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Experience with moving visual stimuli drives the early development of cortical direction selectivity

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Abstract

The onset of vision occurs when neural circuits in the visual cortex are immature, lacking the full complement of connections1,2 and the response selectivity that defines functional maturity3,4. Direction selective responses are particularly vulnerable to the effects of early visual deprivation, but how stimulus driven neural activity guides the emergence of cortical direction selectivity remains unclear. To explore this issue we developed a novel motion training paradigm that allowed us to monitor the impact of experience on the development of direction selective responses in visually naïve ferrets. Using intrinsic signal imaging techniques we found that training with a single axis of motion induced the rapid emergence of direction columns that were confined to cortical regions preferentially activated by the training stimulus. Using 2-photon calcium imaging techniques, we found that single neurons in visually naïve animals exhibited weak directional biases and lacked the strong local coherence in the spatial organization of direction preference that was evident in mature animals. Training with a moving stimulus, but not with a flashed stimulus, strengthened the direction selective responses of individual neurons and preferentially reversed the direction biases of neurons that deviated from their neighbors. Both effects contributed to an increase in local coherence. We conclude that early experience with moving visual stimuli drives the rapid emergence of direction selective responses in visual cortex.

Direction selectivity is the capacity of neurons to respond more significantly to one principal direction of stimulus motion than any other5. In the retino-geniculo-cortical pathway, it is

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Author contributions: Y.L. performed intrinsic imaging experiments and analysis. Y.L. and S.D.V. performed 2-photon experiments, and S.D.V., Y.L., and M.M. analyzed the 2-photon data. Y.L. and S.D.V. contributed equally to this work. Y.L., S.D.V., L.E.W., and D.F. wrote the paper, and all authors discussed the results and commented on the manuscript.

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first expressed at the level of columnar circuits in the primary visual cortex (V1) where it is organized into a map of direction preference6,7. In ferrets, direction columns emerge shortly after the onset of visual experience in a process that dark-rearing experiments indicate requires visual experience3. To gain insight into the mechanisms by which stimulus driven neural activity shapes the emerging properties of cortical neurons we asked whether exposure to a moving visual stimulus was sufficient to induce the development of cortical direction columns and, if so, how this experience altered the response properties of individual cortical neurons.

We began by studying the emergence of direction columns using intrinsic signal imaging techniques in juvenile ferrets (n = 9; postnatal day 30−35) that had less than one day of visual experience. Consistent with previous reports, the visual cortex of these visually naïve ferrets exhibited a well defined system of orientation columns, but lacked the columnar pattern of direction selective responses which normally develops to mature levels within 7−10 days following eye opening3 (Figure 1). To determine whether exposure to a moving visual stimulus was sufficient to induce the emergence of direction selective responses, animals were exposed to a ‘training’ stimulus (a single sine or square wave grating, spatial frequency 0.06−0.08cpd, temporal frequency 4 Hz) drifting back and forth along an axis of motion orthogonal to the grating orientation. As illustrated for two example cases (Figure 1b, d), no changes were apparent for the first 8−10 hours of stimulation; at later time points, however, small domains of directional preference became evident in difference images. This direction difference signal continued to intensify and the number of emergent domains increased across the area of the responsive cortex as the experiments continued. The strength of the direction difference signal was quantified by calculating a direction selectivity index (DSI, supplementary methods), and there was a notable and progressive increase in DSI that accompanied exposure to the training stimulus (Figure 1e).

Evidence that exposure to the training stimulus was responsible for the rapid emergence of direction selectivity came from experiments performed following the training period in which cortical responses to eight directions of motion were examined. Direction selective responses were evident for stimulus directions that matched that of the training stimulus in 7 of 9 animals, but were weak or absent for other directions of motion in all animals that were trained (Figure 1f, g). Compared to the initial conditions, the average DSI values after training were significantly increased for responses to stimuli whose properties matched the training stimulus, but DSI values associated with responses to stimuli orthogonal to the training stimulus were not significantly different from those at the start of training (Fig 1g, t-test $P<0.001$, $P=0.12$). Moreover, at the end of the training period, there was no significant difference in the average response of the trained and untrained orientation columns to a drifting stimulus of the preferred orientation (Supplementary figure S1b), indicating that the emergence of direction columns cannot be attributed to a general increase in the activity of the trained columns. Because orientation selectivity is not a prominent feature of pre-cortical sites in the visual pathway (retina, LGN)8-12, the orientation selectivity of these training effects indicates that the mechanisms responsible for the rapid emergence of direction selectivity must include events that reside at the level of cortical circuits.

To better understand changes at the cellular level that underlie the emergence of direction columns, we employed in vivo 2-photon imaging of calcium signals13 to explore the direction selective properties of individual layer 2/3 neurons (Figure 2a). As a first step, we examined the magnitude of single-neuron direction tuning in visually naïve animals and compared this to direction tuning in animals that had visual experience sufficient to achieve mature levels of direction selectivity as measured by intrinsic signal imaging. Neurons in visually naïve animals were highly responsive and tuned for stimulus orientation, but were at best only weakly selective for direction of motion (Figure 2b,c). The median direction index (DI) value for visually naïve animals was 0.15, and only 6% of neurons in visually naïve animals exhibited a DI value of 0.5, which corresponds to a preferred/opposite response ratio of 2:1 (Figure 2c). In contrast, many neurons in animals with visual experience were well tuned for direction of motion, such that the median value of the DI was 0.56. Thus, the normal emergence of direction columns that ensues following eye opening in ferrets reflects a significant increase in the percentage of neurons that exhibit strong tuning for direction of motion.

We then examined whether the rapid emergence of direction columns that occurs under the influence of a training stimulus is accompanied by an increase in the direction selectivity of individual cortical neurons. In these experiments, we limited our analysis to 262 cells that had significant orientation tuning (vector test, see Supplementary methods) and could be unambiguously identified both before and after the training period (Figure 3a). Because the calcium sensitive dye generally fades over time, the training period was shorter (3−6 hours) than that used for intrinsic signal imaging so that reliable tuning curves could be measured at the conclusion of training (Supplementary methods, Supplementary Figures S2-S5). The distribution of DI values measured after the training period was significantly higher than the values measured in the same neurons prior to training (Figure 3b and c) (KW test, $p<0.001$): across all cases, median DI increased to 0.39, and 36% of cells exhibited DI values above 0.5. On an individual basis, median DI values increased in all five cases. This training effect was sufficiently strong that statistically significant increases in direction selectivity were observed when the responses of individual cells were compared before and after training (Figure 3a, Supplementary Figures S2, S3): 59 cells (23%) distributed among 4 of 5 animals exhibited a statistically significant increase in DI over that found prior to training, and none exhibited a significant decrease in direction selectivity (bootstrap test, see Supplementary methods). To test whether motion of the training stimulus was necessary to induce these rapid changes in direction selective responses, we examined the effects of training with an identical grating stimulus that was flashed (sinusoidal modulation between grating and gray screen, 4Hz). This stimulus was effective in driving cortical activity (Supplemental Figure S5a), but no significant increase in direction selectivity was found after flash training (Figure 3b,c, KW test, $P=0.27$). Both motion and flash training resulted in small increases in the degree of orientation tuning (KW test, $P<0.001$) that were equal in magnitude (KW test, $P=0.29$) (Figure 3c).

These results provide strong evidence that visual experience increases the magnitude of direction selectivity, and by itself, this could explain the rapid emergence of columnar structure visualized with intrinsic signal imaging. However, a comparison of the spatial organization of direction preferences in visually naive and experienced animals suggests that

visual experience also plays a role in determining which direction of motion is preferred by individual cortical neurons (Figure 4a). Animals with visual experience exhibited a robust clustering of neurons with similar direction preference, consistent with descriptions of the fine scale mapping of direction preference in mature cat visual cortex14. In contrast, in visually naïve animals, the spatial organization of direction preferences was weak, and neighboring neurons often exhibited opposite direction preferences. To quantify this difference, we developed a measure of local coherence: for each neuron, we calculated the percentage of neighboring cells with similar direction preferences (within 45°) minus the percentage with opposite preferences (within 45°). The direction preference of weakly selective cells is acutely sensitive to trial-to-trial variability, so we accounted for this variability in local coherence values by employing the bootstrap technique to create 100 simulations of each cell's responses drawn randomly from the original data with replacement. The median local coherence value from these 100 simulations is reported here. In animals with mature direction selectivity and robust columnar structure, average local coherence values were positive and highest for samples within a radius of 50 μm, falling gradually to near zero at distances of 200−250 μm (Figure 4b,c). The local coherence values for visually naïve animals were significantly lower at all distances; nevertheless, they were significantly different from zero, indicating a weak tendency for nearby neurons in visually naïve animals to exhibit similar direction preferences. To determine whether experience with moving stimuli was sufficient to explain this increase in the spatial coherence of direction preference, we examined the local coherence indices in animals that had undergone training with a motion stimulus and those that received flash training. Motion training produced a significant increase in local coherence values over that found for visually naïve animals (KW test, $P<0.001$), while flash training produced a significant decrease (KW test, $P<0.001$).

These results imply that as a result of motion training, the direction of motion preferred by individual cortical neurons changes so that their preference becomes more like that of their neighbors. Examination of the preference of individual neurons before and after training revealed a number of examples where this appeared to be the case (Figure 4d). However, this conclusion rests on the confidence with which one can assign a direction preference to the weakly selective neurons in visually naive animals. From our bootstrap simulations, we derived a measure of uncertainty in direction preference, defined to be the percentage of simulations that differed from the mean direction by more than 90°. We observed a wide range of uncertainty in initial direction preferences. The direction preference of some cells was highly uncertain before motion training but became more certain after training (Figure 4e1). For example, 68 cells whose uncertainty was greater than 25% before motion training, decreased to less than 25% uncertainty after training. Other cells exhibited moderate direction preference biases at the onset of training that could be either strengthened (Figure 4e2) or reversed (Figure 4e3). Of the 148 cells that had an initial uncertainty less than 25%, 74 were likely to have maintained their initial direction preference (likelihood of reversing less than 25%), while 20 were more than 75% likely to have reversed their preference.

We then asked whether the diverse effects of motion training produced a more coherent map of direction preference by building on the weak spatial organization that existed at the onset of training. Consistent with this idea, we found a significant correlation between the

likelihood of direction preference reversal and local coherence prior to training (Figure 4f; regression F test $P < 0.001$, $R^2 = 0.22$), but not with flash training (Figure 4g; regression F test $P=0.34$, $R^2=0.006$). Thus, in regions with strong local coherence, there was a predictable impact of motion training: cells that were surrounded by neighboring neurons expressing the opposite direction preference prior to training (high negative local coherence) were likely to reverse their direction preference, while cells whose neighbors expressed the same preference (high positive local coherence) were unlikely to reverse. Furthermore, the impact of motion training on the likelihood of reversal was unpredictable in regions characterized by weak local coherence (local coherence index values near 0). These findings implicate local cortical interactions in the mediation of the effects of motion training on direction preference. This systematic relationship between the likelihood of preference reversal and initial coherence values also rules out the possibility that changes in uncertainty alone are sufficient to explain the increase in local coherence. Indeed, on average, we estimate that training-induced reversals in direction preference accounted for 53% of the total training-induced increase in spatial coherence, while changes in uncertainty accounted for 34%; the remaining 13% was contributed by slight but significant changes in orientation preference that accompanied motion training (see Supplementary methods, Supplemental Figure S4). Although fewer cells appeared to reverse direction than the number of cells that reduced uncertainty, the impact of a preference reversal on the local coherence index is greater than the impact of a reduction of preference uncertainty.

We conclude that early experience with moving visual stimuli exerts a strong, rapid, and selective impact on response properties of developing cortical neurons, transforming a weakly biased array of poorly selective neurons into a more mature state that exhibits stronger direction selectivity and enhanced spatial coherence of direction preference. The rapid time course of these effects and the fact that they are sensitive to the spatiotemporal structure of the stimulus are consistent with activity-dependent mechanisms of synaptic plasticity15,16. But what differentiates these observations from most previous demonstrations of activity dependent alterations in stimulus preference17-25 is that the information present in the training stimulus is ambiguous: opposite directions of motion are presented and yet neurons rapidly acquire and/or strengthen their preference for a single direction. Thus, the spatiotemporal cues present in a bidirectional motion training stimulus, which is more consistent with the balanced stimulation an animal might receive in nature, are sufficient to drive the development of cortical circuits that represent each direction of motion. Evidently, sufficient asymmetry in functional architecture exists to facilitate symmetry-breaking and seed the formation of direction columns from stimulus patterns that are equally balanced. Our spatial analysis has identified a constructive mechanism that operates locally and is reflected in the preferences of neighbouring neurons in layer 2/3. This mechanism—possibly mediated by local recurrent or feedforward circuits—effectively disambiguates the bidirectional motion energy of the training stimulus by influencing the probability that a neuron's initial preference will be reinforced or reversed. It remains unclear whether the weak direction bias present at the onset of training emerges via visual experience through closed lids26, endogenous activity27,28, or activity-independent mechanisms29,30. Nevertheless, the evidence presented here indicates that the events preceding eye opening are insufficient to account for either the magnitude of direction

selectivity or the preference exhibited by mature cortical neurons, and that early experience with moving stimuli exerts a significant impact on both features.

Methods Summary

Ferrets were anesthetized with ketamine and isoflurane (2% for surgery, 0.08−2% during imaging). 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. For intrinsic signal imaging experiments, cortex was illuminated with 610-nm light and data was acquired using the Imager 2001/3001 (Optical Imaging Inc) 3. For 2-photon experiments, Oregon Green 488 BAPTA-1 AM (Invitrogen) was pressure injected into cortex and changes in calcium fluorescence were monitored with a 2-photon microscope (Prairie Technologies) driven by a mode-locked laser (810nm, Coherent). Stimulation and analysis were performed using custom software for Matlab (Mathworks). See Supplementary Methods for details.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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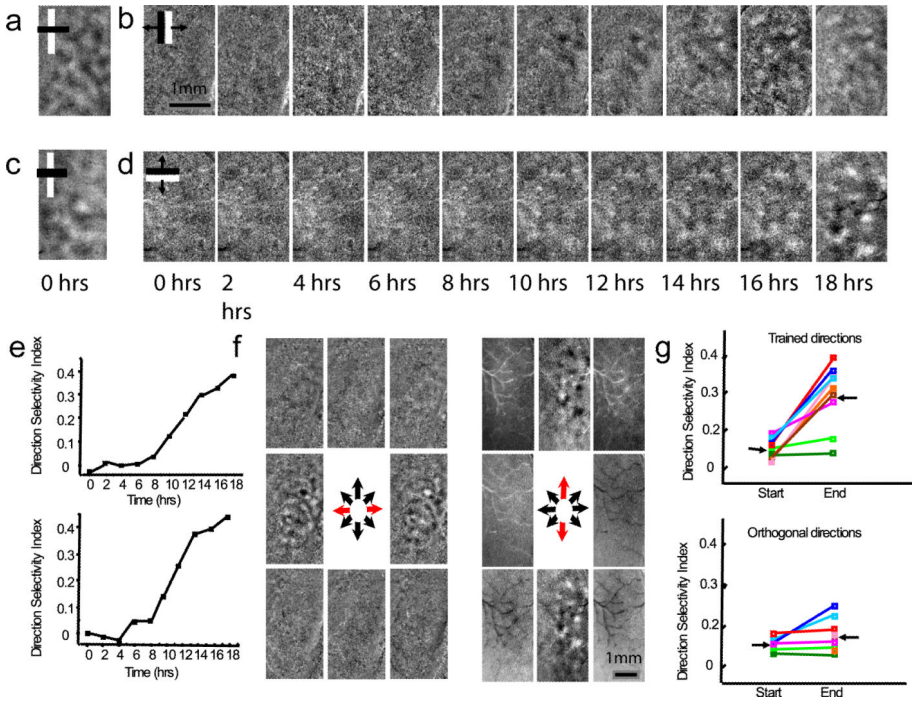

Figure 1.

Rapid emergence of direction columns with motion training. (a-d) Visually naïve animals (postnatal days 34−35) with orientation columns (a,c) were trained using moving vertical (a,b) or horizontal (c,d) gratings. Direction domains emerged after 8 hours. (e) Time course of training-induced increases in direction selectivity (top, b; bottom, d). (f) After training, direction domains were present only for the trained directions of motion (red arrows indicate training directions). (left, b; right, d). (g) Direction selectivity before and after training; colors indicate different animals and arrows indicate median DSI, which increased significantly for trained (t-test, $P < 0.001$, top), but not orthogonal motion directions (t-test, $P = 0.12$, bottom).

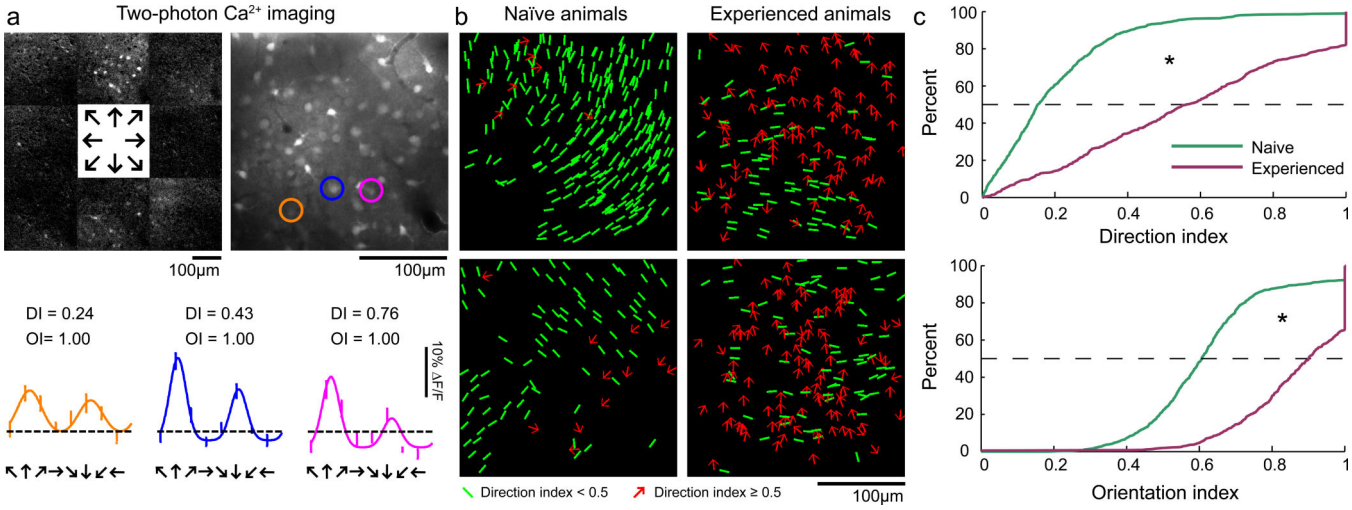

Figure 2.

Direction selectivity of cells in visually naïve and experienced ferrets demonstrated by 2-photon calcium imaging. (a) Left: average responses (ΔF/F) to different directions of motion at 160 μm depth in visual cortex of an animal with 3 weeks experience. Right: OGB-labeled cells at same depth. Bottom: tuning curves and direction/orientation indices (DI/OI) for cells circled in top right figure (dashed lines = mean blank responses). (b) Plots of orientation/direction selective cells in 2-photon multi-depth imaging fields from naïve and experienced animals. Cells with DI < 0.5 are depicted with green bars indicating preferred orientation; cells with DI ≥ 0.5 with red arrows indicating preferred direction. (c) Cumulative histograms of DI and OI for naïve and experienced animals. Differences between groups are significant (KW test, $P<0.001$).

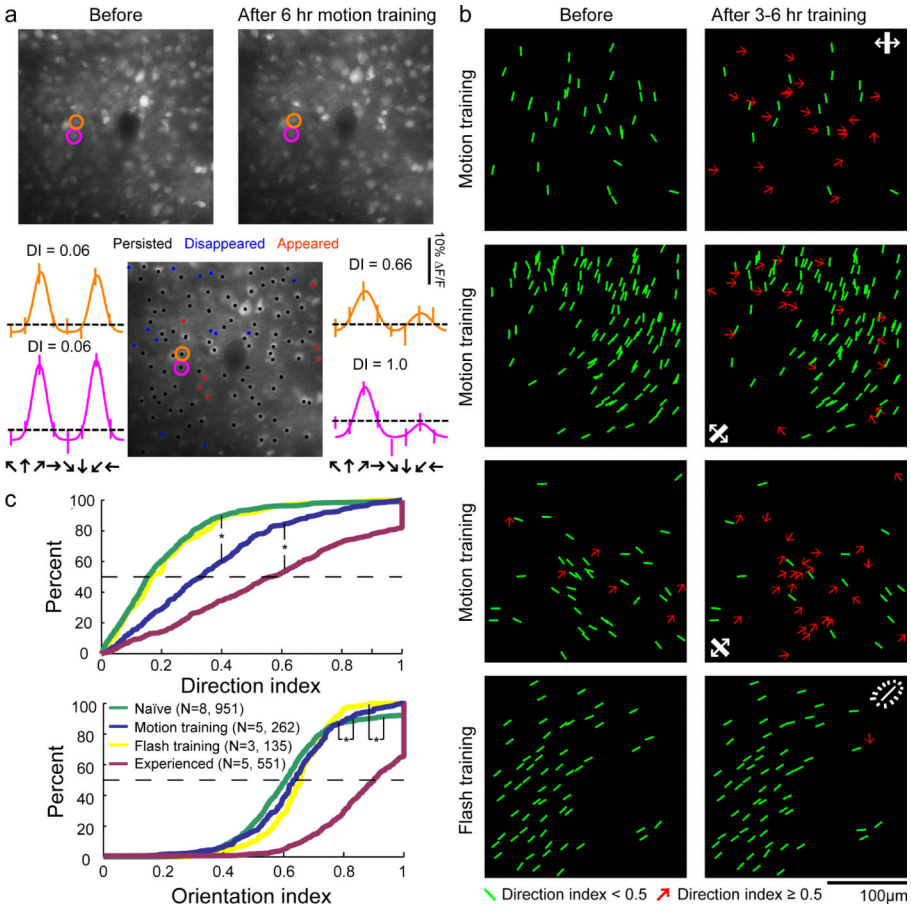

Figure 3.

Motion training increases direction selectivity in individual cells. (a) Representative 2-photon images (135 μm depth) showing labeled cells evident before (top left) and after training (top right). Cell history over the course of training (persistant, disappeared, appeared) is depicted in the bottom middle. Tuning curves and DI values for circled cells are shown at bottom left (before training) and right (after training) (b) Plots of cells from 4 animals before and after motion or flash training; icons indicate training directions or orientation of flashing stimulus. (c) Cumulative histograms of DI and OI for naïve, motion-trained, flash-trained, and experienced conditions. DI increased significantly following motion training (KW test, $P < 0.01$) but not after flash training (KW test, $P = 0.27$).

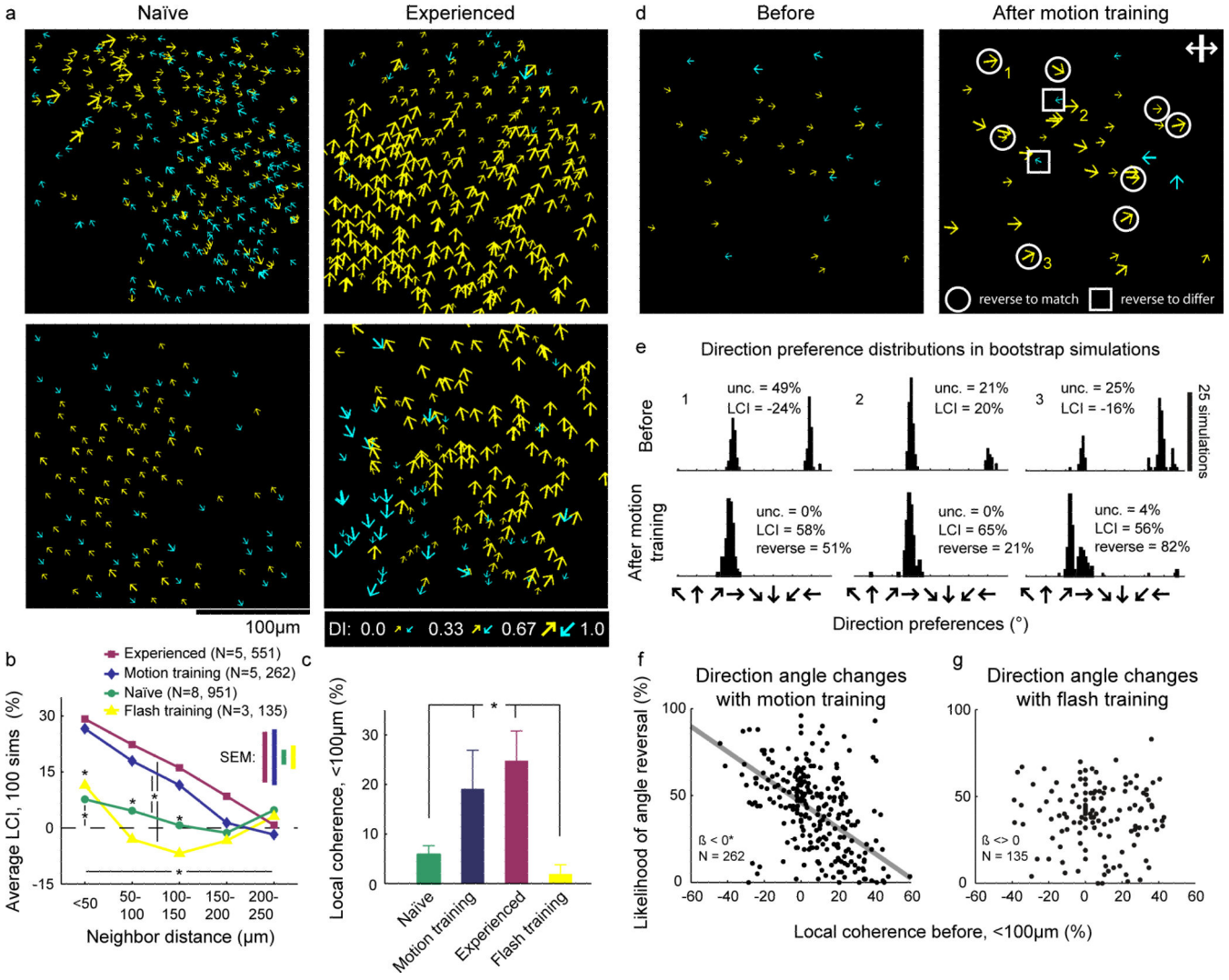

Figure 4.

Impact of normal experience and motion training on direction preference. (a) Plots of direction preference in naïve and experienced animals. Arrows indicate preferred direction, length indicates magnitude. Color differentiates cells with opposite preferences (± 90 degrees). (b,c) Spatial coherence of direction preference increased with experience or motion training, but not with flash training (SEM calculated across animals; N = animal, cell number). Significant relations among curves and with distance (ANOVA), and differences from 0 in naïve and flash traces (sign test) indicated by *. (d) Motion training effects on the direction preference of individual cells (numbers refer to cells in e). Cells in circles and squares appeared to reverse their preference, coming to prefer rightward and leftward motion respectively. (e) Distributions of preferred directions in simulations for 3 cells (unc., preference uncertainty; reverse, likelihood of a preference reversal; LCI, local coherence index). Some cells initially were uncertain but developed a consistent preference after motion training (1); others exhibited biases that strengthened (2) or reversed (3). (f) Influence of initial LCI on motion training effects. Cells whose preferred direction differed

from their neighbors were most likely to reverse (g) No systematic relationship was observed with flash training.