area (C) of the negative-going VEP component. Values in between the tick marks were interpolated. VEP-assessed contrast sensitivity functions based on the interpolated VEP amplitudes (D, black) and VEP area (E, black). Overlaid in red are behaviorally-assessed CSF functions from Prusky and Douglas, 2004.
Supplemental Figure 8. Colorplot of VEP amplitude response (µV) to 40 presentations of phase-reversing gratings at various spatial frequencies and contrast levels. Each panel is the response from one 129/Sv mouse. See figure 23 for the group averaged response. Values in between the tick marks were interpolated. Same scale for all plots.
Supplemental Table 2. Latency to VEP and current sink/source peaks in response to (A) 0.05 and (B) 0.30 cyc/deg gratings. 129/Sv mice.

<table>
<thead>
<tr>
<th></th>
<th>0.05 cyc/deg</th>
<th>0.30 cyc/deg</th>
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<tr>
<td><strong>A</strong></td>
<td></td>
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<tr>
<td>VEP-peak latency (msec)</td>
<td></td>
<td></td>
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<tr>
<td>Negative Component</td>
<td>78 +/- 8</td>
<td>95 +/- 8</td>
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<tr>
<td>Positive Component</td>
<td>159 +/- 17</td>
<td>208 +/- 21</td>
</tr>
<tr>
<td><strong>B</strong></td>
<td></td>
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<td>Current sink-peak latency (msec)</td>
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<tr>
<td>0.05 cyc/deg</td>
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<tr>
<td>Layer 4</td>
<td>71 +/- 5</td>
<td>99 +/- 6</td>
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<tr>
<td>Superficial Layers</td>
<td>151 +/- 12</td>
<td>182 +/- 18</td>
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<tr>
<td>Deep Layers</td>
<td>137 +/- 15</td>
<td>142 +/- 7</td>
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<tr>
<td>0.15 cyc/deg</td>
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<td>Layer 4</td>
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<td>Superficial Layers</td>
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<td>Deep Layers</td>
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Supplemental Figure 9. Correlations among various components of the layer 4 VEP and current sink activity in the different cortical layers of 129/Sv mice (n = 6). Responses to 0.05 and 0.3 cyc/deg were combined. See text for details.
Chapter 3:

VEP Responses to High Spatial Frequency Gratings are Eliminated after Monocular Deprivation
Introduction

The visual cortex as a model system for studying plasticity

The visual cortex has been a model system for studying plasticity ever since Hubel and Wiesel performed a series of single unit recordings in the 1960s to examine the neural response to various stimuli presented to one or both eyes of monkeys and cats. In both species, they observed monocularly driven cells that respond preferentially to visual stimulation of either the contralateral or ipsilateral eye as well as binocular cells that respond equally to both eyes (Hubel, 1988; Hubel and Wiesel, 1962, 1968, 1977). To determine if this distribution of monocular versus binocular driven cells is malleable, Hubel and Wiesel performed a key experiment: they sutured shut the lids of one eye in a one-week old kitten and waited ten weeks before reopening the eye and recording from neurons in the kitten’s cortex. When they reopened the eye, the effect of monocular deprivation (MD) was dramatic: none of the cells they sampled responded to the deprived eye though more cells were responsive to the non-deprived eye (Wiesel and Hubel, 1963). This shift in the response of cortical cells from the deprived eye to the non-deprived eye is known as an ocular dominance (OD) shift. The same results were found when the experiment was repeated in juvenile monkeys (Hubel et al., 1977).

In a follow-up study designed to determine if the age of eye closure is important, they sutured shut the eye of adult cats for long periods (over a year) and observed no loss of the cortical response to stimulation of the previously deprived eye (Wiesel and Hubel, 1965). This suggested to them that there must be a period of plasticity between birth and adulthood during which visual deprivation leads to cortical reorganization.
They found that the period for inducing an OD shift is between four weeks and four months in the cat, and from birth to about one year in the monkey (Hubel and Wiesel, 1970; Hubel et al., 1977). This period of plasticity is known as the critical period.

**Ocular dominance plasticity in mice**

Building on the initial cat OD plasticity experiments, several studies have further characterized and determined the mechanisms of the OD shift induced in the visual cortex by MD. Aside from varying factors like rearing conditions, duration of deprivation and so on, several experiments have looked for MD effects on OD in different species. MD has been shown to lead to an OD shift in ferrets (Issa et al., 1999), rabbits (Van Sluyters and Stewart, 1974), rats (Maffei et al., 1992), hamsters (Emerson et al., 1982), and mice (Drager, 1978). All of these species have poorly developed binocular systems compared with the cat and monkey and therefore, none of them could compete with the cat and monkey as preferred subjects in visual plasticity experiments. However, with the advent of molecular engineering tools, the mouse has become the preferred model system for studying the cellular and molecular mechanisms underlying behavioral and physiological phenomena and has since been increasingly used to study OD plasticity. Following up on Drager’s 1978 experiment, various techniques have been applied to assess ocular dominance plasticity in mouse visual cortex (Figure 27). Using single-unit recordings, Gordon and Stryker (1996) defined the mouse critical period to be between postnatal day 20-32, peaking at postnatal day 28. They further determined that four days of deprivation yields the maximal OD shift in C57BL/6 mice (Figure 27a). Multiple studies published using various techniques such as visually evoked potential (VEP) recordings in awake mice (Frenkel and Bear, 2004; Sawtell et al., 2003), optical imaging
(Hofer et al., 2006) and calcium imaging with two-photon microscopy (Mrsic-Flogel et al., 2007) in anaesthetized mice all provide corroborating evidence that an OD shift can be induced and measured in the mouse visual cortex (see Figure 27b-d). All of these methods, with the exception of the single-unit recordings, used low spatial frequency stimuli ranging from 0.03 – 0.05 cyc/deg. In the single-unit recordings, stimuli were optimized for each cell. Therefore, these results give us insight into the plasticity of the visual pathway with respect to the response to low spatial frequency gratings. As suggested by recent single unit studies (Niell and Stryker, 2008), and by data in the previous chapters, cells in layer 4 of C57BL/6 V1 have a preference for low spatial frequency gratings, but they can respond to spatial frequencies up to at least 0.3 cyc/deg. In this study, we wanted to determine if plasticity after three days of monocular deprivation varied depending on the spatial frequency of the gratings used for testing.

**Results**

We deprived the contralateral eye of juvenile (~P27) C57BL/6 mice for three days and recorded layer 4 VEPs from binocular V1 in response to gratings of three spatial frequencies (0.05, 0.15, and 0.3 cyc/deg; see Methods).

**Deprived (contralateral) versus non-deprived (ipsilateral) eye VEP responses**

Under baseline conditions, the ipsilateral-eye VEP, collapsing across spatial frequencies, was significantly lower in amplitude than the response evoked by binocular (Figure 28a left; repeated measures one-way anova, n = 11 mice, $p = 0.0358$) or contralateral-eye stimulation ($p = 0.0284$) consistent with anatomical findings that layer
4 receives fewer inputs from the ipsilateral eye (Drager, 1974). There was no significant difference between binocular and contralateral-eye VEP amplitudes ($p = 0.9417$) suggesting that the binocular response in non-deprived V1 is dominated by contralateral eye input. After deprivation (Figure 28a right), the VEP response of the ipsilateral-eye, collapsed across spatial frequencies, was not significantly different from baseline ($p = 0.4991$); however, in response to high spatial frequency gratings (0.15 and 0.3 cyc/deg), ipsilateral-eye VEP amplitudes appeared to be higher than contralateral-eye amplitudes after MD. The binocular VEP amplitudes after MD were higher than the contralateral-eye ($p = 0.0725$) and similar to the ipsilateral-eye amplitudes ($p = 0.3791$) suggesting that after MD, the baseline pattern reverses and the binocular response is now dominated by ipsilateral eye input. Since the ipsilateral eye response was not potentiated, it must be the case that the contralateral eye response was depressed after MD. Indeed, contralateral eye VEP amplitudes after MD were significantly lower than baseline ($p = 0.0035$).

**VEP responses to grating stimuli of various spatial frequencies**

There was a significant effect of spatial frequency on the baseline contralateral eye VEP response ($p = 0.0004$), which was still seen after MD ($p = 0.0003$). In general, the largest amplitude VEPs for contralateral-eye stimulation were evoked by grating stimuli of 0.05 cyc/deg spatial frequency. The baseline contralateral-eye VEP response to 0.05 cyc/deg was significantly larger than its baseline response to 0.15 (post-hoc paired t-test, $p = 0.0141$) and 0.3 cyc/deg ($p < 0.0001$). The contralateral-eye VEP amplitudes evoked by 0.15 and 0.3 cyc/deg were not significantly different from each other ($p = 0.2534$). All changes discussed thus far are captured in the VEP waveforms.
which, as displayed in Figure 28b, are averages across all animals. Although 0.05 cyc/deg gratings continued to evoke the largest amplitude VEPs after MD (p < 0.05), the fractional change of the contralateral eye response, calculated as the post-MD response amplitude over the baseline response amplitude (Figure 28c, y-axis), indicates an overall decrease in responsiveness to 0.05 cyc/deg gratings. A comparable fractional change was observed in response to the other spatial frequencies so there was no significant effect of spatial frequency on the amount of contralateral eye depression following 3 days of MD (one-way anova, p = 0.3028). The net effect of these changes for the contralateral eye is that its baseline preference for 0.05 cyc/deg gratings is maintained after MD (Figure 28d, p = 0.6144); preference for the other spatial frequencies decreased, but not significantly. Interestingly in control animals that did not undergo MD (supplemental figure 10d), the contralateral-eye preference for 0.05 cyc/deg gratings was significantly reduced after 3 days (n = 9 mice, paired t-test, p = 0.0316) and preference for 0.15 and 0.3 cyc/deg gratings was increased although not significantly. The decreased preference to 0.05 cyc/deg is due to a decrease in the VEP amplitude after 3 days (supplemental figure 10c, p= 0.0955), which was smaller than the decrease induced by MD (unpaired t-test, p = 0.0149). Likewise, the contralateral-eye preference to 0.15 and 0.3 cyc/deg was enhanced in non-deprived mice because the VEP response to 0.15 and 0.3 cyc/deg gratings increased after 3 days of binocular experience (supplemental figure 10c). It would seem that for the contralateral-eye, binocular experience (BE) enhanced the VEP response to high spatial frequency gratings at the expense of the response to low spatial frequency gratings; MD maintained the preference for low spatial frequency gratings. It is likely that the
preference for low spatial frequency gratings is maintained in mice undergoing MD as they may still be able to experience low spatial frequency stimuli through the closed eyelid (supplemental figure 11).

In comparison to the contralateral deprived-eye response, the effect of spatial frequency on the ipsilateral open-eye VEP was modest before MD (Figure 28a left, \( p = 0.0512 \)) and no effect was found after MD (Figure 28a right, \( p = 0.1727 \)). Baseline ipsilateral-eye VEP amplitudes were generally uniform across the spatial frequencies although 0.15 cyc/deg evoked a larger amplitude response than 0.3 cyc/deg gratings (\( p = 0.0278 \)). The fractional changes of the ipsilateral-eye response (Figure 28c, x-axis) were smaller than the changes in the contralateral-eye response (\( p = 0.007 \)) and did not vary with the spatial frequency of the gratings (\( p = 0.4924 \)). This confirms previously reported data that 3 days of MD does not cause a shift, detectable by VEPs, in the non-deprived (ipsilateral) eye response (Frenkel and Bear, 2004; Sawtell et al., 2003). In mice experiencing binocular vision, the normalized ipsilateral-eye response, collapsed across spatial frequency, was not changed after 3 days (supplemental figure 12 left; one-way repeated measures anova, \( p = 0.9387 \)) although there was a slight increase in the preference for 0.05 cyc/deg gratings and a slight decrease for 0.3 cyc/deg gratings. No significant change was detected in the normalized ipsilateral-eye response of mice whose contralateral eye was monocularly deprived for 3 days (supplemental figure 12 right, \( p = 0.8516 \)), although there was a visible increase in the preference for 0.05 cyc/deg gratings.
Discussion

Monocular deprivation maintains the baseline preference for low spatial frequency gratings

In non-deprived control mice (P27 – P30), there was an increasing preference for high spatial frequency gratings at the expense of the preference for low spatial frequency gratings. However, in contralateral-eye deprived mice, the response to all spatial frequencies decreased and the magnitude of the depression did not significantly vary across the spatial frequency used for testing. Since the baseline VEP response to high spatial frequencies was already low, eliminating this response after MD had a net effect of biasing the visual response, even more so, towards low spatial frequencies. This should effectively reduce visual acuity. Although we did not do a full measure of visual acuity after MD, Prusky and Douglas (2003) have shown that in mice deprived from P19 – P32, the acuity of the deprived eye is significantly reduced, and there is no change in the acuity of the non-deprived eye. In the mouse, motion vision is likely to be more important than high-resolution vision. As a result, in order to maintain low-resolution vision during visual deprivation, the mouse visual system may be more likely to sacrifice responsiveness to higher spatial frequencies. In addition, our experiments also demonstrate that it is also likely that mice can, to some extent, experience low spatial frequency vision through the closed eyelid and this could aid in the maintenance of the response to low spatial frequency gratings.


Figure 27. Methods to assess ocular dominance plasticity in mouse visual cortex. **A**, Top, distribution of OD scores of neurons recorded from the binocular zone of a normal P24 mouse. Lower left, OD distribution of neurons recorded in the binocular zone ipsilateral to the deprived eye of a mouse that underwent MD from P28 to P32. Lower right, OD distribution of neurons from the binocular zone contralateral to the deprived eye. From Gordon and Stryker, 1996. **B**, Contralateral (filled bars) and ipsilateral (open bars) visually evoked potential (VEP) amplitudes measured during a baseline period and again after 3 days of MD initiated at P28. Modified from Sawtell et al, 2003. **C**, Optical imaging response strength of the deprived (contralateral) and open (ipsilateral) eyes in normal juvenile mice and age-matched mice undergoing 4 -10 days MD. Modified from Hofer et al, 2006. **D**, Cumulative histogram of eye-specific calcium transient amplitudes ($\Delta F/F$) of all responsive cells in normal animals and after MD of the contralateral eye. Distributions of contralateral-eye ($D_1$) and ipsilateral-eye ($D_2$) responses tested after various lengths of contralateral eye MD are shown. Modified from Mrsic-Flogel et al, 2008.
Figure 28. Changes in VEP response to gratings of various spatial frequencies after 3 days of monocular deprivation (MD). Baseline VEPs were recorded from awake head-fixed juvenile (P27) mice (n = 11) chronically implanted with electrodes in layer 4 of binocular V1. The amplitude of the negative going VEP component for binocular (blue), contralateral (black), and ipsilateral (red) eye responses was measured before (A, left) and after 3 days MD (A, right). VEP waveforms (B) for each condition are averages across all recorded animals. Thick lines indicate the post-MD condition, thin lines represent baseline. C, Fractional change, calculated as the ratio of the post-MD amplitude to the baseline amplitude, for the deprived eye (DE) is plotted along the y-axis; fractional change for the non-deprived eye (NDE) is plotted along the x-axis. Note the larger fractional change in the DE response to 0.3 cyc/deg. D, Preferred spatial frequency (SF) was determined by normalizing VEP amplitudes in response to each spatial frequency to the amplitude that evoked the maximum response. The baseline preference for 0.05 cyc/deg (thin line) was maintained after MD (thick line).
Supplemental figure 10. Changes in VEP response to gratings of various spatial frequencies after 3 days of binocular experience (BE). Baseline VEPs were recorded from awake head-fixed juvenile (P27) mice (n = 9) chronically implanted with electrodes in layer 4 of binocular V1. The amplitude of the negative-going VEP component for contralateral (black) and ipsilateral (red) eye responses was measured before (A, left) and after 3 days BE (A, right). VEP waveforms (B) for each condition were averaged across all recorded animals. Thick lines indicate the post-BE condition, thin lines represent baseline. C, Fractional change, calculated as the ratio of the post-BE amplitude to the baseline amplitude, for the contralateral eye is plotted along the y-axis; fractional change for the ipsilateral eye is plotted along the x-axis. D, Preferred spatial frequency was determined by normalizing VEP amplitudes in response to each spatial frequency to the amplitude that evoked the largest response. The baseline preference for 0.05 cyc/deg (thin line) was significantly decreased (p = 0.0316) after 3 days of BE (thick line).
Supplemental Figure 11. VEP responses elicited through closed eyelids. VEPs were recorded in response to grating stimuli of varying spatial frequencies at 100% contrast while both eyes were open (black) and following binocular deprivation by lid suture (red). Baseline recordings were made in response to a gray screen of equal luminance (gray line). VEPs were significantly greater than baseline when both eyes were open (post hoc paired t-test, $P < 0.001$). When the eyelids were sutured closed, VEPs in response to 0.05 cyc/deg gratings were significantly different from baseline (post hoc paired t-test, $P < 0.001$; indicated by asterisk). Post hoc statistical comparisons were done after reaching significance with repeated measures 2-way ANOVA ($P < 0.001$). Traces below graph are binocular VEPs from one representative animal prior to (black) and following (red) binocular lid suture. Thin line is noise level. Note the persistence of a VEP in response to 0.05 cyc/deg stimuli following lid suture.
Supplemental figure 12. Preferred spatial frequency for the ipsilateral-eye in non-deprived (left) and contralateral-eye deprived (right) mice under baseline conditions (thin lines) and after three days (thick lines) of monocular deprivation (MD) or binocular experience (BE). Spatial frequency (SF) preference was determined by normalizing VEP amplitudes in response to each spatial frequency to the amplitude that evoked to the maximum response. Collapsed across spatial frequency, the ipsilateral-eye SF preference was not changed after 3 days of binocular experience (one-way repeated measures anova, $p = 0.9387$) or monocular deprivation of the contralateral eye ($p = 0.8516$)
Chapter 4:

Screening the Visual Function of Shank1\(^{-/-}\) Mice
Introduction

With the advent of molecular engineering tools, the mouse has become the preferred model system for studying the cellular and molecular mechanisms underlying behavioral and physiological phenomena. Dozens of mice have been generated with various genetic modifications targeting brain specific genes. Several behavioral paradigms have been developed to assay for behavioral changes resulting from these modifications. For example, if a gene of interest is heavily expressed in the hippocampus, which is a structure thought to be important for learning and memory, then the standard behavioral assays would include tasks like the Morris water maze for spatial learning and memory (Crawley, 2008). These spatial navigation tasks require that the mice be able to see and use external cues in their environment. Therefore, in order to avoid an experimental confound, the genetic modification should not affect the animals’ visual ability. Since the layer 4 VEP measure of visual acuity corresponds with behaviorally measured visual acuity determined by Prusky and Douglas (2004), it can be used to assay the visual function of mutant mice prior to administering behavioral tests. Unlike behavioral tests of vision, VEP assessed visual function does not require training time and is therefore a promising electrophysiological test of visual function that can be administered to a number of animals in a short time. We used this assay to test the visual function of Shank1<sup>−/−</sup> knockout mice.

Shank1 is a post-synaptic density protein associated indirectly with NMDA receptors via GKAP and PSD-95, and with the actin cytoskeleton via Cortactin (Boeckers et al., 1999; Naisbitt et al., 1999). Due to these interactions, Shank1 could potentially translate synaptic activity into structural changes. Overexpressing Shank1 in
cultures of hippocampal neurons resulted in significantly larger spine heads (Sala et al., 2001). The Shank1−/− mouse has significantly smaller spines and reduced post-synaptic density relative to wildtype animals (Hung et al., 2008). The size of the spine head correlates with the size and strength of the synapse (Kasai et al., 2003; Matsuzaki et al., 2001). It is thought that small, thin spines promote plasticity, whereas large stable spines are the anatomical substrates of “memory”(Kasai et al., 2003). If Shank1 is necessary for the formation and/or stabilization of large mushroom type spines, then the knockout should be deficient in learning and/or memory tasks. Recently it was demonstrated that in an eight-arm radial maze task, Shank1−/− mice are able to learn the task and even make significantly less errors than wildtype mice (Hung et al., 2008). However, when re-tested one month later, the mice lose this advantage and make the same number of errors as wildtype mice. It would seem that having smaller, and presumably more plastic, spines resulting from the absence of Shank1 is conducive to learning at the cost of long-term memory. Based on these behavioral data, it is highly unlikely that Shank1−/− mice have impaired vision.

However, electrophysiological experiments identified a deficiency in basal synaptic transmission in Shank1−/− mice. Hung et al. (Hung et al., 2008) investigated excitatory synaptic transmission at the Schaffer collateral/commissural-CA1 synapse in acute hippocampal slices from wild-type and Shank1−/− mice (3–5 weeks of age). AMPA receptor-mediated field EPSPs in the stratum radiatum of Shank1−/− mice were significantly shifted downward compared with wild type (Figure 29a), especially at high stimulus intensities, demonstrating that Shank1 deficiency reduces basal synaptic transmission. The lack of a change in paired pulse facilitation indicated that this
reduction in synaptic transmission is unlikely to be caused by a change in presynaptic release probability. A significant reduction in the frequency of the mEPSC of Shank1^{-/-} mice (Figure 29b) suggests that the decreased basal transmission is primarily caused by a reduction in functional synapses (Hung et al., 2008). Based on these data, we predict that the amplitude of the VEP may be different in Shank1^{-/-} mice.

Results

We determined visual acuity by recording VEPs in response to gratings of various spatial frequencies and contrasts from juvenile (P27 – P30) mice implanted with electrodes in binocular V1 (see Methods). The Shank1^{-/-} mice were in the 129/Sv background (Chapter 2). All analyses were done using measurements of the negative-going VEP component.

Since they were able to perform the eight-arm radial maze task better than wildtype mice, it is unlikely that Shank1^{-/-} mice are visually impaired at the spatial frequencies required to perform that task. Indeed, VEP determined visual acuity was only slightly lower in Shank1^{-/-} mice (0.55 cyc/deg; Figure 30a, red) compared to wildtype mice (0.6 cyc/deg). However, there was a main effect of genotype on the amplitude of the negative-going VEP, which was dampened in knockout mice (three-way anova, p = 0.0116, Figure 30b cf. Figure 23b). A significant interaction between genotype and spatial frequency (p = 0.0225), as well as genotype and contrast (p = 0.0002) suggests that the effect of genotype is more pronounced for particular spatial frequencies and contrast levels. Comparing the wildtype and Shank1^{-/-} colorplots suggests that VEP responses to 0.15 – 0.6 cyc/deg gratings at 50 – 100% contrast are greatly reduced in the knockout while responses from 0.05 – 0.1 cyc/deg grating at low
contrast levels (25%) appear normal. In addition to amplitude, the averaged waveforms in knockout mice were different in other respects from wildtype mice (Figure 31a). For example, there was a significant main effect of genotype on latency to peak negativity (two-way anova, \( p = 0.0002 \)) which was later in knockout mice (85 msecs post-stimulus onset) than in wildtype mice (73 msecs). To illustrate this point, mean peak latencies of VEPs evoked in response to various spatial frequencies at 100% contrast for wildtype and knockout mice are shown in Figure 31b. As the figure demonstrates, there was also a significant effect of spatial frequency on peak latency (\( p = 0.0002 \)) such that higher spatial frequency gratings evoked later peaking responses. There was also an interaction between genotype and spatial frequency (\( p = 0.0535 \)) which means that the effect of genotype on latency depends on the particular spatial frequency. Indeed, genotype appears to affect the latency to peak of low spatial frequency responses more so than the latency to peak of high spatial frequency responses. The positive-going component was also significantly reduced in knockout mice (\( p < 0.0001 \); Figure 31c cf Figure 24c) and the significant interaction between genotype and spatial frequency (\( p = 0.0006 \)) as well as genotype and contrast (\( p = 0.0059 \)) means that this reduction was in response to particular spatial frequencies. Comparing the wildtype and Shank1-/- colorplots indicates that the positive-going component was most reduced in response to spatial frequencies greater than 0.1 cyc/deg and contrast levels above 12%. One other featured difference between the VEP waveforms of wildtype and Shank1+/- is that in knockout mice in response to higher spatial frequency stimuli, the initial negative going component was not clearly separated from the second negative going component by a positive going component (Figure 31a).
Discussion

The deficiency in basal transmission previously reported by Hung et al (2008) may explain why VEP amplitudes were lower and peak latencies were longer in Shank1-/- mice compared to wildtype mice. We have previously shown that the negative-going VEP component reflects current sinks in layer 4 (Chapter 1 and 2), which are in turn likely generated by synaptic activity at the site of direct geniculate input onto layer 4 (Mitzdorf, 1985). The paired-pulse facilitation results reported by Hung et al (2008) suggest that the decrease in basal transmission is not caused by a change in presynaptic release probability. Rather, a significant reduction in the frequency of mEPSC from Shank knockout mice suggests that the decreased basal transmission is largely due to a reduction in functional synapses (Hung et al., 2008). If synaptic function is reduced in Shank knockout mice, this should also lead to a reduction in the layer 4 current sink amplitude and consequently, a reduction in the VEP amplitude. The longer latency to peak VEP negativity likely reflects longer layer 4 current-sink latency due to weaker coherence of the synaptic events (Mitzdorf, 1985).

The reduced VEP negative-going amplitudes in Shank1-/- did slightly reduce VEP assessed visual acuity in these mice (0.55 cyc/deg versus 0.6 cyc/deg in wildtype mice). However, this probably did not manifest as a deficit in their performance in the eight-arm radial maze task, which uses large-sized cues that can be detected with low spatial frequency vision (Hung et al., 2008). Acuity measured using the negative-going component indicates that in the knockout, layer 4 is receiving geniculate input for spatial frequencies up to 0.55 cyc/deg, however, this does not mean that the rest of cortex is processing the input. The positive-going component of the VEP in 129/Sv mice corresponds in time with the peak of current sinks occurring in superficial layers.
(Chapter 2). In wildtype 129/Sv mice, superficial layer current sinks are more pronounced in response to high spatial frequency gratings. We suggested therefore, that in 129/Sv mice, the cortical pathway for processing high spatial frequency gratings involves layer 4 and superficial layers. Since the positive-going component, which likely reflects current sink activity in superficial layers, was lower in Shank1<sup>−/−</sup> knockout mice, it is possible that the LGN input into layer 4 is not being relayed effectively in these mice. Therefore a behavioral test for vision must be performed in order to determine whether or not the visual function of Shank1<sup>−/−</sup> mice is normal. In addition, current source density analysis to study the laminar pattern of current sink activity in response to gratings of various spatial frequencies and contrasts may give further insight into the strength of synapses across the laminae of V1.


Figure 29. Decreased synaptic strength in Shank1 mutant mice. A, Left, Sample traces (average of 10 consecutive responses) represent the responses evoked with seven different stimulus intensities from wild-type (+/++) or Shank1^-/- hippocampal slices. The same sample traces are shown at two different scales. Stimulus artifacts were truncated. Right, Summary graph of the input–output relationships of field EPSPs of wild-type mice (-/-; n = 15 slices from 9 mice) and Shank1^-/- mice (-/-; n = 12 slices from 7 mice). Symbols indicate the mean +/- SEM. The input–output relationship of Shank1^-/- is significantly weaker than that of wild-type mice (*p < 0.05; Student’s t test). B, Left, Top and Middle, Two consecutive sample mEPSC traces from wild-type and Shank1^-/- mice. Left, Bottom, Averaged mEPSC from wild-type mice (left) and Shank1^-/- mice (center) (average of 300 traces) and superimposed traces (sup; right). Note that the time course of the events is the same. Right, Summary graphs of the amplitude and frequency of mEPSCs in wild-type (n = 12 cells/6 mice) and Shank1^-/- (n = 13 cells/6 mice) animals. There was no significant difference in mEPSC amplitude (NS, not significant). The frequency of mEPSCs in Shank1^-/- mutants is significantly less than that of wild-type mice (*p < 0.02; Student’s t test). From Hung et al, 2008.
Figure 30. VEP assessed visual function of Shank1−/− mice. Mice (n = 13) were exposed to 40 presentations of horizontal or vertical phase reversing (1 Hz) gratings that varied in contrast levels and spatial frequency (see Methods). A, Contrast sensitivity function of Shank1−/− (red squares), and wildtype (gray circles). Acuity is slightly decreased in Shank1−/− mice. B, Colorplot of the group averaged negative-going VEP amplitude response (µV) to gratings of various spatial frequencies and contrast levels. VEP amplitudes in Shank−/− mice were significantly smaller than wildtype (C, repeated from Figure 23b, p = 0.0116). Values in between the tick marks were interpolated.
Figure 31. The VEP waveform is altered in Shank1−/− mice. A, Group averaged VEP waveforms from Shank−/− mice in the 129/Sv background (red) are overlaid on wildtype 129/Sv waveforms (black) to highlight the differences between wildtype and Shank−/− VEP responses to gratings of various spatial frequencies and contrasts. Note the increase in latency to peak negativity, particularly in response to low spatial frequency gratings. For example, at 100% contrast, B, latency to peak was significantly later in Shank−/− mice in response to 0.05 – 0.15 cyc/deg gratings (p <0.05). C, The positive-going VEP component was significantly smaller in knockout mice (p < 0.0001) compared with wildtype mice (D, repeated from Figure 24c). Values in between tick marks were interpolated.
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“All scientific knowledge is held to be teachable, and what is scientifically knowable is capable of being learned.”
- Aristotle, Nichomachean Ethics, Book 6

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“The perfect form of friendship is that between the good,
and those who resemble each other in virtue...

Such friendships are of course rare,
because such men are few.

Moreover they require time and familiarity:
as the saying goes,
you cannot get to know a man till you have
consumed the proverbial amount of salt in his company.”

- Aristotle, *Nicomachean Ethics*, Book 8

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