Development/Plasticity/Repair

Visual Stimulus Speed Does Not Influence the Rapid Emergence of Direction Selectivity in Ferret Visual Cortex

Neil J. Ritter,[1] Nora M. Anderson,[1] and Stephen D. Van Hooser[1,2,3]

[1]Department of Biology, [2]Volen Center for Complex Systems, and [3]Sloan-Swartz Center for Theoretical Neurobiology, Brandeis University, Waltham, Massachusetts 02454

Sensory experience is necessary for the development of some receptive field properties of neurons in primary sensory cortical areas. However, it remains unclear whether the parameters of an individual animal's experience play an instructive role and influence the tuning parameters of cortical sensory neurons as selectivity emerges, or rather whether experience merely permits the completion of processes that are fully seeded at the onset of experience. Here we have examined whether the speed of visual stimuli that are presented to visually naive ferrets can influence the parameters of speed tuning and direction selectivity in cortical neurons. Visual experience is necessary for the development of direction selectivity in carnivores. If, during development, cortical neurons had the flexibility to choose from among different inputs with a range of spatial positions and temporal delays, then correlation-based plasticity mechanisms could instruct the precise spatiotemporal selectivity that underlies speed tuning and direction selectivity, and the parameters of an individual animal's experience would influence the tuning that emerges. Alternatively, the tuning parameters of these neurons may already be established at the onset of visual experience, and experience may merely permit the expression of this tuning. We found that providing different groups of animals with either slow (12.5 deg/s) or fast (50 deg/s) visual stimuli resulted in emergence of direction selectivity, but that speed tuning and direction selectivity were similar in the two groups. These results are more consistent with a permissive role for experience in the development of direction selectivity.

Key words: area 17; motion; recurrent connections; striate cortex; thalamocortical

Significance Statement

The proper development of brain circuits and neural response properties depends on both nature (factors independent of experience) and nurture (factors dependent on experience). In this study, we examined whether the quality of visual experience of an individual animal influences the development of basic sensory detectors in primary visual cortex. We found that, although visual experience is required for the development of direction selectivity, tuning for stimulus speed could not be altered by specific experience with slow or fast stimuli. These results suggest that the tuning parameters for direction selectivity are specified independently of an animal's sensory experience, and that a range of experiences can promote the proper mature expression of direction selectivity in primary visual cortex.

Introduction

Receptive field properties of neurons early in the visual pathway undergo substantial changes as the neural circuits responsible for perception and behavior assemble and mature. Much of the initial wiring is governed by internal processes, independent of sensory experience, that sketch out characteristic feature selectivity, such as retinotopic organization, ocular dominance, and orientation selectivity (Chapman et al., 1996; Horton and Hocking, 1996; McLaughlin and O'Leary, 2005; Huberman et al., 2008). Some of these features are present at the onset of visual experience (Frégnac and Imbert, 1978; Chapman and Stryker, 1993), but other features, such as direction selectivity, require visual experience for their formation (Li et al., 2006). It remains unclear what role experience plays in the formation and maturation of neural circuits.

The development of direction selectivity in neurons in primary visual cortex (V1), the ability to respond differently to two directions of motion, has been established as a model system for

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Correspondence should be addressed to Dr. Stephen D. Van Hooser, Brandeis University, 415 South Street MS008, Waltham, MA 02454. E-mail: vanhooser@brandeis.edu.

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exploring how experience influences the development of neural circuits. Kittens (Zhou et al., 1995) and ferrets (Li et al., 2006) deprived of visual experience for the first few months of life do not develop direction selectivity in V1. Neurons in V1 of kittens reared under 8 Hz stroboscopic illumination also do not develop direction selectivity (Cynader and Chernenko, 1976). The deficit is permanent despite subsequent exposure to normal visual experience (Humphrey and Saul, 1998; Li et al., 2006) and is reflected in behavioral deficits in direction discrimination in cats (Pasternak et al., 1985; Pasternak and Leinen, 1986) and in humans (Ellemberg et al., 2002). Further, the artificial introduction of moving stimuli to anesthetized visually naive ferrets is sufficient to cause the rapid emergence of direction selectivity within 3–6 h (Li et al., 2008; Van Hooser et al., 2012).

Although experience is required for the development of direction selectivity, it remains unclear whether experience has an instructive influence on the tuning properties that emerge, or rather, whether experience merely permits the completion of processes that are fully seeded at the onset of experience. Direction selectivity requires that neurons respond to visual stimulation at different spatial positions with different temporal delays (Barlow and Levick, 1965; Adelson and Bergen, 1985), but it is unclear whether the quality of an animal's early experience can influence the set of spatial positions and temporal delays that provide inputs to cortical neurons.

To address this question, we examined whether experience that was limited to particular speeds could influence the speed and direction tuning of developing visual cortical neurons in naive ferret visual cortex around the age of eye opening (~p32) when direction selectivity begins to develop. Ferrets have a visual system similar to that of the cat and other carnivores (Law et al., 1988) but are born at a comparatively early developmental stage, making the ferret a physiologically robust preparation for long recording sessions. In addition, direction selectivity can be rapidly induced in the laboratory with 3–6 h of stimulation with sinusoidal gratings (Li et al., 2008; Van Hooser et al., 2012) or via direct optogenetic cortical stimulation (Roy et al., 2016), which allows the influence of experience to be explored parametrically.

Here, we measured speed tuning and direction selectivity before and after several rounds of artificial visual stimulation. One set of animals received stimulation with sinusoidal gratings that moved at 12.5 deg/s; a second group of animals received stimulation with sinusoidal gratings that moved at 50 deg/s; and a third group of control animals received stimulation with a gray screen. Both sets of animals that received grating stimulation exhibited increases in direction selectivity and, further, exhibited similar speed tuning (peak tuning: 25 deg/s) preferences regardless of the speed of their experienced visual stimulation. This evidence is consistent with the idea that the mixture of spatial positions and temporal delays that underlie direction selectivity are already established independent of experience, and that experience has a permissive influence on the development of direction selectivity.

Materials and Methods

General design. All experimental procedures were approved by the Brandeis University Animal Care and Use Committee and performed in compliance with National Institutes of Health guidelines. Ferrets (*Mustela putorius furo*; $n = 15$ females, age postnatal day [P] 31–34) were used in terminal electrophysiological experiments designed to influence visual direction selectivity. Female animals were used exclusively because animals were cohoused with sexually mature females, and cohousing with males causes stress. Ferrets were split into two primary study groups. Experimental animals ($n = 12$) underwent 9 h of visual training with drifting gratings moving at different speeds. Control animals ($n = 3$) underwent the same procedure as experimental animals but were exposed to a static gray screen for 9 h instead of drifting gratings.

Surgical procedures. Ferrets were sedated with ketamine (20 mg/kg i.m.). Atropine (0.16- 0.8 mg/kg i.m.) and dexamethasone (0.5 mg/kg i.m.) were administered to reduce bronchial and salivary secretion and to reduce inflammation, respectively. The animal was anesthetized with a mixture of isoflurane, oxygen, and nitrous oxide through a mask while a tracheostomy was performed. Animals were ventilated with 1.5%–3% isoflurane in a 2:1 mixture of nitrous oxide and oxygen. A cannula was inserted into the intraperitoneal cavity for delivery of neuromuscular blockers and Ringer's solution ($3\ ml \cdot kg^{-1} \cdot h^{-1}$), and the animal was inserted in a custom stereotaxic frame that did not obstruct vision. All wound margins were infused with bupivacaine. Silicone oil was placed on the eyes to prevent corneal damage. A craniotomy (4×4 mm) was made in the right hemisphere, and the dura was removed with a 31 gauge needle. Next, ferrets were paralyzed with the neuromuscular blocker gallamine triethiodide ($10–30\ mg \cdot kg^{-1} \cdot h^{-1}$) through the intraperitoneal cannula to suppress spontaneous eye movements, and the nitrous oxide-oxygen mixture was adjusted to 1:1. The animal's ECG was continuously monitored to ensure adequate anesthesia, and the percentage of isoflurane was increased if the ECG indicated any distress. Body temperature was maintained at 37°C.

Electrophysiology. The 32 channel silicon probes (NeuroNexus, A1xP32-poly2-10 mm-50-177) were used to record from all layers of ferret primary visual cortex. The probe was positioned approximately perpendicularly to the surface of the brain and lowered until all pads were inserted (900–1100 μm), and 3%–4% agarose was applied to prevent brain pulsation. Mineral oil was applied to the agarose at regular intervals to prevent agarose from drying. Recordings were done at the start of the experiment and after every 3 h of training up to 9 h. Signals were collected in two ways:

For most of the experiments ($n = 11$), signals were amplified with a preamplifier/amplifier system by Multichannel Systems. Data from all 32 channels were acquired with custom software in LabVIEW and a National Instruments 6071e data acquisition board. The remaining experiments ($n = 4$) signals were acquired with a headstage (RHD2132) by Intan Technologies.

For both data collection methods, individual spike waveforms were extracted using 5 SDs as a threshold. All spikes on a given channel were grouped together as multiunit clusters because signals that could be reliably attributed to single units were rarely encountered with these electrodes.

Visual stimulation and data analysis. Visual stimuli were created in MATLAB (The MathWorks) with the Psychophysics Toolbox (Brainard, 1997; Pelli, 1997) on a Macintosh Pro running OSX and displayed on a Sony monitor (GDM-520) positioned 45 cm in front of the ferret. Stimuli were full-field, high-contrast sine wave gratings with direction of motion (0°, 30°, 60°, 90°, 120°, 150°, 180°, 210°, 240°, 270°, 300°, 330°) perpendicular to grating orientation. Spatial frequency of grating stimulus was always 0.08 and temporal frequency varied.

For each site on the electrode, we examined mean response to drifting grating stimulation (F0). Sites were only included in analysis if they passed the following three exclusion criteria: (1) exhibited significant variation across all stimuli by an ANOVA test; (2) had at least a 1 Hz response to 25 deg/s grating stimulation; and (3) had a preferred orientation within 30° of the optimal orientation (see below). All recordings in each experiment had at least 5 sites pass these exclusion criteria.

The fit-less vector measures circular variance (CV) and directional circular variance (DCV) were calculated as described previously (Ringach et al., 2002; Mazurek et al., 2014) when responses to a full range of directions sampled in 30° increments were available (see Fig. 2*B*, Stimulus 1).

Responses across a range of speeds were collected only for motion in the two directions perpendicular to a single optimal orientation (see Fig. 2*B*, Stimulus 2). The optimal orientation was chosen at the start of each experiment based on the responses to an initial assessment of orientation selectivity at 25 deg/s. Ferret primary visual cortex contains a columnar map of orientation preference (Chapman et al., 1996), and we were able to record from many neurons with similar orientation selectivity simultaneously by inserting the linear electrode array perpendicular to the

surface of the brain. Previous work has shown that orientation selectivity is invariant to speed in ferret V1 (Moore et al., 2005). A direction selectivity index (DSI) was defined to be $(R_p - R_n)/R_p$, where R_p is the response in the preferred direction and R_n is the response in the direction that is 180° opposite the preferred direction. Because preferred direction can change with speed (Basole et al., 2003; Moore et al., 2005), preferred direction was defined here as the direction that had the greatest summed response to 12.5, 25, and 50 deg/s grating stimulation.

Illustrative modeling for Figure 1. An example simple feedforward circuit model was built to illustrate possible schemes for the development of direction selectivity across a range of speeds in Figure 1. Velocity tuning curves were computed for a single cortical cell receiving inputs from a pool of 65 LGN cells representing 13 different positions in space separated by 1° and 5 different latencies: 0, 20, 40, 60, and 80 ms. To achieve realistic speed tuning in V1 neurons, LGN input was modulated by a factor of 2 using a Gaussian function that peaked at 25 deg/s. LGN inputs with different spatial positions and response latencies were selected deliberately to generate example speed tuning curves (see Fig. 1*F*,*H*). Computer simulations were performed with MATLAB.

Bootstrap analysis. A change index was calculated for orientation and direction selectivity, tuning width, and firing rate using bootstrapping. All individual site index values for each animal at each time point were sampled with replacement, and bootstrapped animal average or median index values for each time point were generated. These values were fit with a linear model to find a rate of change in index value as follows:

$$\mathrm{IND}(tc, t, an) = \alpha_{an} + m \times t$$

Where *tc* is the type of visual training the animal received (12.5 deg/s, 50 deg/s, or control gray screen), *t* is time (in hours) that the animal has been trained, *an* is animal number, α_{an} is the bootstrapped initial index value for a particular animal, and *m* is the bootstrapped rate of change in index for the training condition. This equation allowed us to determine the average influence of each stimulus condition over time while allowing a parameter that described repeated measures of an individual (α_{an}). By pooling across time, this measure gives us more statistical power than a repeated-measures ANOVA.

Results

Development of direction selectivity in primary visual cortex

In juvenile ferrets around the age of eye opening (~p30), V1 neurons are selective for the orientation of edges but not their direction of motion (Li et al., 2006). Direction selectivity develops naturally over subsequent weeks through a process that requires visual experience (Li et al., 2006, 2008), but the biological mechanism of direction selectivity and how it develops are unknown.

A direction-selective cell must respond to stimuli at different spatial positions with different temporal delays (Reichardt, 1961; Adelson and Bergen, 1985), but it is unclear whether experience influences the positions and delays that are sampled in mature neurons. The requirement for visual experience raises the possibility that visual stimuli might play an instructive role in the development of direction selectivity (Li et al., 2008). That is, experience might dictate the spatial positions and delays that provide input to a cortical neuron. An illustration of such a model, where neurons that respond to different positions in space and different latencies are imagined to arise within the LGN, is shown in Figure 1*A* (Possible Juvenile State I). Moving visual stimuli will activate the LGN neurons in particular patterns that, through a learning rule, results in a pattern of connections that is direction-selective (Fig. 1*C*, Adult State).

Alternatively, the potential spatial and temporal inputs to cortex may be fixed at the onset of experience (Fig. 1*A*, Possible Juvenile State II) and direction selectivity may be acquired through changes, such as an increase in synaptic strength of fixed connections (Fig. 1*G*,*H*) or a general increase in cortical inhibition (Garkun and Maffei, 2014; Van Hooser et al., 2014). In these cases, visual experience would be permissive rather than instructive. It is difficult to distinguish between these possibilities by the activity of V1 neurons, as juvenile connection schemes I and II posit similar V1 neuron responses to moving visual stimuli (Fig. 1*B*,*D*).

A prediction of the instructive model is that the parameters of visual stimuli will influence the resulting mature state. If the mature state is the same regardless of the experienced visual stimuli, then one would conclude that V1 is not using visual experience to instruct the construction of direction selection circuits. For example, in the instructive model, if a juvenile ferret were to experience only visual stimuli moving at either slow or fast speeds, the V1 neuron would develop direction selectivity at the slow or fast speeds, respectively (Fig. 1*E*). Alternatively, in a permissive model, training with slow or fast speeds will lead to development of direction selectivity with a common predetermined set of speed preferences (Fig. 1*G*). Responses of a V1 neuron to a bar moving up and down at different speeds before and after experience for these two possibilities are shown in Figure 1*F*, *H*.

To test the hypothesis that the speed of visual stimulation influences the development of direction selectivity, we took advantage of the fact that direction selectivity can be acquired rapidly in a laboratory setting by providing several hours of visual stimulation with a motion stimulus (Li et al., 2008; Van Hooser et al., 2012). In previous experiments, exposure to a stimulus moving back and forth at 50 deg/s for 3–6 h caused the rapid increase of direction selectivity in naive, anesthetized ferrets that had recently opened their eyes.

We first sought to examine the influence of different training speeds on the development of orientation and direction-selective tuning properties at 25 deg/s. We wanted to establish that (1) multiunit recording methods used here would recapitulate results of the previous 2-photon experiments, and (2) to examine whether experience of different speeds altered tuning curve shapes.

We first examined how the response of V1 neurons to drifting gratings of different orientations moving at 25 deg/s (Fig. 2*B*; Test Stimulus 1) change throughout 9 h of training with drifting gratings moving at 50 deg/s, 12.5 deg/s, or to a static gray screen control (Fig. 2*B*; Training Stimuli α, β, or γ).

DSI develops at 25 deg/s with 12.5 and 50 deg/s training

Direction tuning curves were measured at 25 deg/s (Fig. 2*B*; Test Stimulus 1) before training (0 Hours) and then again after 3, 6, and 9 h of training for each of the three training conditions (Fig. 3). Responses were normalized and rotated such that the maximum response of a site had a value of 1 in the upward direction. These curves were used to calculate measures of orientation and direction selectivity.

Direction selectivity was quantified using 1 − Directional Circular Variance (1 − DCV). 1 − DCV values were initially similar across the three training conditions (Fig. 4*A*; Kruskal–Wallis test, $H(2) = 0.58$, $p = 0.748$) with a mean value of 0.105 ± 0.017. Training with both 12.5 and 50 deg/s drifting gratings had a significantly greater rate of increase in animal average 1 − DCV at 25 deg/s compared with control (Fig. 4*A*; bootstrap test; see Materials and Methods). Individual site values of 1 − DCV for each condition throughout training are shown in Figure 4*B*–*D*. There was a significant increase in median 1 − DCV with 12.5 deg/s training (Fig. 4*B*; Kruskal–Wallis test, $H(2) = 33.97$, $p < 0.001$) and 50 deg/s training (Fig. 4*C*; Kruskal–Wallis test, $H(2) = 33.00$, $p < 0.001$), but not in control (Fig. 4*D*; Kruskal–Wallis test, $H(2) = 1.6$, $p = 0.206$).

Orientation selectivity was quantified with 1 − Circular Variance (1 − CV). The 1 − CV values were initially similar across all

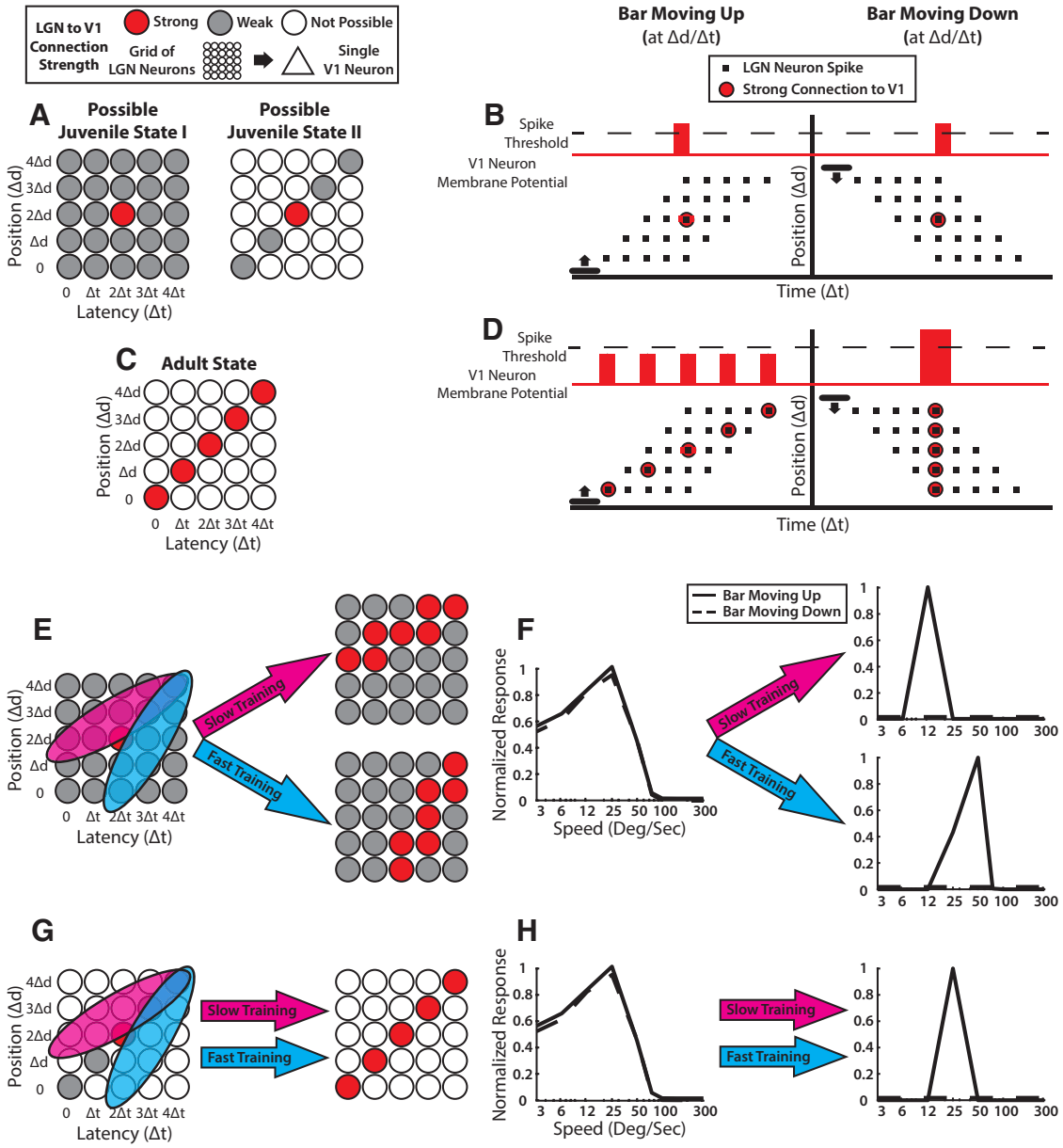

Figure 1. Hypotheses outline: models of the development of direction selectivity in primary visual cortex. ***A***, Two possible juvenile connection schemes between a group of LGN neurons that respond to combinations of 5 adjacent positions in space at 5 different latencies and a single V1 neuron. The single V1 neuron is illustrated only in the figure key above ***A***. LGN neuron color key: red (strong) represents V1 connection exists and produces a strong response in the V1 neuron; gray (weak) represents V1 connection exists but only produces a weak response in the V1 neuron; white (not possible) represents V1 connection does not exist and cannot produce a response in the V1 neuron. ***B***, Firing times of LGN neurons from ***A*** to a bar moving up and a bar moving down. LGN neurons with a strong connection to the V1 neuron cause a deflection in the V1 neuron's membrane potential that will lead to a spike if a threshold is crossed. ***C***, Adult pattern of connections between LGN neurons and a single V1 neuron. ***D***, Firing times of LGN neurons from ***C*** to a bar moving up and a bar moving down. The threshold for firing in the V1 neuron has increased from the juvenile state in ***B***, and activity from multiple LGN neurons with strong connections is required for the V1 neuron to fire. The pattern of connections from LGN shown here results in the V1 neuron being selective for a bar moving down. ***E***, ***F***, If visual experience plays an instructive role in the development of direction selectivity, (***E***) we would expect training of Possible Juvenile State 1 with visual stimuli moving at fast and slow speeds to result in different patterns of connections between LGN and V1. ***F***, Model responses of a V1 neuron to a bar moving up and a bar moving down for Juvenile State 1 before and after training with fast and slow speed visual stimuli. Before training, the cortical response largely reflects speed tuning of LGN cells. After training, the speed tuning is dominated by the pattern of connections. ***G***, ***H***, If visual experience plays a permissive role in the development of direction selectivity, (***G***) we would expect training of Possible Juvenile State 2 with visual stimuli moving at fast or slow speeds to result in identical patterns of connections between LGN and V1. ***H***, Model responses to a bar moving up and a bar moving down for Juvenile State 2 before and after training with fast or slow speed visual stimuli. Speed tuning of the V1 neuron is dictated by constraints in the pattern of connections.

three training conditions (Fig. 4*E*; Kruskal–Wallis test, $H(2) = 2.48$, $p = 0.290$) with a mean value of 0.383 ± 0.036. No difference in rate of increase in animal average $1 - CV$ was found between training conditions and control (Fig. 4*E*; bootstrap test; see Materials and Methods). Individual site values of $1 - CV$ for each condition throughout training are shown in Figure 4*F–H*. There was a significant increase in median $1 - CV$ in all conditions: with 12.5 deg/s training (Fig. 4*F*; Kruskal–Wallis test, $H(2) = 7.24$, $p = 0.007$), 50 deg/s training (Fig. 4*G*; Kruskal–Wallis test, $H(2) = 29.22$, $p < 0.001$), and control (Fig. 4*H*; Kruskal–Wallis test, $H(2) = 10.14$, $p = 0.001$).

To assess tuning width, direction tuning curves were fit (data not shown) with a double Gaussian function as described previously (Swindale, 1998; Carandini and Ferster, 2000; Mazurek et

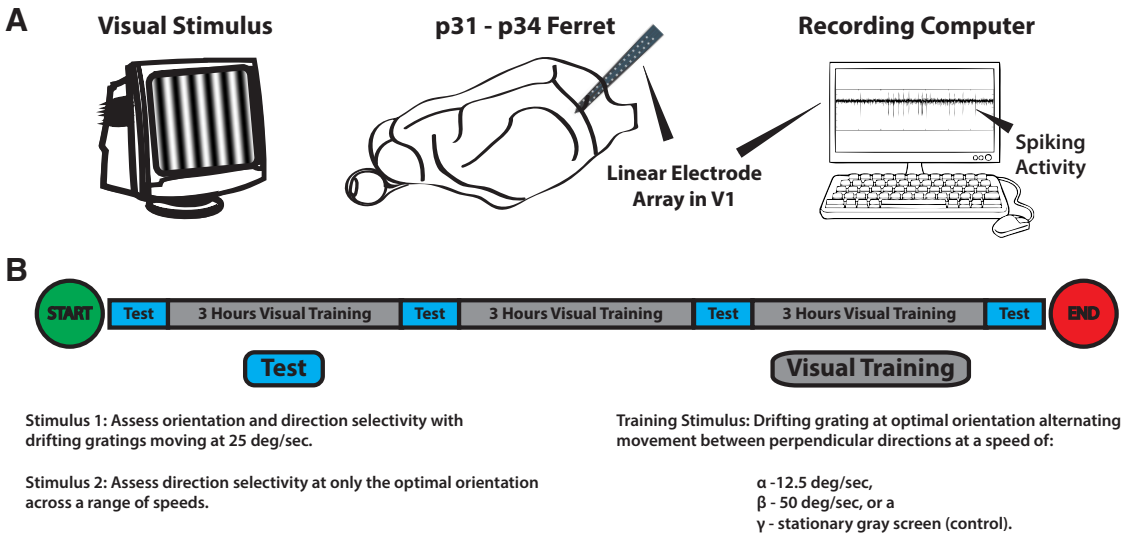

Figure 2. Experiment design. ***A***, Electrophysiology setup. A 32-channel linear electrode array (NeuroNexus, A1x32-Poly2) is inserted into V1 of an anesthetized juvenile ferret (p31–p34). A receptive field center is found manually for placement of the visual stimulus monitor. Raw voltage data are recorded, and spike times are extracted for analysis. ***B***, Time course of experiment. Experiment starts with a test phase to identify an optimal stimulus orientation at 25 deg/s (Stimulus 1) and response across a range of speeds to a bar moving bidirectionally in the two directions perpendicular to the optimal orientation (Stimulus 2). The test phase alternates for the duration of the experiment with one of three different 3 h visual training conditions: α, sinusoidal grating at optimal orientation moving bidirectionally at 12.5 deg/s; β, sinusoidal grating at optimal orientation moving bidirectionally at 50 deg/s; or γ, static gray screen control.

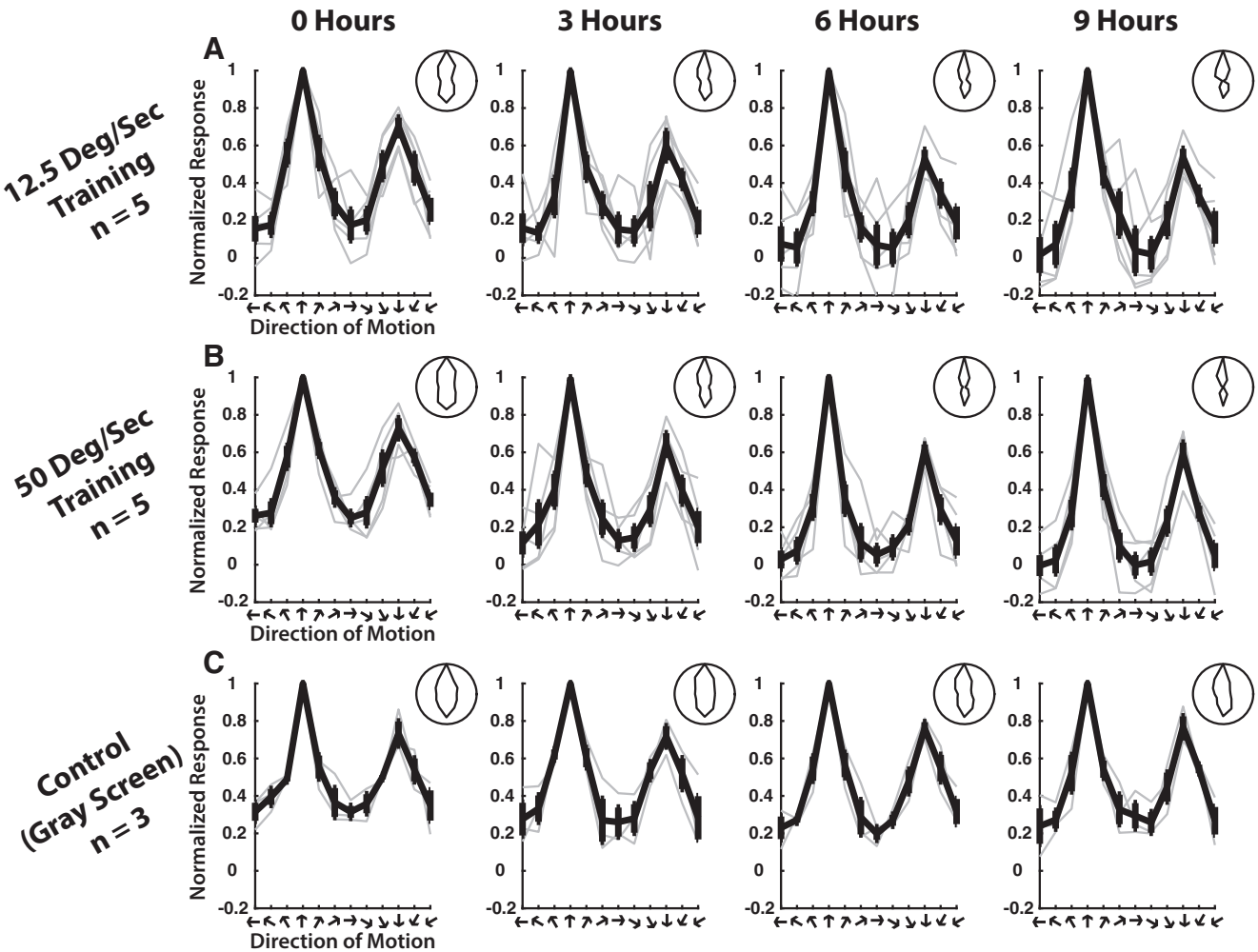

Figure 3. Cortical cells respond best to visual stimuli moving at 25 deg/s at a particular orientation and direction of motion. Average responses (by animal) of V1 sites to Stimulus 1 (Fig. 2*B*) before and after 3, 6, and 9 h of training with one of the three training stimuli: (***A***) 12.5 deg/s, (***B***), 50 deg/s, and (***C***) control. Responses are normalized and aligned such that upward motion has the largest response. Black line indicates condition average. Gray lines indicate individual animal averages. Insets, Condition average polar plots with up being 90 degrees. Error bars indicate SEM across animals.

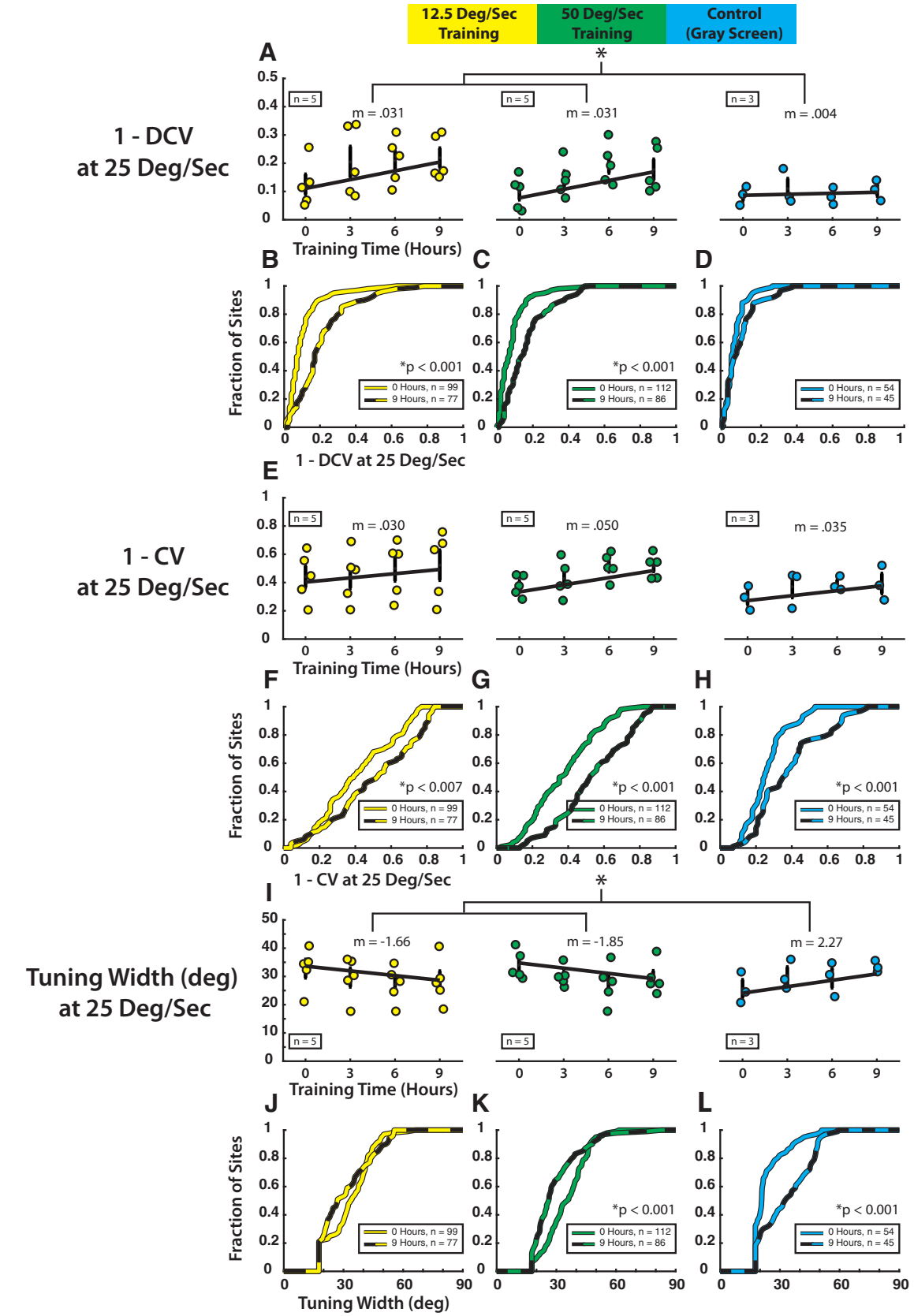

Figure 4. Cortical cells exhibit rapid increases in direction selectivity measured at 25 deg/s with either 12.5 or 50 deg/s training. ***A***, ***E***, Color indicates training condition: yellow represents 12.5 deg/s; green represents 50 deg/s; blue represents control. ***A***, Average responses (by animal) of 1 − DCV at 25 deg/s for each of the three training conditions. Black line indicates estimate of rate of change in 1 − DCV over time and is reported as *m* in units of Δ1 − DCV/h. *$p < .05$ (Bootstrap test). ***B–D***, Cumulative histograms of 1 − DCV for all sites for each of the three training conditions. V1 sites in animals that received training exhibited a progressive increase in direction selectivity, whereas V1 sites in control animals did not. ***E***, Animal averages of 1 − CV for each of the three training conditions. Black line indicates estimate of rate of change in 1 − CV over time and is reported as *m* in units of Δ1 − CV/h. ***F–H***, Cumulative histograms of (*Figure legend continues.*)

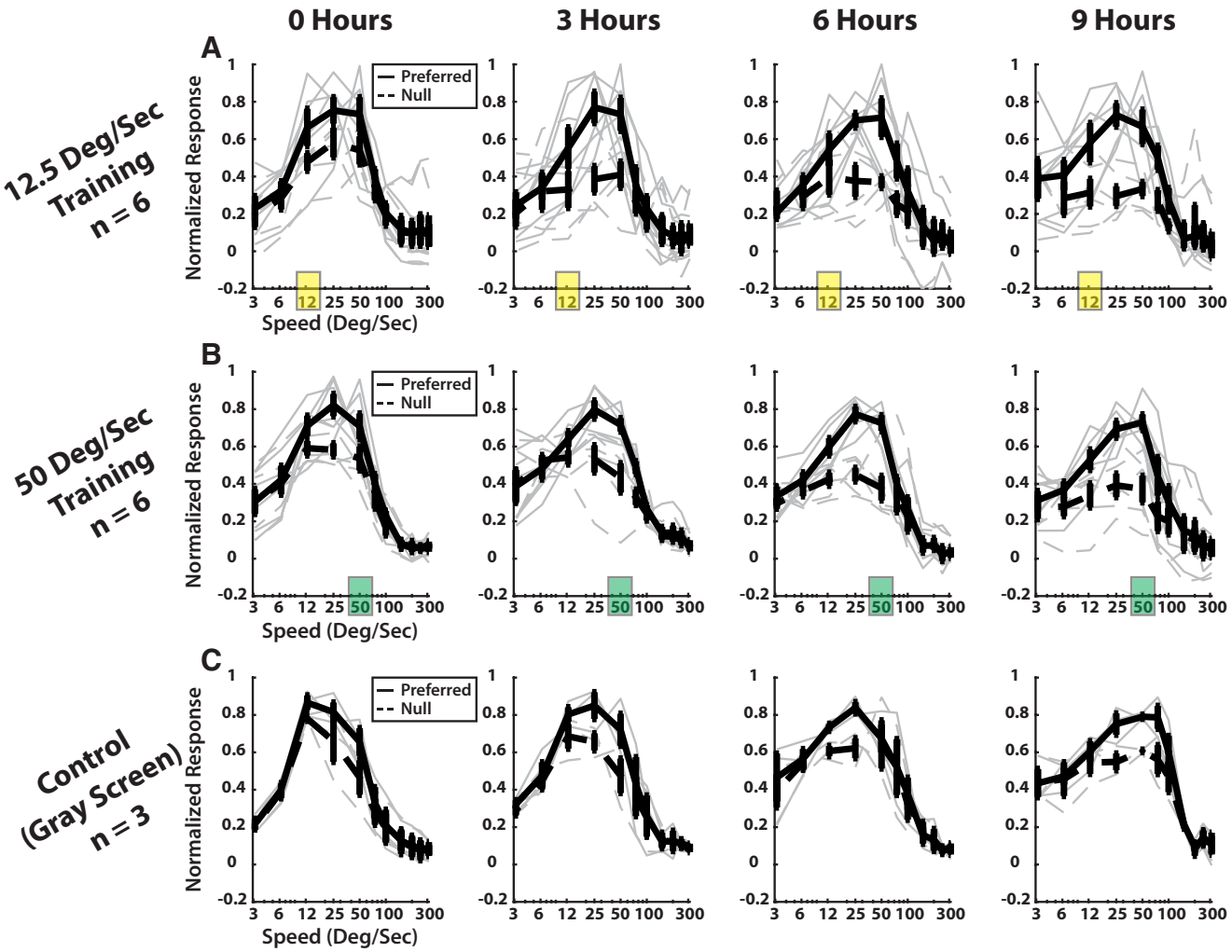

Figure 5. Cortical cells respond to optimally oriented visual stimuli moving across a range of speeds. Average responses (by animal) of V1 sites to Stimulus 2 (Fig. 2*B*) before and after 3, 6, and 9 h of training with one of the three training stimuli (***A***) 12.5 deg/s, (***B***), 50 deg/s, and (***C***) control. Responses are normalized. Solid black line indicates condition average response in the preferred direction. Solid gray lines indicate individual animal averages. Dashed black line indicates condition average response in the null direction. Dashed gray lines indicate individual animal averages. Error bars indicate SEM.

al., 2014). Tuning width was initially similar across all three conditions (Fig. 4*I*; Kruskal–Wallis test, $H(2) = 2.84$, $p = 0.237$) with a mean value of 31.6 ± 1.8 degrees. Training with both 12.5 and 50 deg/s drifting gratings had a significantly greater rate of decrease in animal average tuning width compared with control (Fig. 4*I*; bootstrap test; see Materials and Methods). Individual site values of tuning width for each condition are shown in Figure 4*J–L*. Changes in median tuning width were different between all conditions: with 12.5 deg/s training there was no change (Fig. 4*J*; Kruskal–Wallis test, $H(2) = 1.6$, $p = 0.207$), with 50 deg/s training there was a decrease (Fig. 4*K*; Kruskal–Wallis test, $H(2) = 16.35$, $p < 0.001$), and in control there was an increase (Fig. 4*K*; Kruskal–Wallis test, $H(2) = 12.69$, $p < 0.001$).

In summary, training with both 12.5 and 50 deg/s drifting gratings produced a greater rate of increase in direction selectivity and a greater rate of decrease in tuning width at 25 deg/s compared with control. This indicates that the two motion training conditions did not differently influence direction selectivity at the intermediate speed of 25 deg/s. Orientation selectivity increased in all conditions, including control. Tuning width increased in control but either did not change, or decreased, with 12.5 or 50 deg/s training.

←

(*Figure legend continued.*) 1 − CV for all sites for each of the three training conditions. (***I***) Animal averages of tuning width for each of the three training conditions. Black line represents estimate of rate of change of tuning width over time and is reported as m in units of degrees/hour. (***J–L***) Cumulative histograms of tuning width for all sites for each of the three training conditions.

DSI across a range of speeds in juvenile ferrets

The direction tuning results told us how responses of V1 neurons changed with 12.5 and 50 deg/s training at the intermediate speed of 25 deg/s, but we wanted to know how responses changed across a range of speeds. If the speed that an animal experienced influenced the speed tuning of V1 neurons, then we would predict that animals exposed to slow speeds would acquire selectivity for slow speeds, and animals exposed to fast speeds would acquire selectivity for fast speeds. To address this hypothesis, speed tuning curves were also measured (Fig. 2*B*; Test Stimulus 2) before training (0 Hours), and then again after 3, 6, and 9 h of training for each of the three training conditions (Fig. 5). Responses were normalized, and a preferred direction was defined as the direction that had the greatest summed response to 12.5, 25, and 50 deg/s drifting grating stimulation. The opposite direction was defined as Null. These curves were used to calculate measures of speed tuning and direction selectivity across a range of speeds.

A DSI was calculated for responses to 12.5, 25, and 50 deg/s drifting gratings (Fig. 6). Here, DSI is defined as follows:

$$DSI = (PR - NR)/PR$$

where *PR* is the blank subtracted response in Hz to the preferred direction, and *NR* is the blank subtracted response in Hz to the null direction. We could not use 1 − DCV, the circular measure of direction selectivity used in Figure 4, as only responses to the preferred and null directions of motion were collected across a range of speeds. A rate of increase in DSI over time was calculated for each training condition (bootstrap test; see Materials and Methods).

We found no systemic influence of the speed of the training stimulus on direction selectivity. Neurons trained with either slow or fast gratings did not exhibit increases in direction selectivity as assessed at 12.5 deg/s compared with control (Fig. 6*A*). Neurons trained with either slow or fast gratings exhibited equal increases in direction selectivity assessed at 25 deg/s or 50 deg/s compared with control (Fig. 6*B*,*C*). We did not observe enhanced selectivity at 12.5 deg/s in animals that experienced gratings at 12.5 deg/s, nor did we observe enhanced selectivity at 50 deg/s in animals that experienced gratings at 50 deg/s.

We also examined whether the speed of the visual training stimulus altered the speed preference of neurons. One might imagine that neurons in animals that received experience with slow gratings might exhibit preferences for slower speeds, and that neurons in animals that received experience with fast gratings might prefer higher speeds. This was not the case. We found no change in preferred speed with 12.5 deg/s training (Fig. 6*D*; Kruskal–Wallis test, $H(2) = 0.55$, $p = 0.459$) or 50 deg/s training (Fig. 6*D*; Kruskal–Wallis test, $H(2) = 0.01$, $p = 0.935$). There was a small increase in preferred speed in the preferred direction in the control (Fig. 6*D*; Kruskal–Wallis test, $H(2) = 3.97$, $p = 0.046$). Similarly, we found no change in preferred speed with 12.5 deg/s training (Fig. 6*E*; Kruskal–Wallis test, $H(2) = 0.55$, $p = 0.459$), 50 deg/s training (Fig. 6*E*; Kruskal–Wallis test, $H(2) = 0.01$, $p = 0.935$), or control (Fig. 6*E*; Kruskal–Wallis test, $H(2) = 3.33$, $p = 0.068$).

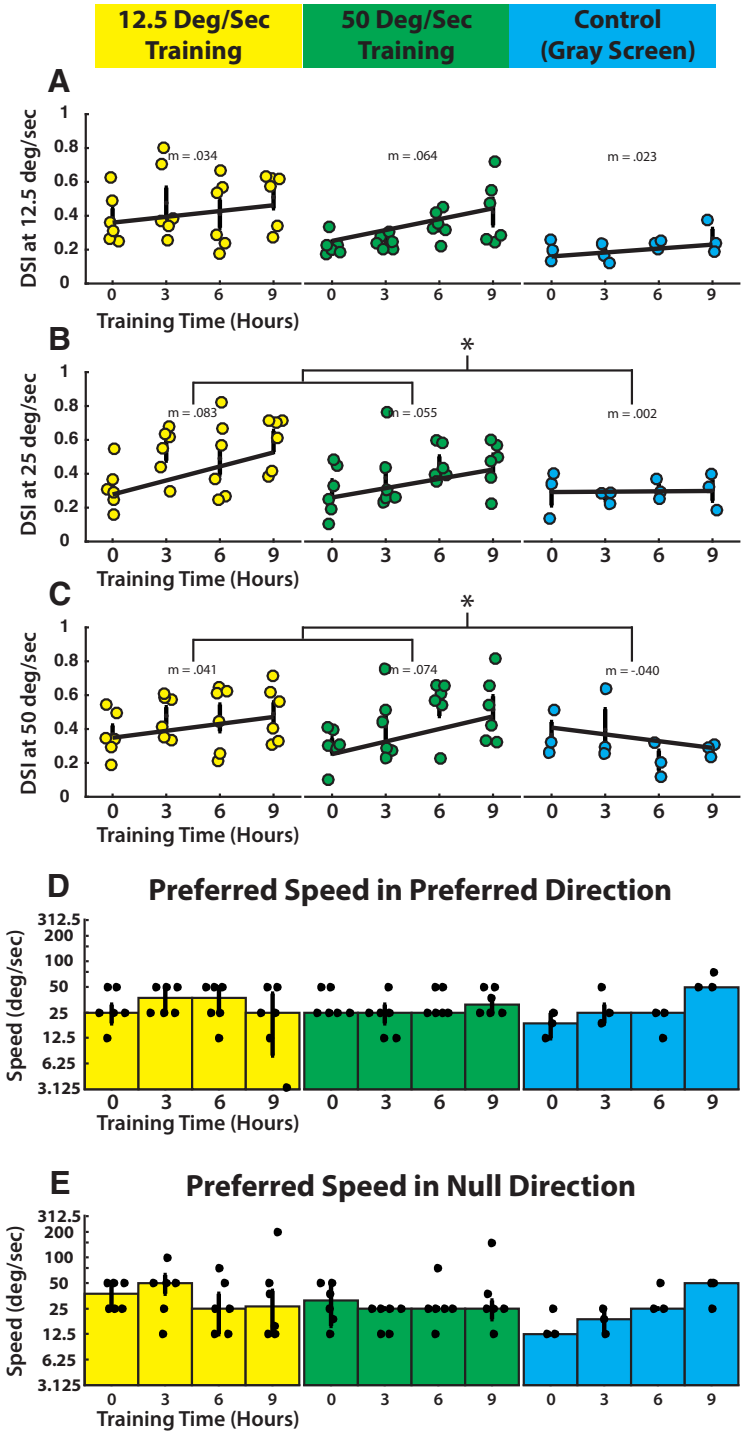

Figure 6. Cortical cells exhibit rapid changes in direction selectivity across a consistent range of speeds with either 12.5 or 50 deg/s training. ***A–C***, Condition average DSI values at each time point at three different test speeds: (***A***) 12.5 deg/s, (***B***) 25 deg/s, and (***C***) 50 deg/s. Error bars indicate SEM. Color indicates training condition: yellow represents 12.5 deg/s; green represents 50 deg/s; blue represents a static gray screen (control). Black line indicates estimate of average change in DSI over time and is reported as *m* in units of ΔDSI/h. $*p < .05$ (Bootstrap test). On average, cortical neurons acquired speed tuning preference for 25 and 50 deg/s regardless of the speed of the training stimulus. ***D***, ***E***, Condition median speed tuning, or speed that elicited the maximum response, in the preferred (***D***) and null (***E***) directions. Error bars indicate median average deviation. Training had no influence on speed tuning in either the preferred or null direction.

In summary, training with either 12.5 or 50 deg/s drifting gratings similarly resulted in increases in direction selectivity assessed at 25 or 50 deg/s, but not at 12.5 deg/s, compared with control. The speed of the training stimulus had no influence on preferred speed.

Cortex remained responsive throughout training

Systemic changes in activity levels in cortex could influence direction selectivity or other receptive field properties measured here. These changes could result from training or be the result of failing animal health or prolonged anesthesia. To monitor this

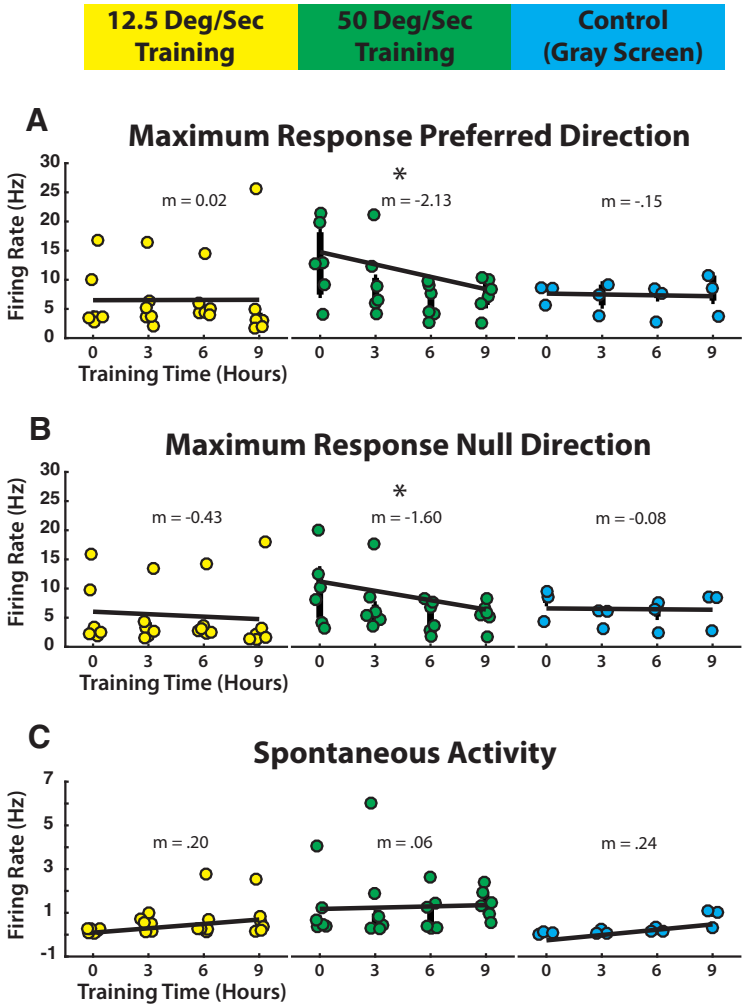

Figure 7. Maximum stimulus-evoked response in the preferred and null directions, and spontaneous activity for each of the three training conditions over time. ***A***, ***B***, Maximum firing rate in the preferred (***A***) and null (***B***) directions. ***C***, Spontaneous activity. Error bars indicate at condition median value, and error bars indicate median absolute deviation. Points are individual animal median values. Black line indicates estimate of average change in firing rate over time and is reported as *m* in units of ΔHz/h. $^*p < .05$ (Bootstrap test). Rate of change in response to the preferred and null direction was similar within training conditions. Training at 50 deg/s had a significantly different rate of change in firing rate (a decrease) in both the preferred and null directions compared with 12.5 deg/s training and control. There was no difference in rate of change of spontaneous activity between training conditions.

possibility, we looked for changes in maximum firing rate in the preferred and null directions and spontaneous activity over the duration of the experiment.

We observed only small changes in responses over the course of the experiment. Response rates to the preferred and null stimuli showed a modest but significant drop for animals that received 50 deg/s training (bootstrap test; see Materials and Methods) but did not change significantly for animals that received 12.5 deg/s training or for control animals (Fig. 7*A*,*B*). Spontaneous firing rates did not change across any of the conditions.

In summary, we found that cortex remains active for the duration of the experiment with little or no change in maximum response to the preferred or null direction, or in spontaneous activity. This suggests that cortex remained healthy for the duration of the experiment.

Discussion

Here we assessed the responses of neurons in V1 to stimuli presented at a range of speeds in naive ferrets that were trained with drifting gratings moving at either 12.5 or 50 deg/s. We found that training with visual stimuli moving at either speed had no influence on speed tuning and had a similar rate of increase in direction selectivity across a range of speeds compared with control. This suggests that the developing visual system is not using specific parameters of experienced stimuli (in this case, speed) to construct a direction-selective circuit as has been suggested in some models. These results are more consistent with a permissive, rather than instructive, role for experience in the development of direction selectivity in V1 neurons.

Role of experience: permissive versus instructive

It is unclear what role visual experience plays in the development of direction-selective neurons in V1. Deprivation experiments have shown that experience is necessary (Zhou et al., 1995; Li et al., 2006), but not all forms of experience are sufficient. Kittens reared under stroboscopic illumination (Cynader and Chernenko, 1976) and naive ferrets exposed to flashing grating stimuli (Li et al., 2008) do not develop direction selectivity in V1. These results could be consistent with a requirement for space-time correlations in neural activity that are produced by moving stimuli, and that visual experience might be playing an instructive role in the development of direction selectivity.

Many models have explored how visual experience through correlation-based plasticity mechanisms can drive formation of a precise arrangement of connections from nonselective inputs that result in a direction-selective cell (Buchs and Senn, 2002; Wenisch et al., 2005; Van Hooser et al., 2014). In these models, the cell that becomes direction-selective has initial access to a pool of inputs representing different positions in space with different latencies (Fig. 1*A*; Possible Juvenile State I). The role of visual experience is to instruct the selection of inputs that result in direction selectivity.

If experience is instructive, that is, if the developing brain is using information present in visual stimuli to guide formation of neural circuits, then we would expect the parameters of visual experience to influence the final state of the circuit. Alternatively, if experience is playing a permissive role in the development of direction selectivity, we would expect the final state of the circuit to be the same and be formed independent of the parameters of visual experience.

We tested this hypothesis here by looking to see whether the speed of visual stimulation that an animal experiences influences the resulting tuning of direction selectivity (Fig. 1). We first found that training with either 12.5 or 50 deg/s drifting gratings results in similar changes in orientation and direction tuning preferences at the intermediate speed of 25 deg/s (Fig. 4). We also found that training with either 12.5 or 50 deg/s drifting gratings resulted in similar direction tuning across a range of speeds (Fig.

6). The speed of the training stimulus did not influence resulting the resulting speed preferences.

These results clarify and extend the interpretation of a recent series of experiments that are all consistent with a permissive role of visual experience in the development of direction selectivity. Li et al. (2008) showed that, in naive cortex, cells exhibited very weak direction selectivity but exhibited small but significant spatial clustering according to their (weak) preferred directions. These small initial biases were predictive of the eventual direction preference acquired after experience (Li et al., 2008), suggesting that initial conditions, derived independently of experience, determined direction preference. Further, Roy et al. (2016) found that unpatterned direct optogenetic stimulation of naive visual cortex was sufficient to produce an increase in direction selectivity. These results are also consistent with the idea that sufficient information is already present in the naive cortex to determine eventual direction preference. Finally, Van Hooser et al. (2012) provided naive animals with motion training in a single direction (unidirectional training), and found that the eventual direction preference that was acquired depended upon a cell's position within the emerging direction map. Cells that were surrounded by cells that were slightly biased toward the trained direction exhibited robust increases in selectivity for the trained direction, and cells that were in regions that were biased toward the opposite direction exhibited no average increase in selectivity. Thus, cells in regions that were biased toward the opposite direction were not converted to the stimulated direction, suggesting that experience cannot overwrite strong initial biases. It should be noted that cells in intermediate regions were converted to prefer the trained direction more than would be expected by chance, indicating that, at the margins, there is some evidence for instructive processes, but a majority of the parameters of spatiotemporal selectivity (direction selectivity and speed tuning) appear to be determined at the onset of experience, only requiring experience for its expression.

Species differences

Visual deprivation (by dark rearing, binocular lid suture, or bilateral congenital cataracts) has a profound impact on the development of direction selectivity in ferrets and cats (Pasternak et al., 1981; White et al., 2001; Li et al., 2006) and on visual motion perception in humans (Ellemberg et al., 2002). These results may not extend to all species. In particular, mice (Sun et al., 2002; Weng et al., 2005) and rabbits (Barlow et al., 1964; Swadlow and Weyand, 1985), unlike carnivores (Cleland and Levick, 1974) and primates (De Monasterio and Gouras, 1975), exhibit relatively high percentages of direction-selective retinal ganglion cells that project to the LGN relay cells (Cruz-Martín et al., 2014), and there is evidence that direction selectivity is present in dark-reared mice that lack any visual experience (Rochefort et al., 2011). Therefore, the influence of experience on direction selectivity as speed tuning may differ between rodents and lagamorphs and carnivores and primates.

Speed tuning of V1 neurons

The origins of speed tuning in the visual cortex remain unclear. Because direction selectivity and speed tuning are examples of spatiotemporal selectivity, one could have imagined that speed tuning would be adjustable by experience over the same timescale as is direction selectivity. Direction selectivity can be rapidly induced in naive animals by 6 h of visual experience (Li et al., 2008; Van Hooser et al., 2012), although here we have shown that speed tuning remains fixed, regardless of the speed of the stimulus that was provided to the animal.

These experiments do not allow us to infer what might have occurred if we had provided experience with slow or fast stimuli over days or weeks. A few hours of experience (3–6 h) is sufficient to cause a substantial increase in direction selectivity (Li et al., 2008; Van Hooser et al., 2012), so the only conclusion we can draw here is that the mechanisms that are producing enhanced direction selectivity over this time frame do not also impact speed tuning.

Fixed speed tuning of V1 neurons: mechanisms

The fact that speed is not influenced by experience suggests that there is some fixed process (or processes) that produces speed tuning in cortical neurons. Center-surround retinal ganglion cells exhibit very broad selectivity to stimulus speed/stimulus temporal frequency, whereas LGN neurons exhibit slightly filtered tuning (Frishman et al., 1983). Cortical neurons exhibit substantially filtered speed/temporal frequency preferences compared with LGN neurons, with cortical neurons exhibiting weaker responses to high temporal frequencies compared with LGN neurons (Hawken et al., 1996; Heimel et al., 2005; Van Hooser et al., 2013). Speed tuning often differs in the two opposite directions (Moore et al., 2005), so it is possible that the mechanisms that mediate speed tuning in the preferred and null directions may differ. The results presented here suggest that whatever process or processes are causing the temporal filtering that was observed, they do not depend on the animal's experience.

These processes could include a predetermined set of positions and delays that can provide input to the cortex (Fig. 1*A*), the filtering of membrane time constant of neurons (Carandini et al., 2007), synaptic depression at thalamocortical or corticocortical synapses (Chance et al., 1998; Priebe and Ferster, 2012), or fixed delays or amplification of propagation within the cortical circuit, such as the delay between feedforward excitation and feedforward inhibition or feedforward processing and feedback processing (Shon et al., 2004; Wenisch et al., 2005; Honda et al., 2011). It is possible that prolonged experience with slow or fast speeds could alter these mechanisms, but we found no evidence for changes over the timescale studied here, which, by contrast, produces strong changes in direction selectivity.

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