Title: An early phase of instructive plasticity in visual cortex before the typical onset of sensory experience

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Abstract

While early experience with moving stimuli is necessary for the development of direction selectivity in visual cortex of carnivores, it is unclear whether experience exerts a permissive or instructive influence. To test if the specific parameters of the experienced stimuli could instructively sculpt the emergent responses, visually naive ferrets were exposed to several hours of experience with unusual spatiotemporal patterns. In the most immature ferrets, cortical neurons developed selectivity to these patterns, indicating an instructive influence. In animals that were 1-7 days more mature, exposure to the same patterns led to a developmentally-typical increase in direction selectivity. We conclude that visual development progresses via an early phase of instructive plasticity, when the specific patterns of neural activity shapes the specific parameters of the emerging response properties, followed by a late phase of permissive maturation, when sensory-driven activity merely serves to enhance the response properties already seeded in cortical circuits.

Introduction

In the developing visual system, molecular cues[1,2] and early spontaneous activity[3-5] lay down the foundation of initial circuitry that exhibits many of the properties that are found in the mature animal, including retinotopic organization and orientation selectivity[6-10]. During a subsequent phase of experience-dependent development, visually-driven activity further shapes these response properties, providing enhanced cortical acuity[11], binocular matching of inputs from the 2 eyes[12], and, in carnivores and primates, the emergence of direction-of-motion selectivity[13,14]. It is of particular interest to understand how early visual activity interacts with, and alters, the immature circuit. Do the circuit connections established before the onset of experience commit cortex to a developmental path with pre-destined response properties, such that subsequent sensory experience merely permits maturation of these pre-seeded properties? Or is the cortical circuit malleable enough so that the particular patterns of visually-driven activity can instructively sculpt the responses according to the quality of the specific stimuli experienced?

Direction selectivity – a preference for stimulus movement in 1 direction as opposed to all others – typically develops in ferret visual cortex over a period of 1-2 weeks after eye opening through a process that requires visual experience[13,15,16], and does not form in dark-reared[13] or strobe-reared[17-20] animals. Direction selectivity can also be rapidly induced in the laboratory by providing an anesthetized ferret kit with 3-9 hours of experience with drifting gratings[15,16,21,22]. While exposure to such smooth spatiotemporal motion increases direction selectivity, many parameters of direction tuning are invariant to the specific parameters of the gratings used for visual stimulation. For example, orientation selectivity is barely malleable during motion exposure: only columns whose orientation preference match the provided stimulus exhibit increases in direction selectivity[15], and the orientation preferences of neurons that initially prefer other orientations are changed only very slightly[15]. Direction angle preference is also relatively unchangeable: stimulation with gratings that move in only one direction cause a dramatic increase in direction selectivity for cells whose initial biases match the stimulated direction, but do not cause an increase in selectivity for cells whose initial biases match the opposite direction[21]. Speed / temporal frequency tuning is also invariant: stimulation with either slow or fast moving

stimuli causes an increase in direction selectivity, but does not alter tuning for speed / temporal frequency[22]. These results suggest that experience with drifting gratings fails to modify many of the parameters of direction tuning (orientation/direction preference angle, speed/ temporal frequency etc.), thereby implying that visual experience is only necessary to permissively increase selectivity and acuity of the tuning.

While the above results suggest a limit to the extent to which the experienced stimulus can shape cortical tuning properties, no experiment to date has directly tested if the nascent visual cortex can be induced to develop selective responses to irregular spatiotemporal patterns, which would be a strong test of whether selectivity is instructed by activity. In all visual motion stimulation experiments to date, young ferrets were exposed to smoothly moving gratings, in which an oriented grating is moved along a smoothly progressing sequence of spatial phases in time. According to the spatiotemporal receptive fields of neurons in the typically-developed visual cortex, such stimuli are ideally suited for driving cortical neurons[23-25]. In addition, the vast majority of ferret kits examined in prior studies already had visual experience for 1-3 days at the time of each experiment, making it difficult to rigorously assess if activity before or around the time of natural eye opening cortex could instructively modify the cortex.

To address these issues, we directly manipulated early visual experience by prematurely opening the eyes of young ferrets and exposing them to grating stimuli that were animated with scrambled spatiotemporal phase sequences. We reasoned that if the patterns of early activity in visual circuits were instructive, then we should be able to induce increased responses to these phase-scrambled grating stimuli through repeated visual exposure. On the other hand, if the cortical circuitry were already committed to developing selectivity for smooth motion, then providing phase-scrambled stimulation should merely increase direction selectivity.

We found evidence for a transition of the influence of early activity in the visual cortex – from instructive to permissive – that occurred around the time of natural eye opening. When the eyes were opened prematurely, or if the state of the cortex was very immature as assessed by levels of orientation selectivity, animals developed increased selectivity to the artificial phase-scrambled stimulus that was experienced.

Animals that were slightly more mature did not acquire increased selectivity to the phase-scrambled patterns but instead exhibited a developmentally-typical increase in direction selectivity, consistent with a permissive influence of visual stimulation. These data provide evidence that the early activity in visual cortex that occurs before and at eye opening – which includes spontaneous activity[3-5], low resolution visual stimulation through the closed lids[26,27], and higher resolution vision through the slowly opening eyes – provides an instructive signal for neural circuit construction. Later activity, after the normal onset of visual experience, is necessary for the maturation of direction selectivity, but only in a permissive manner.

Results

Neurons in carnivore primary visual cortex respond strongly to oriented gratings moving in one direction following a smoothly progressing sequence of spatiotemporal phases. We wanted to test if early exposure to gratings moving with irregular spatiotemporal patterns could modify the cortical circuitry and induce neurons to respond selectively to irregular motion. For this purpose, we designed a stimulus family of gratings moving with scrambled spatiotemporal phase sequences. To create such phase-scrambled visual stimuli, we varied the typical oriented grating stimuli that drive the cortex well. We discretized grating phase into 8 steps (**Figure 1**), defined forward and backward stimuli as phase sequences [1 2 3 4 5 6 7 8] and [8 7 6 5 4 3 2 1], respectively, and approximated a viewing temporal frequency of 2 Hz by showing each phase for 1/(8*2Hz) = 0.0625 s. We selected a family of 10 stimuli that ranged across this spatiotemporal space that we labeled *S1*…*S10*: forward motion (*S1*), backward motion (*S2*), 6 sequences that exhibited varying degrees of correlation with forward and backward motion (S3-S8), and counterphase stimuli at 2 spatial phases labeled *S9* and *S10*, respectively (**Figure 1abcd; Supplementary Videos 1-10**). Stimuli *S3*-*S8* contain spatiotemporal energy at multiple spatial and temporal frequencies (**Supplementary Figure S1**), while stimuli *S1*, *S2*, *S9*, and *S10* contain energy around a single spatial and temporal frequency.

We developed 2 selectivity measures to quantify neural responses to this stimulus family, which are 10-dimensional. The first measure, called the Selectivity Index (*SI*) for stimulus *n*, is equal to the response of the neuron to that stimulus divided by the sum of the responses to stimuli *S1*…*S10*. We also developed a second

measure that considered the fact that *S1…S10* are correlated with one another that we termed the Response Projection Index (*RPI*). We can imagine linear receptive field kernels (*KSn*) that would give a maximal response to each stimulus (*Sn*), as shown in **Figure 1e**, and we can compute the responses of these kernels to each of the 10 stimuli. The RPI describes how close the response of a measured neuron, in 10-dimensional response space, is to the responses that would be expected from an ideal kernel (*KSi*) relative to another ideal kernel (*KSj*) (**Figure 1f**). A neuron that gives responses identical to *KS1* would have an *RPI(KS1 vs KS2)* value of -1, whereas a neuron that gives responses that are identical to *KS2* would have an *RPI(KS1 vs KS2)* value of +1. Stimulus *S8* is uncorrelated with *S1* and *S2*, and *KS8* has an *RPI(KS1 vs KS2)* value of 0 (**Figure 1f**).

We prepared young ferrets for 2-photon imaging of virally-expressed GCaMP6-s in visual cortex[28]. We began each experiment by measuring responses to drifting gratings in order to assess the initial orientation and direction tuning. A well-represented orientation preference was selected to be the orientation angle for stimuli *S1…S10*, and responses to these stimuli were measured. Next, stimulus *S6* or *S8* (stimuli that were poorly correlated with *S1* and *S2*) was selected for 6-9 hours of prolonged visual stimulation[15,21,22]. Finally, responses to stimuli *S1…S10* and traditional orientation and direction tuning were re-assessed using sinusoidal gratings.

Example responses from a ferret whose eyes were opened prematurely are plotted in **Figure 2**. After 6 hours of exposure to stimulus *S6*, there is a clear enhancement of the response of the imaging field to stimulus *S6* **(Figure 2abdefgh)**. To characterize the degree to which neural responses were similar to that of a neuron that is perfectly selective to the trained stimulus *S6*, we computed the RPI for stimulus *S1* vs *S2* and *S1* vs *S6*. There is a clear upward shift in *RPI S1 vs. S6*, indicating that neural responses are more selective for stimulus *S6* after exposure than before (**Figure 2dh**). Despite the fact that the ferret received stimulation with the relatively broadband motion stimulus *S6*, traditional direction selectivity index values for this animal exhibited a decrease (**Figure 2cij**), which is opposite to what we would have expected for animals that have naturally opened their eyes[15,21,22].

Responses in slightly more mature ferrets resembled the example in **Figure 3**. After 6 hours of exposure to stimulus *S6*, there was no increase in selectivity to stimulus *S6* **(Figure 3abdefgh)**. Instead, this animal exhibited an increase in direction selectivity index values **(Figure 3cij)**, as would be expected for a permissive role of visual experience for the development of direction selectivity.

Individual ferrets mature at different rates (**Figure 4ab**), and we examined which factors determined whether a ferret showed increased selectivity to the phase-scrambled training stimulus or showed increased direction selectivity. Animals whose eyes were opened prematurely were highly likely (4/4) to exhibit increased selectivity to the phase-scrambled stimulus (**Figure 4cd**), but the best hallmark of immaturity that predicted increased selectivity to the phase-scrambled stimulus was the animal's initial orientation selectivity index (OSI) value (**Figure 4e**). While orientation selectivity is evident at the time of eye opening, OSI values are relatively small in naïve animals and increase substantially over the first 1-2 weeks of visual experience[13,15]. Animals with weak initial OSI values showed large increases in selectivity for the phase-scrambled stimulus and lacked increases in direction selectivity, while animals with stronger initial OSI values (>0.3) generally lacked increases in selectivity for the phase-scrambled stimulus (7/8) and instead exhibited robust increases in direction selectivity (7/8) (**Figure 4ef**). Primary data for all animals in the study are shown in **Supplementary Figure 2**.

While these data showed that the least mature animals acquired receptive fields that were more correlated with the phase-scrambled training stimulus, it remained possible that we were merely pushing the brain circuitry into an unnatural configuration that, while producing increased responses to the phase-scrambled training stimulus, was simply another allowable developmental configuration but not one that was truly instructed by the training stimulus. To understand how responses were altered relative to the full stimulus family, we projected the 10-d responses of these animals onto a reduced 2-d representation using principal component analysis (**Figure 4g**). In each case where we observed a significant training effect (full 95% range >0 in **Figure 4ce**), the mean responses of these animals moved closer to the training stimulus. Further, we performed a pairwise examination of the change in RPI for each stimulus compared to the training stimulus in these animals (**Figure 4h**). For most stimuli (*S1*,*S2*,*S3*,*S5*,*S6*,*S9*,*S10*), the actual responses moved significantly

closer to those of a hypothetical neuron that would respond optimally to the training stimulus. For other stimuli (*S4*, *S7*, *S8* when it was not the training stimulus) that were located near to the training stimuli (*S6*, *S8*) in 10-dimensional space (**Figure 4g**), the average tuning moved about equally close to hypothetical optimal responses for the reference stimulus and the training stimulus. In total, these results indicated that the neural responses were becoming more like those of hypothetical neurons optimally tuned to the training stimulus, as expected for an instructive influence.

These results show that the spatiotemporal tuning of neurons was modified by visual experience provided to the premature cortex. However, all response properties were not malleable, indicating that the influence of premature or very early experience has limits. Orientation preference, for example, was not altered by this experience (**Supplementary Figure 3**), suggesting that either some features of the circuit are fixed even at our earliest point of examination, or that longer stimulation would be required to alter these properties. Nevertheless, the spatiotemporal response profile of these cells was modified through experience with a stimulus that was specific to the individual animal in a manner that was not possible just a few days later.

Discussion

We have shown that ferret primary visual cortex can acquire selectivity to a phase-scrambled grating stimulus after several hours of exposure to this stimulus, but only when the cortex is very immature as assessed by its orientation selectivity. Animals for which we prematurely opened the eyes or that exhibited very low orientation selectivity index values acquired increased selectivity to the phase-scrambled stimulus that was experienced. By contrast, animals that exhibited stronger but still immature orientation selectivity index values responded to the visual stimulation by acquiring developmentally-typical increases in selectivity to direction-of-motion.

To our knowledge, this is the first time that cortical neurons have been induced to become selective to an irregular spatiotemporal stimulus through visual stimulation alone. In the disease amblyopia, a poor alignment of the 2 eyes or poor resolution in 1 of the 2 eyes causes a substantial drop in receptive field

acuity/resolution and poor matching of receptive field properties across the 2 eyes[11], which reflects degradation of receptive field structure but not the formation of a new, precise spatiotemporal receptive field. Other studies have imposed new receptive field structure, but have done so by pairing visual stimuli with external feedback control of visual[29] or somatosensory[30] cortex. The neurons in our study exhibited responses unlike those found in typically-developing animals in that they showed specific selectivity for an unnatural, phase-scrambled grating stimulus, which is a strong demonstration of instructive plasticity. This selectivity also differs from the interesting induction of sequence selectivity in visual cortex[31,32] in that the selectivity introduced here is to a stimulus that is compact in space and time, with a cycle frequency of 4 Hz. The ability of cortex to acquire such unusual selectivity suggests that the cortex is particularly malleable in the face of activity in this very early window.

While we have shown that visual activity in the window from a few days before eye opening to just after eye opening drives strong plasticity in spatiotemporal selectivity, it remains unclear how exactly this plasticity is used by the developing animal under typical developmental conditions. Spontaneous activity, which is necessary for the development of orientation selectivity[7], dominates in the week before eye opening[3,10,33], and it may be the case that the patterns of this spontaneous activity "instruct" the formation of the cortical circuitry that reflects visual tuning parameters such as selectivity to smoothly moving stimuli. This spontaneous activity is sufficient for formation of orientation selectivity and the initial biases for direction angle preference because both still form in dark-reared animals[13,21]. In addition, very early visual experience through the closed lids drives visual activity[26,27] and this activity, in addition to experience in the hours after eye opening, may instruct the development of smooth spatiotemporal receptive fields under typical conditions. Differences in the quality and patterning of activity that typically occurs in this early window may underlie species differences in functional architecture such as ocular dominance patches or receptive field parameters such as the fraction of cells that exhibit direction selectivity[34].

We conclude that the influence of neural activity on the formation of visual circuits exhibits a sharp transition from instructive to permissive that occurs around the onset of natural visual experience. This

conclusion builds on the prior knowledge that spontaneous activity before experience is necessary for proper development of visual circuits[3,7] by suggesting that the quality and patterning of early activity sculpts the circuitry that supports the parameters of tuning such as spatiotemporal selectivity that are later revealed when selectivity is amplified through experience. After this transition, the net influence of activity-dependent circuit mechanisms must be qualitatively different, because a variety of patterns of neural activity drive the formation of typical smooth direction selectivity, with tuning parameters that cannot be greatly altered.

This developmental transition also mirrors a physiological transition observed in rats and in preterm humans, where flashes of light given before the typical onset of natural visual experience produce prolonged bursts of cortical activity, but these prolonged bursts fade around the onset of natural visual experience (P12 in rats, 36 gestational weeks in humans)[35]. The circuit changes underlying this transition are still unclear, but changes in cortico-thalamic loops and cortical inhibition may contribute[35,36]. Emerging research suggests that preterm humans exhibit higher rates of poor acuity later in life[37] that cannot be explained by the acuity of the eyes[38]. The mechanisms underlying this poor acuity are not understood and may be varied. Brain damage could contribute[37]. But it is also possible that the premature cortex could be vulnerable to certain types of premature visual experience that could impact the formation of the initial brain circuitry. Future research on the influence of visual stimulation and neural activity on the premature brain may inform best practices for care of the earliest preterm infants.

Methods Summary

Ferrets were anesthetized with ketamine and isoflurane (2% for surgery, 0.08-2% during imaging). GCaMP6-s was introduced to the cortex with an AAV virus in a prior survival surgery. Changes in calcium fluorescence were monitored with a 2-photon microscope (Prairie Technologies) driven by a mode-locked laser (920nm, Mai Tai Deep See, Spectra-Physics). The training protocol consisted of 5s stimulation followed by 10s interstimulus interval; the protocol continued for 20min followed by 10min of no stimulation and this entire procedure was repeated for several hours. Stimulation and analysis were performed using custom software for Matlab (Mathworks). See **Supplementary Information** for details.

Figure legends

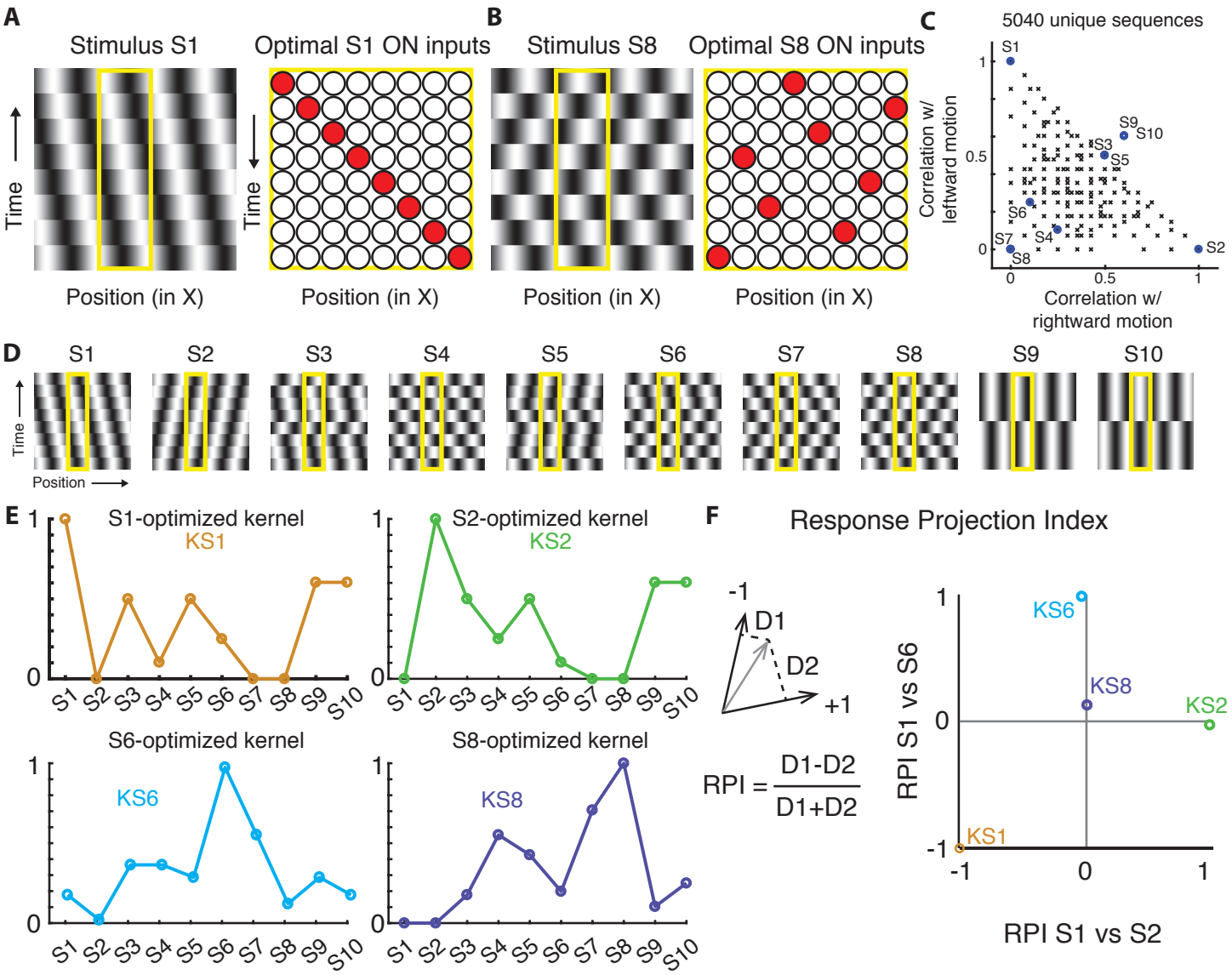

Figure 1. **Design of a spatiotemporal stimulus family. A)** Left: X-T (space-time) view of a vertical sinusoidal grating shifting to the left at each phase step, termed stimulus *S1*. Each strip represents a video frame. The phase progression of this grating is denoted as [1 2 3 4 5 6 7 8]. Yellow box indicates hypothetical location of a receptive field. Right: Hypothetical X-T inputs (ON only) that depicts positions and latencies of inputs that would drive an optimal response to stimulus S1. **B)** Left: X-T view of a vertical sinusoidal grating advancing with scrambled phase steps ([8 3 6 2 7 4 1 5]), termed stimulus *S8*. Right: Hypothetical X-T inputs (ON only) that depicts positions and latencies of inputs that would drive an optimal response to stimulus *S8*. **C)** Plot of the best-aligned correlation with leftward (*S1*) and rightward (*S2*) motion for all 5040 possible unique phase

sequences (black x) and our selections for stimuli named "*S#*". *S3*-*S8* are phase-scrambled stimuli that deviate substantially from left or right motion. *S9* and *S10* are counterphase stimuli at 2Hz (phase progression [1 1 1 1 5 5 5 5] and [3 3 3 3 7 7 7 7], respectively). **D)** Video frame strips of *S1*-*S10*. **E)** Responses of hypothetical cells with input kernels optimized for indicated stimuli. A cell optimized for *S1* (*KS1*, orange) responds strongly to *S1*, but not to *S2*, *S7*, or *S8*. A cell optimized for *S2* (*KS2*, green) responds strongly to *S2* but not *S1*, *S7*, or *S8*. Cells optimized for either *S6* (*KS6*, cyan) or *S8* (*KS8*, purple) respond weakly to *S1* and *S2* and relatively poorly to each other's optimal stimulus. **F)** Response Projection Index (RPI) indicates how well the tuning curve of a given cell matches that expected by a cell optimized for 2 particular stimuli. *Left*: Each cell's normalized response curve in 10-dimensional space. The response is compared to the responses expected from hypothetical reference neurons that are optimized for 2 given stimuli (such as *S1* and *S2*). The distance in vector space between the actual response (gray vector) and the vector line that defines the 2 reference neurons is calculated (D1 and D2), and an index is calculated RPI = (D1-D2)/(D1+D2). If the cell's responses match that expected by a hypothetical neuron that is optimized for the first reference stimulus, then RPI is -1. If the cell's responses match that expected by a hypothetical neuron that is optimized for the second stimulus, RPI is 1. *Right*: Scatter plot of RPI index values for kernels optimized for the particular stimuli indicated. X axis is RPI relative to S1 and S2 and Y axis is RPI relative to S1 and S6.

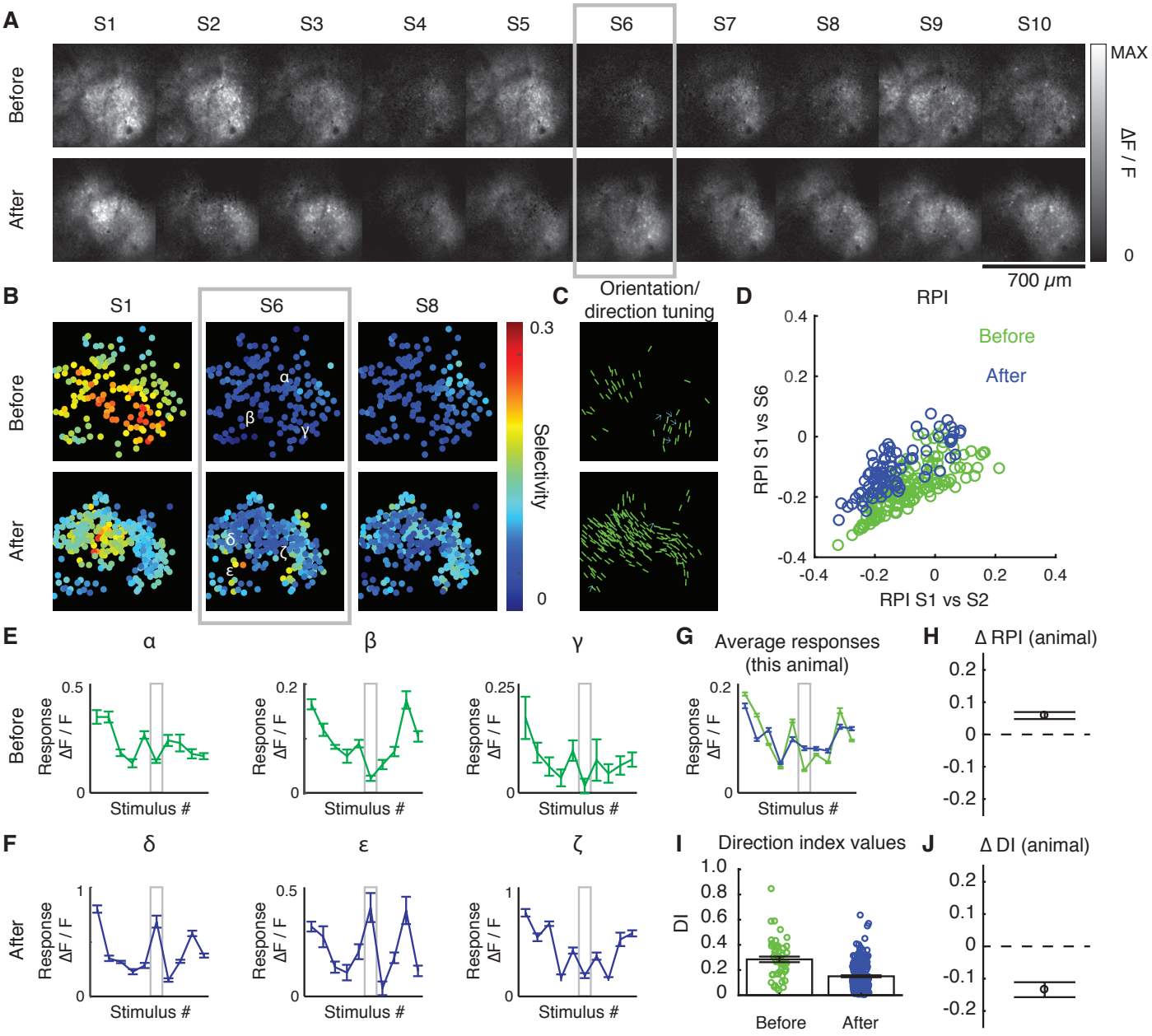

Figure 2. **In a visually naïve ferret, 6 hours of experience with a phase-scrambled grating pattern caused an increase in selectivity for that pattern. A)** Pixel map of GCaMP6-s responses to a family of spatiotemporal stimuli before and after 6 hours of training with pattern *S6*, indicating a substantial increase in selectivity for *S6*. The animal's eyes were opened prematurely on P30. **B)** Single cell Selectivity Index (SI) values for different stimuli (*S1* – a phase-regular, unscrambled direction stimulus, and *S6*/*S8* – phase-scrambled stimuli). Selectivity for smooth motion (*S1*) decreases, while selectivity for stimulus *S6* increases in many cells. **C)** Orientation and direction tuning in single cells before and after training. Green bars represent orientation-selective but not direction-selective cells (DI<0.5), blue dots indicate visually responsive but poorly tuned cells, and cyan arrows

indicate direction-selective cells. **D)** Response Projection Index (RPI) for *S1* vs *S2* (X axis) and *S1* vs *S6* (Y axis) for cells measured before (green) and after (blue) 6 hours of experience with *S6*. There is a substantial upward shift on the Y axis, indicating that cells exhibit responses that are more like a cell that is optimized to respond to *S6*. **E)** Responses to 3 example cells (indicated in B) before experience. **F)** Responses to 3 example cells (indicated in B) after experience. Cells δ and ε exhibit strong responses to stimulus *S6*. **G)** Grand average of responses before and after 6 hours exposure to *S6*. On average, there is an enhancement of the response to *S6*. **H)** Estimated difference in cell RPI (*S1* vs. *S6*) before and after experience (95% confidence intervals), indicating a significant increase in selectivity to stimulus *S6*. **I)** Direction index values of cells before and after exposure to *S6*. Direction index values decreased slightly after exposure to *S6*. **J)** Estimated difference in DI of cells before and after experience (95% confidence intervals), indicating a significant decrease in DI with *S6* experience.

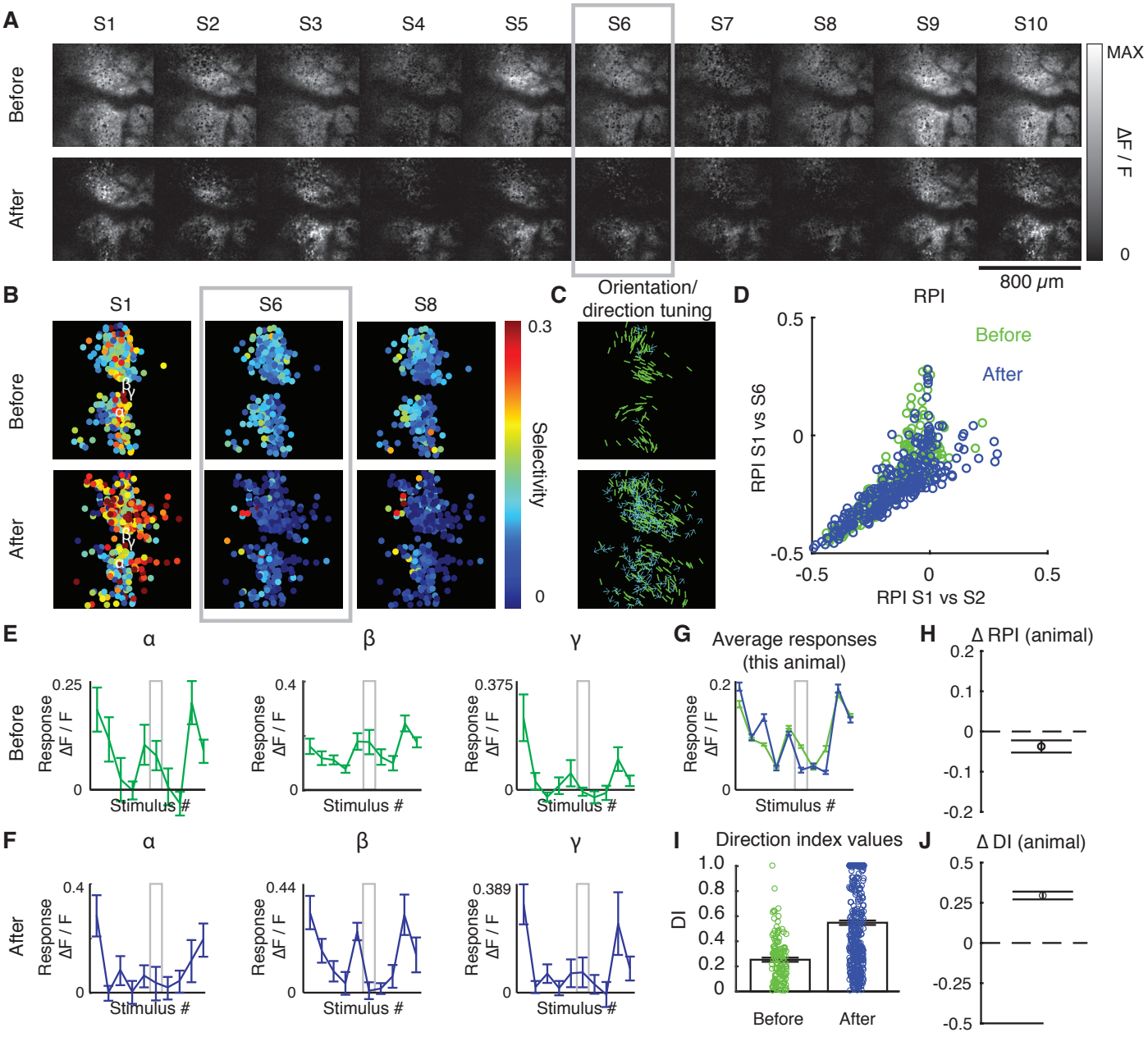

Figure 3. **In a ferret with 3 days of visual experience, 6 hours of experience with a phase-scrambled grating pattern caused an increase in direction selectivity rather than selectivity for the phase-scrambled pattern. A)** Pixel map of GCaMP6-s responses to a family of spatiotemporal stimuli before and after 6 hours of training with pattern *S6*. Responses appear to increase for stimuli S1 and S2 as opposed to pattern *S6*. The animal's eyes opened naturally on P33 and was examined on P36. **B)** Single cell Selectivity Index (SI) values for different stimuli (*S1* – a phase-regular, unscrambled direction stimulus, and *S6*/*S8* – phase-scrambled stimuli). Selectivity for smooth motion (*S1*) increases, while selectivity for stimulus *S6* decreases. **C)** Orientation and direction tuning for single cells before and after training. Green bars represent orientation-

selective but not direction-selective cells (DI<0.5), blue dots indicate visually responsive but poorly tuned cells, and cyan arrows indicate direction-selective cells. **D)** Response Projection Index (RPI) for *S1* vs *S2* (X axis) and *S1* vs *S6* (Y axis) for cells measured before (green) and after (blue) 6 hours of experience with *S6*. There is no apparent increase in Response Projection Index for the trained pattern *S6*. **E)** Responses to 3 example cells (indicated in B) before experience. **F)** Responses to 3 example cells (indicated in B) after experience Cells lacked increases in responses to *S6* and cells α and β exhibit increased responses to stimulus *S1* as compared to *S2*. **G)** Grand average of cell responses before and after 6 hours exposure to *S6*. On average, there is an enhancement of the response to *S1*, but a decrease in responses to stimulus *S6*. **H)** Estimated difference in single cell RPI (*S1* vs. *S6*) before and after experience (95% confidence intervals), indicating no significant changes in selectivity to stimulus *S6*. **I)** Direction index values for single cells before and after exposure to *S6*. Direction index values were increased substantially after visual stimulation. **J)** Estimated difference in DI before and after experience (95% confidence intervals), indicating a significant increase in DI with *S6* experience.

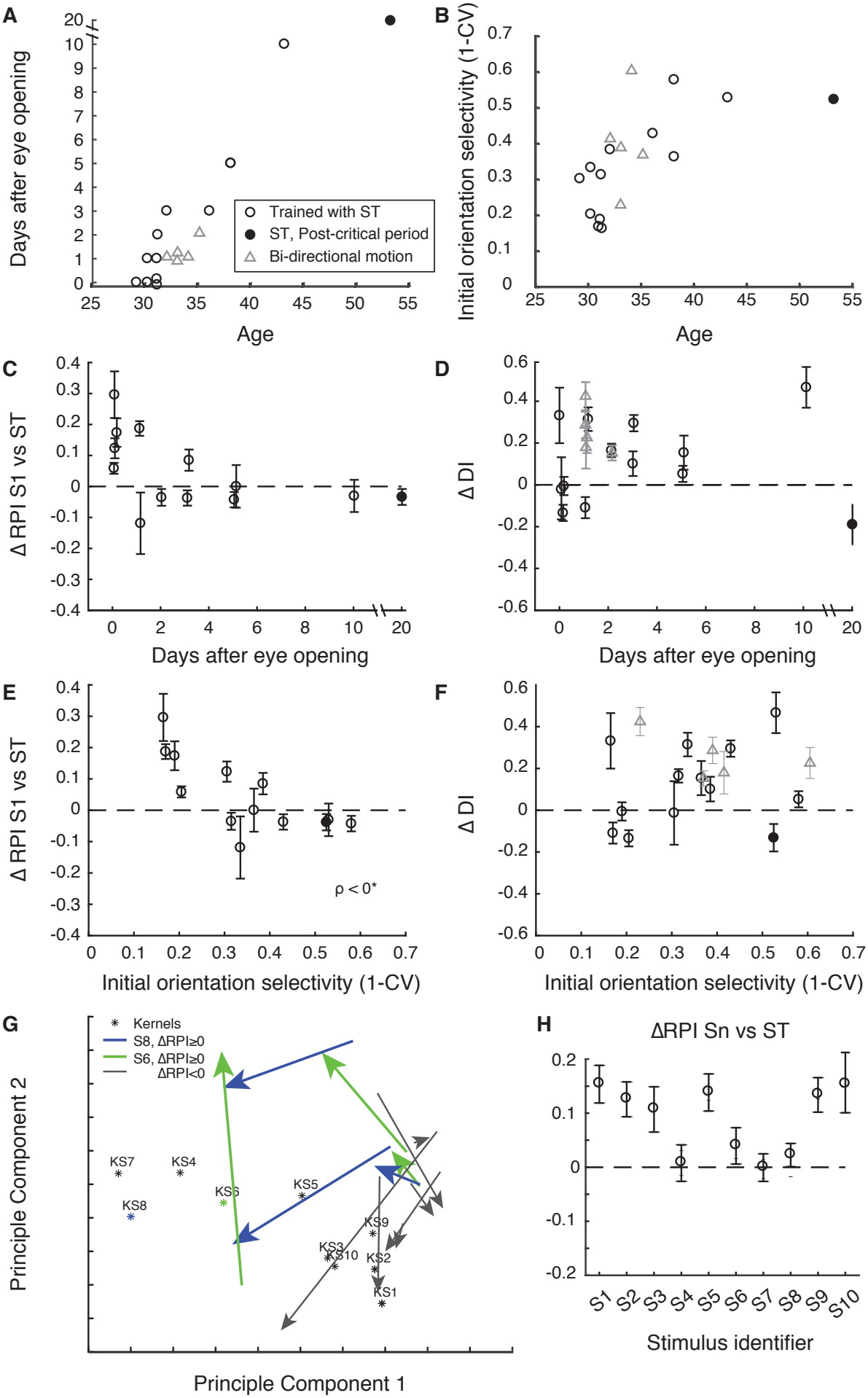

Figure 4. **Relationship between changes in visual selectivity after phase-scrambled stimulus exposure and parameters that are related to animal maturity**. **A)** Relationship between animal age and days after eye opening; ferrets exhibit a range of eye opening from P29 – P34. *ST* indicates animals that were trained with *S6* or *S8*. Triangles indicate animals from Li/Van Hooser et al. (2008) that were trained with bidirectional moving stimuli. Filled circle is a single animal that had emerged from the critical period for direction selectivity development (about 2 weeks after eye opening). **B)** Relationship between animal age and initial orientation selectivity as quantified by 1 minus the circular variance (CV). On average, orientation selectivity becomes stronger with age, but there is a range of initial selectivity in the youngest animals, which likely reflects the range of cortical maturity achieved. **C)** Difference in RPI for *S1* vs the trained stimulus (denoted *ST*; *S6* or *S8*, depending) (Y axis) before and after training (95% confidence intervals indicated) plotted against days of visual experience (days after eye opening). **D)** Same, but difference in direction index values is plotted. **E)** Difference in RPI vs. initial orientation selectivity that was measured at the beginning of the experiment (before training stimulus exposure). Animals with the lowest orientation selectivity exhibit strong changes in RPI and become more selective for the arbitrary training stimulus. $\rho<0$* indicates significant negative correlation ($p<0.009$). **F)** Same, but for DI. **G)** Principle component projection from 10-dimensional space to 2-dimensional space of mean responses (for each animal) to stimuli *S1*…*S10*, before and after training, with vectors indicating the transition from the mean response before training to after training (arrow points at mean state after training). Responses of hypothetical neurons optimized for each stimulus (*KS1*…*KS10*) shown. Animals that exhibited significant ΔRPI (*S1* vs. *ST*) are indicated (trained with *S6* green, *S8* blue). In this reduced view, average responses of significant animals moved closer to *KST*, while animals (8/8) that exhibited no significant effect moved to be near to *KS1*, *KS2*, *KS9*, *KS10* (typical V1 receptive fields). **H)** Change in RPI for significant animals with each stimulus used as a reference with the training stimulus (*Sn* vs. *ST*). For animals trained with *S6* or *S8*, values of RPI (*S6* vs. *S6*) or RPI (*S8* vs. *S8*) were excluded from the average as it is 0 by definition. Changes in responses became more like a hypothetical neuron tuned to the training stimulus *KST* than stimuli *S1*, *S2*, *S3*, *S5*, *S6*, *S9*, and *S10*, and changes in responses remained equally close to stimuli *S4*, *S7*, and *S8* (when *S8* was not the training stimulus) on average. As responses changed in 10-dimensional space, they moved closer

to *KST* for most stimuli while moving no closer or farther from *KS4*, *KS7*, and *KS8*. This is consistent with the idea that the training stimulus provided an instructive influence on receptive field properties in this early developmental period.

References

1 Fukuchi-Shimogori, T. & Grove, E. A. Neocortex patterning by the secreted signaling molecule FGF8. *Science* **294**, 1071-1074, doi:10.1126/science.1064252 (2001).

2 Cang, J. *et al.* Selective disruption of one Cartesian axis of cortical maps and receptive fields by deficiency in ephrin-As and structured activity. *Neuron* **57**, 511-523, doi:10.1016/j.neuron.2007.12.025 (2008).

3 Katz, L. C. & Shatz, C. J. Synaptic activity and the construction of cortical circuits. *Science* **274**, 1133-1138 (1996).

4 Torborg, C. L. & Feller, M. B. Spontaneous patterned retinal activity and the refinement of retinal projections. *Prog Neurobiol* **76**, 213-235, doi:10.1016/j.pneurobio.2005.09.002 (2005).

5 Wong, R. O. Retinal waves and visual system development. *Annu. Rev. Neurosci.* **22**, 29-47 (1999).

6 Hubel, D. H. & Wiesel, T. N. Receptive Fields Of Cells In Striate Cortex Of Very Young, Visually Inexperienced Kittens. *J Neurophysiol* **26**, 994-1002 (1963).

7 Chapman, B. & Stryker, M. P. Development of orientation selectivity in ferret visual cortex and effects of deprivation. *J Neurosci* **13**, 5251-5262 (1993).

8 Fregnac, Y. & Imbert, M. Early development of visual cortical cells in normal and dark-reared kittens: Relationship between orientation selectivity and ocular dominance. *J. Physiol.* **278**, 27-44 (1978).

9 Wiesel, T. N. & Hubel, D. H. Ordered arrangement of orientation columns in monkeys lacking visual experience. *J Comp Neurol* **158**, 307-318 (1974).

10 Smith, G. B., Hein, B., Whitney, D. E., Fitzpatrick, D. & Kaschube, M. Distributed network interactions and their emergence in developing neocortex. *Nat Neurosci* **21**, 1600-1608, doi:10.1038/s41593-018-0247-5 (2018).

11 Kiorpes, L. Visual development in primates: Neural mechanisms and critical periods. *Dev Neurobiol* **75**, 1080-1090, doi:10.1002/dneu.22276 (2015).

12 Wang, B. S., Sarnaik, R. & Cang, J. Critical period plasticity matches binocular orientation preference in the visual cortex. *Neuron* **65**, 246-256, doi:10.1016/j.neuron.2010.01.002 (2010).

13 Li, Y., Fitzpatrick, D. & White, L. E. The development of direction selectivity in ferret visual cortex requires early visual experience. *Nat Neurosci* **9**, 676-681 (2006).

14 Hatta, S. *et al.* Nasotemporal directional bias of V1 neurons in young infant monkeys. *Invest Ophthalmol Vis Sci* **39**, 2259-2267 (1998).

15 Li, Y., Van Hooser, S. D., Mazurek, M., White, L. E. & Fitzpatrick, D. Experience with moving visual stimuli drives the early development of cortical direction selectivity. *Nature* **456**, 952-956, doi:10.1038/nature07417 (2008).

16 Smith, G. B. *et al.* The development of cortical circuits for motion discrimination. *Nat Neurosci* **18**, 252-261, doi:10.1038/nn.3921 (2015).

17 Kennedy, H. & Orban, G. A. Response properties of visual cortical neurons in cats reared in stroboscopic illumination. *J Neurophysiol* **49**, 686-704 (1983).

18 Cremieux, J., Orban, G. A., Duysens, J. & Amblard, B. Response properties of area 17 neurons in cats reared in stroboscopic illumination. *J Neurophysiol* **57**, 1511-1535 (1987).

19 Humphrey, A. L. & Saul, A. B. Strobe rearing reduces direction selectivity in area 17 by altering spatiotemporal receptive-field structure. *J. Neurophysiol.* **80**, 2991-3004 (1998).
20 Humphrey, A. L., Saul, A. B. & Feidler, J. C. Strobe rearing prevents the convergence of inputs with different response timings onto area 17 simple cells. *J. Neurophysiol.* **80**, 3005-3020 (1998).
21 Van Hooser, S. D. *et al.* Initial neighborhood biases and the quality of motion stimulation jointly influence the rapid emergence of direction preference in visual cortex. *J Neurosci* **32**, 7258-7266, doi:10.1523/JNEUROSCI.0230-12.2012 (2012).
22 Ritter, N. J., Anderson, N. M. & Van Hooser, S. D. Visual Stimulus Speed Does Not Influence the Rapid Emergence of Direction Selectivity in Ferret Visual Cortex. *J Neurosci* **37**, 1557-1567, doi:10.1523/JNEUROSCI.3365-16.2016 (2017).
23 DeAngelis, G. C., Ohzawa, I. & Freeman, R. D. Spatiotemporal organization of simple-cell receptive fields in the cat's striate cortex. II. Linearity of temporal and spatial summation. *J. Neurophysiol.* **69**, 1118-1135 (1993).
24 DeAngelis, G. C., Ohzawa, I. & Freeman, R. D. Spatiotemporal organization of simple-cell receptive fields in the cat's striate cortex. I. General characteristics and postnatal development. *J. Neurophysiol.* **69**, 1091-1117 (1993).
25 Priebe, N. J. & Ferster, D. Direction selectivity of excitation and inhibition in simple cells of the cat primary visual cortex. *Neuron* **45**, 133-145, doi:S0896627304008402 [pii]

10.1016/j.neuron.2004.12.024 (2005).
26 Krug, K., Akerman, C. J. & Thompson, I. D. Responses of neurons in neonatal cortex and thalamus to patterned visual stimulation through the naturally closed lids. *J Neurophysiol* **85**, 1436-1443 (2001).
27 Akerman, C. J., Smyth, D. & Thompson, I. D. Visual experience before eye-opening and the development of the retinogeniculate pathway. *Neuron* **36**, 869-879 (2002).
28 Chen, T. W. *et al.* Ultrasensitive fluorescent proteins for imaging neuronal activity. *Nature* **499**, 295-300, doi:10.1038/nature12354 (2013).
29 Meliza, C. D. & Dan, Y. Receptive-field modification in rat visual cortex induced by paired visual stimulation and single-cell spiking. *Neuron* **49**, 183-189 (2006).
30 Jacob, V., Brasier, D. J., Erchova, I., Feldman, D. & Shulz, D. E. Spike timing-dependent synaptic depression in the in vivo barrel cortex of the rat. *J Neurosci* **27**, 1271-1284, doi:10.1523/JNEUROSCI.4264-06.2007 (2007).
31 Gavornik, J. P. & Bear, M. F. Learned spatiotemporal sequence recognition and prediction in primary visual cortex. *Nat Neurosci* **17**, 732-737, doi:10.1038/nn.3683 (2014).
32 Xu, S., Jiang, W., Poo, M. M. & Dan, Y. Activity recall in a visual cortical ensemble. *Nat Neurosci* **15**, 449-455, S441-442, doi:10.1038/nn.3036 (2012).
33 Chiu, C. & Weliky, M. Spontaneous activity in developing ferret visual cortex in vivo. *J Neurosci* **21**, 8906-8914 (2001).
34 Horton, J. C. & Adams, D. L. The cortical column: a structure without a function. *Philos Trans R Soc Lond B Biol Sci* **360**, 837-862 (2005).
35 Colonnese, M. T. *et al.* A conserved switch in sensory processing prepares developing neocortex for vision. *Neuron* **67**, 480-498, doi:10.1016/j.neuron.2010.07.015 (2010).
36 Murata, Y. & Colonnese, M. T. An excitatory cortical feedback loop gates retinal wave transmission in rodent thalamus. *eLife* **5**, doi:10.7554/eLife.18816 (2016).
37 Gallo, J. E. & Lennerstrand, G. A population-based study of ocular abnormalities in premature children aged 5 to 10 years. *Am J Ophthalmol* **111**, 539-547 (1991).
38 Slidsborg, C. *et al.* Cerebral damage may be the primary risk factor for visual impairment in preschool children born extremely premature. *Arch Ophthalmol* **130**, 1410-1417, doi:10.1001/archophthalmol.2012.1393 (2012).