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Metaplasticity Governs Natural Experience-Driven Plasticity of Nascent Embryonic Brain Circuits

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SUMMARY

During embryogenesis, brain neurons receiving the same sensory input may undergo potentiation or depression. While the origin of variable plasticity in vivo is unknown, it plays a key role in shaping dynamic neural circuit refinement. Here, we investigate effects of natural visual stimuli on neuronal firing within the intact, awake, developing brain using calcium imaging of 100 s of central neurons in the *Xenopus* retinotectal system. We find that specific patterns of visual stimuli shift population responses toward either potentiation or depression in an N-methyl-D-aspartate receptor (NMDA-R)-dependent manner. In agreement with Bienenstock-Cooper-Munro metaplasticity, our results show that functional potentiation or depression can be predicted by individual neurons' specific receptive field properties and historic firing rates. Interestingly, this activity-dependent metaplasticity is itself NMDA-R dependent. Furthermore, network analysis reveals increased correlated firing of neurons that undergo potentiation. These findings implicate metaplasticity as a natural property regulating experience-dependent refinement of nascent embryonic brain circuits.

INTRODUCTION

During early periods of embryonic brain development, brief sensory experience plays a direct role in shaping neural circuit structure, connectivity, and function. Plasticity of neuronal firing during this critical period of development can have long-lasting effects on network growth to influence normal and abnormal brain function later in life. Unlike mammalian embryos, frog and fish larvae provide an accessible developing brain circuit to study this stage of neuronal growth, in which afferent input can be driven by well-controlled visual stimuli for study of activity-dependent central circuit formation and refinement. In these systems, visual experience affects dendritic and axonal growth (Haas et al., 2006; Ramdya and Engert, 2008; Ruthazer et al., 2003; Sin et al., 2002), synaptic efficiency (Engert et al., 2002; Zhang et al., 2000), and excitability (Aizenman et al., 2002, 2003) of central neurons. Single-neuron recordings in open brain preparations have demonstrated NMDA-R-dependent synaptic long-term potentiation (LTP) and long-term depression (LTD) at retinotectal synapses after electrical or visual stimulation (Engert et al., 2002; Vislay-Meltzer et al., 2006; Zhang et al., 1998, 2000; Zhou et al., 2003). Interestingly, the same visual experience can induce either LTP or LTD (Zhou et al., 2003) simultaneously in different tectal cells. We hypothesize an explanation for such variable plasticity responses is provided by the Bienenstock-Cooper-Munro (BCM; Bienenstock et al., 1982) theory which suggests that a neuron's firing rate prior to plasticity induction may directly affect that cell's ability to exhibit subsequent synaptic LTP or LTD. In this form of plasticity regulation, termed metaplasticity (Abraham, 2008), neurons with distinct initial states can respond differently to the same presynaptic stimulus. The BCM learning rule has been shown to be valid for plasticity induced changes in synaptic efficiency as well as neuronal excitability (Daoudal et al., 2002; Wang et al., 2003), both of which may be reflected in long-term changes in a neuron's functional response properties. It is presently unclear whether metaplastic rules govern synaptic plasticity of individual neurons during normal brain development or if metaplasticity occurs in the absence of experimental priming such as dark rearing or monocular deprivation. Proper testing of these theories requires simultaneous monitoring of activity in large populations of neurons within intact systems to determine whether individual neuronal pretraining firing rates predict variable plasticity results.

To measure endogenous activity and visual response properties of large neuronal ensembles, we utilized in vivo imaging of spontaneous and visually evoked calcium events. This technique allows simultaneous probing of the visual properties of hundreds of tectal neurons in the awake brain with single-cell resolution. Visual receptive field (RF) responses were probed both prior to and up to 1 hr following visual training. Using population imaging and network analysis of activity, we find experience-driven plasticity in the retinotectal system causes variable functional plasticity of individual tectal neurons, driving functional RF responses of neurons toward long-lasting potentiation or depression. Plasticity is specific to the RF properties evoked during training and shows evidence for BCM metaplasticity, in that pretraining activity predicts plasticity outcome. Increasing activity with visual experience during pretraining also shifts the plasticity threshold. Together, our results demonstrate that natural sensory input plays a profound and lasting role in the functional development of intact brain circuits, subject to each neuron's previous history.

RESULTS

To noninvasively investigate how sensory experience alters circuit function within the intact and awake developing brain,

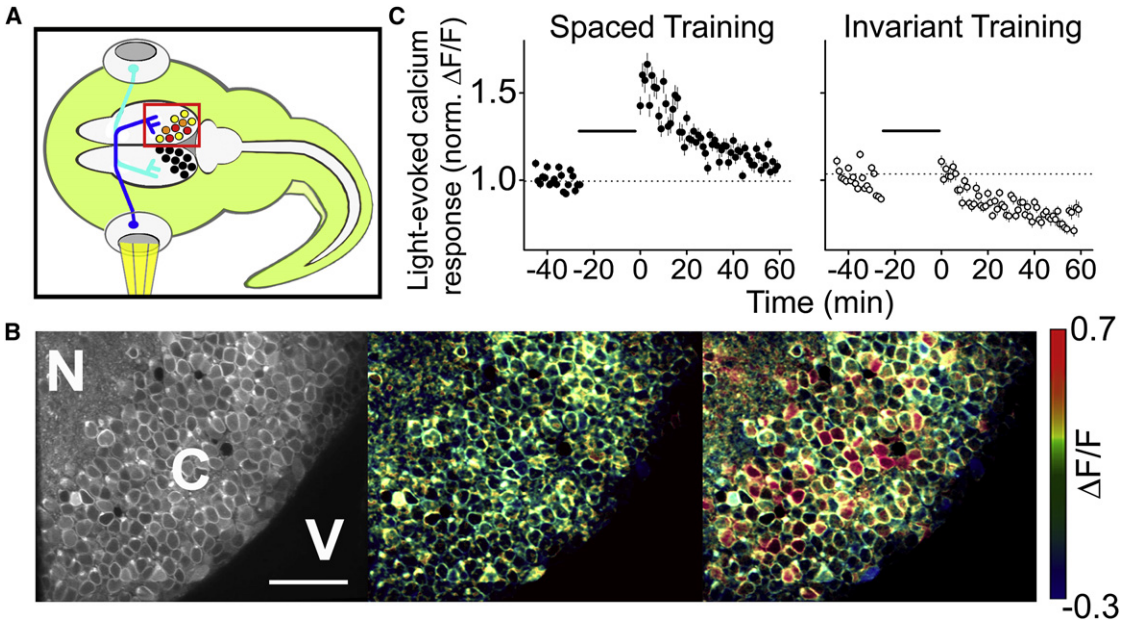

Figure 1. Visually Driven Plasticity of Evoked Ca^{2+} Events in the Intact and Awake Brain

(A) Diagram of the experimental set-up: visual stimuli are projected into the tadpole eye while performing two-photon imaging of contralateral optic tectum (red box, image area shown in B).

(B) Left: fluorescence optical section through tectum demonstrating neuropil (N), cell body region (C), and ventricle (V) (average of five images; scale bar = 140 μm). Middle: Ca^{2+} responses immediately before visual stimulus. Right: visually evoked calcium responses induced by brief, 50 ms, OFF stimulus. Pseudo-color image using scale of fractional change in fluorescence intensity relative to average baseline levels.

(C) Mean SCEP recordings (±SEM) of all cells after spaced training (left; n = 6 tadpoles, 168 cells; 14% ± 1%, $p < 10^{-11}$, 40–60 min posttraining, t test) and invariant training (right; n = 5 tadpoles, 106 cells; −20% ± 1%, $p < 10^{-12}$, 40–60 min posttraining, t test). Bar denotes training periods.

we simultaneously monitored the activity of hundreds of neurons within the optic tectum of unanaesthetized stage 50 tadpoles (Nieuwkoop and Faber, 1967) in response to wide-field light ON or OFF stimuli (Gaze et al., 1974; Zhang et al., 1998) using in vivo two-photon time-lapse single-cell-excitability-probing (SCEP) (Johenning and Holthoff, 2007) of calcium dynamics (Brustein et al., 2003; Stosiek et al., 2003) (Figure 1A). Because the amplitudes of Ca^{2+} transients in individual neurons are correlated with action potential firing (Niell and Smith, 2005; Smetters et al., 1999), SCEP allows monitoring of the plasticity of functional RF responses within the intact circuit. We find that more than 45% of cells in a single optical section of the tectum demonstrate clear evoked calcium responses to 50 ms OFF stimuli (Figure 1B and see Figures S1 and S2 available online). Fifty milliseconds OFF stimuli trigger somatic action potentials in tectal neurons without residual ON responses (Tao et al., 2001; Zhang et al., 2000), allowing us to probe changes in a neuron's OFF RF. Amplitudes of visually evoked wide-field responses remained stable over 1 hr 45 min of probing at 60 s intervals in 69% of the visually responsive cells.

Long-Lasting Functional Plasticity in Central Neuronal Ensembles Driven by Natural Sensory Stimuli

Can brief patterned visual training affect network activity and circuit RF plasticity in the intact and awake developing tadpole brain? While patterned input appears unnecessary for early RF refinement in the intact tectum of zebrafish (Niell and Smith, 2005), exposure to specific patterns of repeated visual stimuli can induce long-lasting synaptic changes in single tectal neurons recorded using patch clamp electrophysiology in open brain preparations of developing *Xenopus* (Vislay-Meltzer et al., 2006; Zhou et al., 2003). To assess functional RF plasticity throughout the tectal circuit in response to visual training, we probed visually evoked calcium responses to 50 ms OFF stimuli before and after a 25 min "spaced training" paradigm composed of repeated trains of high-frequency 50 ms OFF stimuli (Figures 3A and S3). High-frequency stimulation significantly increases cell activity compared to pretraining probing (Figure 2A). Average calcium transient amplitude is also greater than pretraining probing (28% ± 6%, $p << 0.01$, t test). Spaced training induced long-lasting potentiation of visual evoked responses to OFF stimuli, evident from a significant increase in the ensemble average firing rate measured 30–60 min post-spaced training (Figure 1C).

If spaced training can cause mean potentiation of tectal neuron responses in the developing brain, is it possible for other patterns of brief visual experience to cause functional depression? Homosynaptic depression can be induced in vivo during weak afferent activity from the retina (Bear et al., 1987; Rittenhouse et al., 1999). BCM theory predicts this effect, postulating that low levels of presynaptic activity will depress active synapses (Bienenstock et al., 1982) and intrinsic excitability (Daoudal and Debanne, 2003), while high levels of presynaptic activity above a modification threshold, θ_m, will lead to postsynaptic strengthening. To

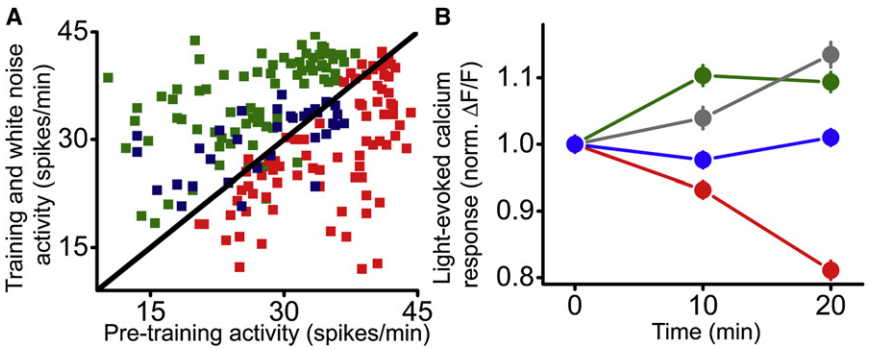

Figure 2. Activity during Training

(A) Scatter plot of activity during high frequency (0.3 Hz) spaced training (ST, green squares), invariant ON light stimulation (Inv, red squares) and white noise stimulation (see Figure 7; WN, dark blue squares) of individual cells versus activity during pretraining SCEP probing. Black line, pretraining and training (or WN) activity are equal. Mean activities are significantly different from pretraining: ST, 1.4 ± 0.04 spikes/min (n = 80 cells in two tadpoles); Inv, 0.8 ± 0.02 spikes/min (n = 80 cells in two tadpoles); WN, 1.14 ± 0.05 spikes/min (n = 33 cells in two tadpoles).

(B) Spaced training plasticity effects are additive with each presentation of high-frequency OFF stimuli. Mean activity of different plasticity groups (±SEM; three tadpoles): long-lasting potentiation (n = 20, green circles), short-term potentiation (n = 8, gray circles), no change (n = 48, blue circles), and long-lasting depression (n = 10, red circles).

reduce presynaptic activity, we presented an unchanging light stimulus to the immobilized eye. Because retinal ganglion cells (RGCs) respond most strongly to changes in the pattern of illumination rather than to steady states of uniform illumination (Wade and Swanston, 2001), "invariant" light stimulation elicits significantly less neuronal firing than baseline probing (Figure 2A). There is no change, however, in average posttraining calcium transient amplitude compared to pretraining probing ($p = 0.47$, t test). Indeed, training with 25 min of invariant light stimulation induced significant long-lasting depression of ensemble visually evoked calcium responses 30–60 min posttraining (Figure 1C).

Variable Plasticity of Individual Central Neurons to the Same Sensory Training Paradigm

Single-cell excitability probing of visually evoked calcium responses allowed us to determine the long-term plasticity effects of natural visual stimulation on individual tectal neurons during both spaced and invariant training (Figure 3). The amplitudes of visually evoked calcium responses in all visually responsive cells showed one of four types of plasticity: long-lasting potentiation (Figures 4A and 4E), short-term potentiation (Figures 4B and 4F), no change from pretraining levels (Figures 4C and 4G), and long-lasting depression (Figures 4D and 4H). Such variable RF plasticity is consistent with visually driven synaptic plasticity previously observed in the retinotectal system (Zhou et al., 2003) and highlights the intrinsic complexity of natural plasticity induction in the intact developing brain. In response to spaced training, over 50% of neurons showed a short or long-lasting potentiation to the probed stimulus, and 12% exhibited long-lasting depression (Figure 4K). Probing during spaced training demonstrates plasticity is additive over periods of high frequency stimulation (Figure 2B). Similar to spaced training, invariant training induced varied plasticity in neurons throughout the tectal circuit (Figures 4F–4H); however, the largest population of cells, 45%, exhibited long-lasting depression (Figure 4K). Among cells showing persistent depression, the amount of depression was significantly greater after invariant than following spaced training (invariant = −33 ± 1%, spaced training = −26% ± 1%, $p = 0.00001$, t test). Taken together, our results show that brief episodes of natural visual experience can cause long-lasting functional changes within the intact, awake, developing brain, with specific patterns of stimulation favoring induction of either potentiation or depression. Training did not produce potentiation or depression in all cells; rather, a varied amount and type of plasticity was observed throughout the tectal circuit, with the specific training paradigm preferentially shifting the majority of cells toward potentiation or depression. Potentiation or depression of functional responses may reflect the plasticity of synaptic inputs (Debanne et al., 2003; Powers et al., 1992) or altered neuronal excitability (Aizenman et al., 2003; Campanac and Debanne, 2008; Daoudal and Debanne, 2003; Daoudal et al., 2002; Wang et al., 2003) .

Synaptic Mechanisms Underlying Visually Induced Tectal RF Plasticity

Activation of *N*-methyl-D-aspartate receptors (NMDA-Rs), a subtype of glutamate receptors, is required for induction of LTP and LTD at retinotectal synapses (Engert et al., 2002; Zhang et al., 1998; Zhang et al., 2000). We tested whether blockade of NMDA-Rs by injection of D-aminophosphovalerate (D-APV; 50 μM), a specific NMDA-R antagonist, interferes with experience-induced plasticity of visually evoked tectal Ca^{2+} responses. NMDA-R blockade significantly reduced visually driven potentiation by spaced training (Figure 4I) and depression after invariant training, albeit to a lesser degree (Figure 4J). Residual depression may be due to other non-NMDA-R-dependent forms of LTD, such as mGluR-mediated depression (Daoudal and Debanne, 2003). Mean evoked calcium responses 30–60 min posttraining demonstrated no significant difference between a continuous probing control and either spaced training + APV or invariant training + APV. APV injection did not affect visually evoked calcium response amplitudes directly (Figure S4), suggesting that the elimination of training induced plasticity was not due to an APV-induced reduction of activity during the training period. Injection of vehicle control before training did not affect plasticity induction (Figure S5). These results support an NMDA-R dependent mechanism mediating induction of visually driven functional plasticity in the awake tadpole optic tectum.

RF Mapping across Tectal Circuit and Specificity of Plasticity to the Characteristics of the Training Sensory Stimuli

Is spaced training induced plasticity specific to the properties of the training stimulus? Because 50 ms OFF spaced training elicits

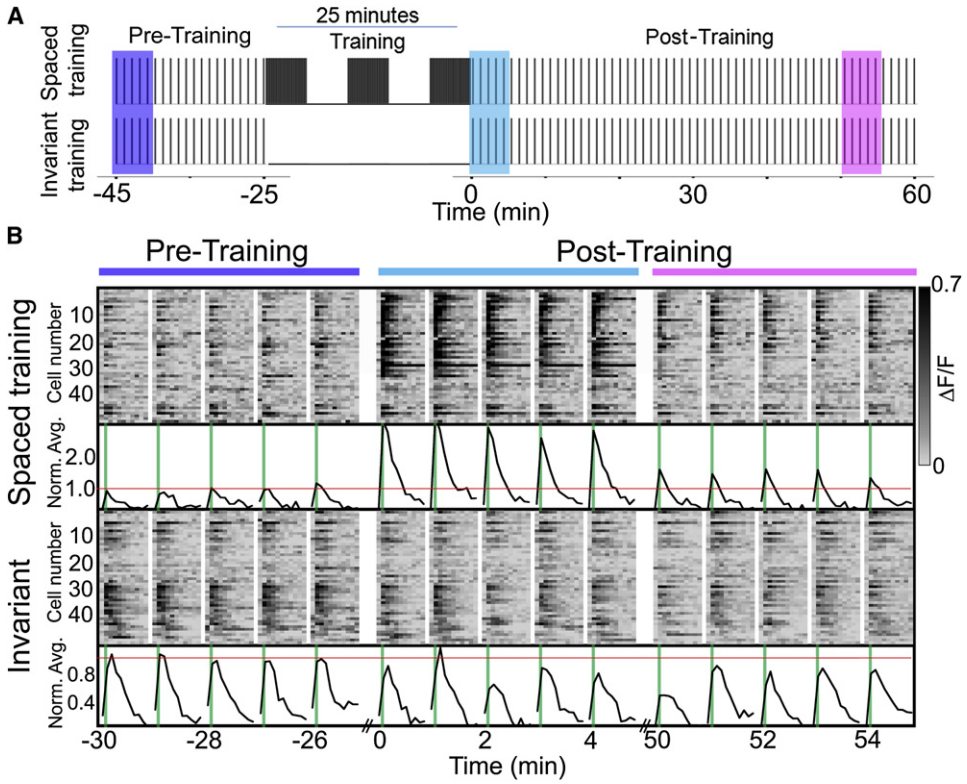

Figure 3. Natural Visual Stimuli Induce Variable Functional Plasticity in the Embryonic Brain

(A) SCEP recording paradigm to probe visual OFF response properties and training stimulation. SCEP is presented every 60 s: 20 min pretraining, 60 min post-training (black bars, 50ms OFF stimuli). Training stimulation: spaced training, three sets of high-frequency (0.3 Hz) repetitive 50 ms OFF stimuli spaced by 5 min ON stimulation (top) and invariant ON light stimulation (bottom). Colored boxes correspond to raster plots shown in (B).

(B) Raster plot of the amplitude of Ca^{2+} transients for 100 randomly selected tectal neurons before and after spaced training (top) and invariant training (bottom). Each vertical section of the plot shows only 11 imaging frames; white lines separate time gaps. Green lines denote 50 ms OFF stimuli. Means of all 50 Ca^{2+} transients, normalized to average pretraining peak values, are shown below raster plot.

only OFF responses, only OFF RFs should show plasticity if training specificity is true. Probing with 60 s OFF stimuli allowed us to map the wide-field OFF (stimulus onset) and ON (stimulus offset) RF properties of neurons throughout the tectum (Figure S6). Approximately half of visually responsive tectal cells were purely OFF dominated without detectable ON response, 5% were purely ON dominated, and the remainder responded in varying degrees to both ON and OFF stimuli. Interestingly, analysis of network responses revealed significant anatomical clustering of cells with ON- and OFF-dominated RFs compared to random reassignments of RF values (Figures 5A and 5B). Clustering of ON- and OFF-center afferents is predicted in computational models (Miller, 1992, 1994) and has been demonstrated in mammalian primary visual cortex (Jin et al., 2008; Zahs and Stryker, 1988). By probing both ON and OFF responses pre- and post-spaced training, we found that plasticity induced by 50 ms OFF spaced training is specific to OFF responses. Eighty-six percent of cells demonstrate no change in ON response after spaced training ($p < 10^{-10}$, compared to OFF no change, chi-square test). Moreover, the amplitude of OFF response plasticity increased with the degree of RF OFF domination (Figure 5C). These results clearly demonstrate specificity of plasticity to characteristics of the training stimuli as a defining characteristic of spaced training with wide-field ON and OFF stimuli in the intact tectum. Furthermore, we find that spontaneous activity of long-lasting potentiated cells is reduced after spaced training (Figure S7), suggesting spaced training induced potentiation is not due to a global increase in cell excitability.

Metaplastic Rules Predict Visually Induced RF Plasticity of Individual Neurons

Do neurons within intact brain circuits exhibit metaplasticity, such that pretraining intrinsic properties of individual neurons influence their responses to training? A second feature of BCM theory is that the value of the modification threshold, θ_m, which determines the degree and direction of synaptic efficiency (Bienenstock et al., 1982) and intrinsic excitability (Daoudal and Debanne, 2003) changes, is not fixed but instead dependent on each neuron's averaged firing rate during the recent past. If the averaged firing rate is high, θ_m rises; if the averaged firing rate is low, θ_m falls (Figure 6A). The intact *Xenopus* retinotectal preparation in combination with uniform dye uptake after bulk loading (Figure S8; Garaschuk et al., 2006; Yasuda et al., 2004) allowed us to indirectly monitor the firing rate of individual tectal neurons during pretraining by measuring spontaneous calcium events driven by endogenous brain circuit activity (Zhou et al.,

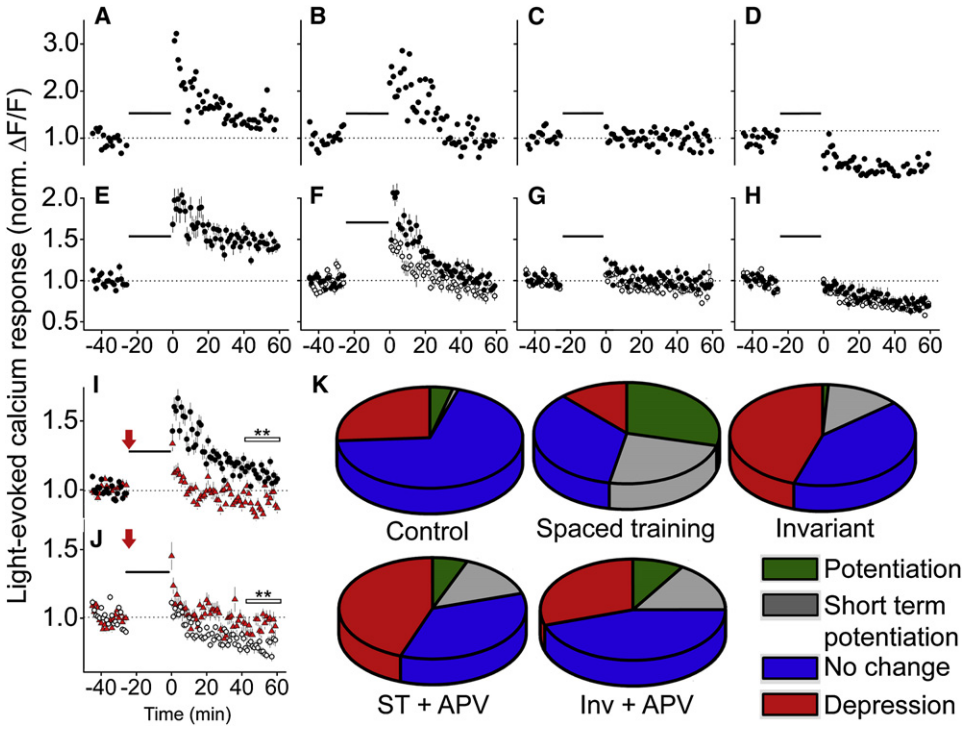

Figure 4. In Vivo Long-Lasting Plasticity of Single Neurons and Ensemble Populations using Visually Evoked Single-Cell Excitability Probing (SCEP)

(A–D) SCEP recordings from single tectal neurons exhibiting (A) long-lasting potentiation, (B) short-term potentiation, (C) no change, and (D) long-lasting depression to the probed, 50 ms OFF stimulus after spaced training (ST).

(E–H) Mean SCEP recordings (±SEM) of all cells exhibiting similar plasticity after ST (closed circles) and invariant training (Inv) (open circles); sample size same as (K).

(I and J) Mean SCEP recordings (±SEM) of all cells after (I) ST and (J) Inv. Plasticity is blocked by tetal injection of APV (red triangles). Mean amplitudes 40–60 min posttraining are significant: (I) n = 3 tadpoles, 79 cells, $p < 10^{-18}$; (J) n = 3 tadpoles, 67 cells, $p < 10^{-14}$ (t tests). Bar denotes training periods and red arrow APV injection.

(K) Percentage of neurons exhibiting long-lasting potentiation (ST 29%, Inv 1%**, control 4%**, ST + APV 6%*, Inv + APV 9%**), short-term potentiation (ST 24%, Inv 13%*, control 1%**, ST + APV 14%, Inv + APV 16%), no change (ST 35%**, Inv 41%**, control 69%, ST + APV 49%**, Inv + APV 45%**), and long-lasting depression (ST 12%**, Inv 45%, control 26%**, ST + APV 39%, Inv + APV 30%*), after various training. ST, n = 6 tadpoles, 168 cells; Inv, n = 5 tadpoles, 106 cells; control, n = 4 tadpoles, 168 cells; ST + APV, n = 3 tadpoles, 79 cells; Inv + APV, n = 3 tadpoles, 67 cells. * $p < 0.05$, ** $p < 0.01$, significant difference (chi-square test) compared to underlined sample.

2003). Calcium transients reflect both slow cell firing rates through their frequency and burst firing rates through their amplitudes. Transient amplitudes have been shown to scale with the number of action potentials fired in a burst in multiple systems and organisms (Brustein et al., 2003; Fetcho et al., 1998; Johenning and Holthoff, 2007; Niell and Smith, 2005;

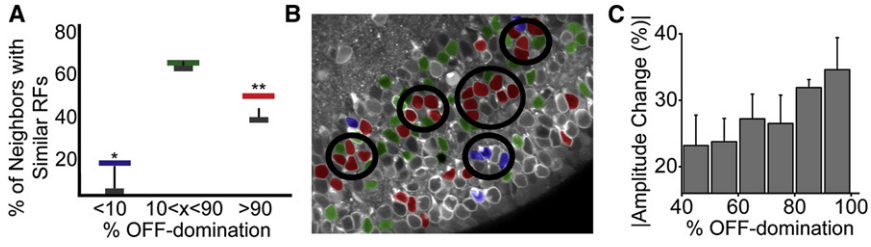

Figure 5. Tectal Neurons Cluster Based on RF Properties and RF Plasticity Is Preferential to the Trained Stimulus

(A) Purely ON- and OFF-dominated RFs cluster anatomically. Colored bars denote measured fraction of nearest anatomical neighbors with similar off-responsiveness; black bars and tails denote bootstrapped means, and 95% confidence intervals for fraction of nearest neighbor pairs within similar off-responsiveness under random reassignment of observed receptive field values (see Experimental Procedures); n = 3 tadpoles, 254 cells *p = 0.04, **p = 0.001, significant difference (one-tailed t test).

(B) Anatomical distribution of RFs in a single tadpole: neurons can be identified as purely OFF-dominated (red), purely ON-dominated (dark blue), or responsive to both ON and OFF stimuli (green) using 60 s OFF stimulus probing. Black circles highlight anatomical clusters.

(C) The induced plasticity correlates to strength of neuronal response to OFF versus ON stimuli (mean ± SEM of 10% bins, n = 3 tadpoles, 254 cells).

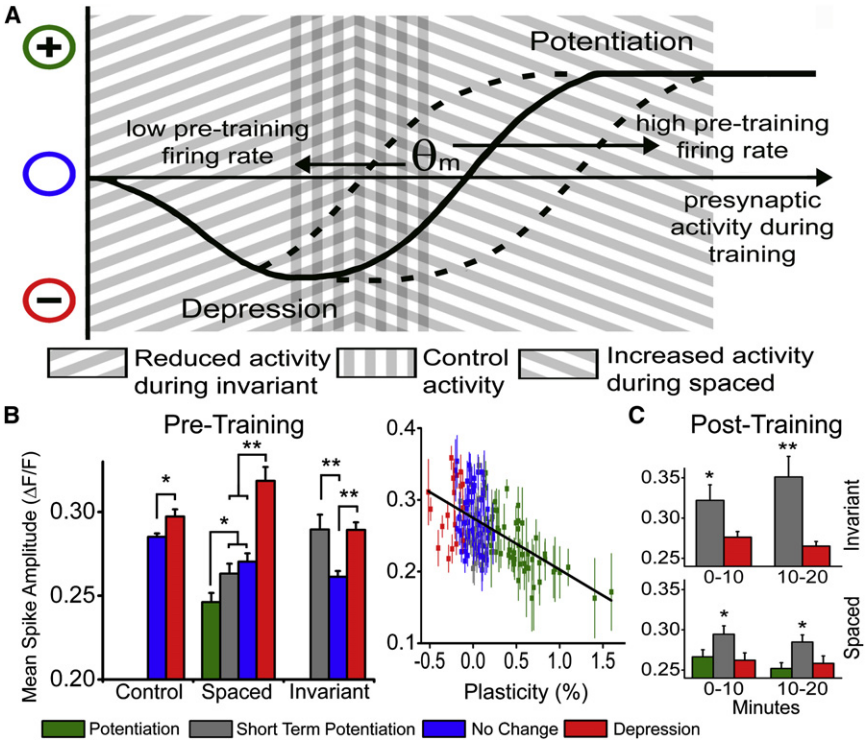

Figure 6. Metaplasticity and Stabilization of Visually Induced Neuronal Plasticity

(A) BMC theory schematic. Horizontal axis, neuronal activity during training: determined as the product of presynaptic activity and synaptic efficiency. Vertical axis, neuronal plasticity. A neuron's modification threshold during training, θ_m, is dependent on its average postsynaptic firing during pretraining. Neurons with high firing levels pretraining shift θ_m to the right, making potentiation more difficult and depression easier to induce. Neurons with low firing levels pretraining show the opposite effect. Reduced pre-synaptic activity during invariant training limits neuronal activity to below the modification threshold.

(B) Mean amplitudes (±SEM) of spontaneous Ca^{2+} events predict plasticity outcomes (left). Spontaneous activity during pretraining is significantly greater in cells that undergo long-lasting depression (red) regardless of the training paradigm. Cells that undergo long-lasting potentiation (green) after spaced training show significantly less pretraining spontaneous activity than those of other plasticity types. Short-term potentiated cells (gray) and cells showing no plasticity change (blue) after spaced training show no significant difference; whereas, short-term potentiated cells after invariant training show no significant difference from depressed cells (*$p < 0.05$, **$p < 0.01$, t tests). Mean amplitudes (±SEM) of spontaneous pretraining activity of individual neurons shows significant correlation to plasticity outcomes (right) (spaced training; black line, linear regression $R^2 = -0.55$; slope = -0.07 ± 0.019).

(C) Mean amplitudes (±SEM) of spontaneous activity posttraining is significantly lower in neurons exhibiting long-lasting plasticity (*$p < 0.05$, **$p < 0.01$, t test). n for all data sets in Figure 6 are the same as in Figure 4.

Ramdya et al., 2006; Smetters et al., 1999; Sumbre et al., 2008; Yaksi and Friedrich, 2006). While we observed no significant differences in the frequency of pretraining spontaneous calcium transients between plasticity groups, the amplitudes of spontaneous calcium transients were significantly different (Figure 6B). Cells that exhibited long-lasting functional potentiation after spaced training had significantly lower pretraining spontaneous calcium transient amplitudes than other plasticity types, while cells demonstrating functional depression had significantly higher amplitudes. These results are predicted by BCM theory (Bear, 2003; Beggs, 2001; Daoudal and Debanne, 2003; Figure 6A). Moreover, potentiation and depression are unlikely to be caused by activity differences during the training period (Figure S9). The BCM model is also supported by our findings that cells demonstrating depression following invariant training exhibited high pretraining calcium transient amplitudes. In this case, minimal presynaptic input during training prohibits neuronal activity from passing the modification threshold, preventing potentiation. In turn, neurons with up-shifted θ_m values due to high levels of pretraining activity are more likely to undergo depression (Figure 6A). Both examples provide evidence of metaplasticity within the intact developing brain, where intrinsic spontaneous firing predisposes expression of future plasticity. While these theories do not address induction of short-term plasticity, increased spontaneous firing has been shown to rapidly reduce synaptic plasticity in the tectum (Zhou et al., 2003). Here, we find that cells that undergo short-term RF plasticity have significantly higher spontaneous firing rates posttraining than posttraining firing rates of those exhibiting long-lasting plasticity, suggesting that enhanced spontaneous activity posttraining reverses functional RF potentiation (Figure 6C).

Experimentally Increased Pretraining Activity Shifts Spaced Training Plasticity Outcomes toward Depression through an NMDA-R-Dependent Mechanism

Spontaneous activity in tectal neurons consists of random single spikes and bursts of spikes (Zhou et al., 2003). White noise light stimulation, such as flashes of randomly patterned ON and OFF checker boards (Zhou et al., 2003) or rapidly varying wide-field light intensities (used here; Ramdya et al., 2006), can readily induce enhanced firing rates (Figure 2A) in tectal neurons with similar properties to endogenous spontaneous tectal activity (Zhou et al., 2003). Average calcium transient amplitude is also increased compared to pretraining probing (17% ± 9%, $p = 0.03$, t test). We presented wide-field white noise stimuli at 5 Hz to experimentally increase cell firing for 1 hr prior to SCEP imaging. After white noise stimulation, pretraining probing, spaced training, and posttraining probing were presented the same as previous experiments. White noise stimulation did not induce significant plasticity effects (59% unchanged; no significant difference from continuous probing control, chi-square test). However, enhanced activity via white noise stimulation did shift subsequent spaced training-induced plasticity

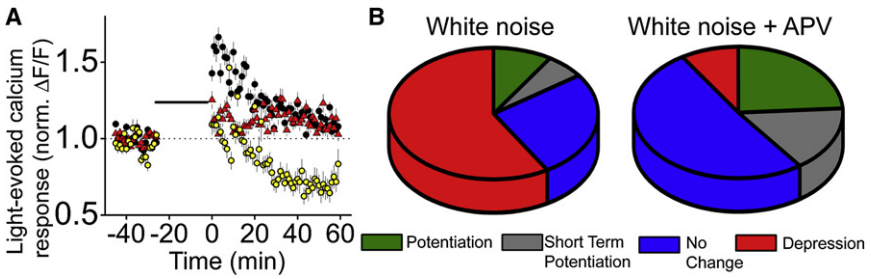

Figure 7. Increased Activity Prior to Training Shifts Plasticity Outcomes toward Depression through an NMDA-R-Dependent Mechanism

(A) Mean SCEP recordings (±SEM) of all cells after spaced training (ST, black circles), white noise priming 1 hr prior to SCEP (WN, yellow circles), and WN priming + APV injection (WN+APV, red triangles). Forty to sixty minutes posttraining: ST, 14% ± 1%; WN, 13% ± 1%; WN+APV, −26% ± 1%. ST and WN+APV show no significant difference, p = 0.44, t test. Bar denotes training periods.

(B) This shift is blocked by APV injection during the metaplasticity phase. Percentage of neurons exhibiting long-lasting potentiation (WN 9%, WN+APV 24%*), short-term potentiation (WN 6%, WN+APV 16%), no change (WN 26%, WN+APV 51%**), and long-lasting depression (WN 58%, WN+APV 9%**), after various training. ST and WN+APV show no significant difference, p = 0.1, chi-square test. ST, n = 6 tadpoles, 168 cells; WN, n = 3 tadpoles, 53 cells; WN+APV, n = 3 tadpoles, 70 cells. *p < 0.05, **p < 0.01, significant difference (chi-square test).

outcomes toward depression (Figure 7). BCM theory predicts this change, since historical enhanced firing rates will shift the modification threshold to the right (Figure 6A). These results strongly support BCM metaplasticity as a plasticity regulator in the awake embryo.

Significantly, the shift in plasticity outcome due to enhanced activity can be abolished by injection of D-APV (50 μM) directly before white noise priming during the metaplasticity phase (Figure 7). Spaced training plasticity results after white noise + APV show no significant difference from spaced training alone without priming (p = 0.1, chi-square test). The absence of reduction in potentiation compared to regular spaced training suggests that APV was washed out prior to training stimulation. Our results reveal NMDA-Rs as an endogenous mechanism for metaplasticity in the awake developing brain.

Potentiation Is Associated with an Increase in Spontaneous Correlated Firing

Does spaced training induced plasticity alter intrinsic ensemble network activity? Analysis of spontaneous activity shows significant correlated firing between tectal cells (Figure 8). Correlated firing is more common among nearest anatomical neighbors, suggesting shared afferent input or local interconnections (Tao et al., 2001) (Figure 8A). Spaced training induced a significant increase in correlated spontaneous activity 0–10 min posttraining (Figure 8B). Increased correlations of cells exhibiting long-lasting potentiation were significantly greater than other plasticity types. Cells showing long-lasting depression following spaced training showed no change in network correlation. Control tadpoles and tadpoles exposed to invariant training exhibited no change in network correlations.

DISCUSSION

Together, our results provide a circuit analysis of the effects of natural sensory experience on neural network function within the intact, awake, developing brain. Noninvasive functional imaging of sensory-induced plasticity expands upon previous electrophysiological studies (Engert et al., 2002; Pratt et al., 2008; Tao and Poo, 2005; Zhou et al., 2003) by simultaneously linking single cell and ensemble plasticity. We find that individual neurons within intact embryonic central circuits respond to plasticity-inducing sensory input in a complex manner, resulting in functional potentiation, depression, or no change toward a probed RF stimulus (Figure 4). While individual neurons show variable plasticity after visual training, mean population responses to RF stimuli can be potentiated or depressed depending on the training paradigm.

Spaced training composed of repeated trains of high-frequency OFF visual stimuli preferentially shifted cell response

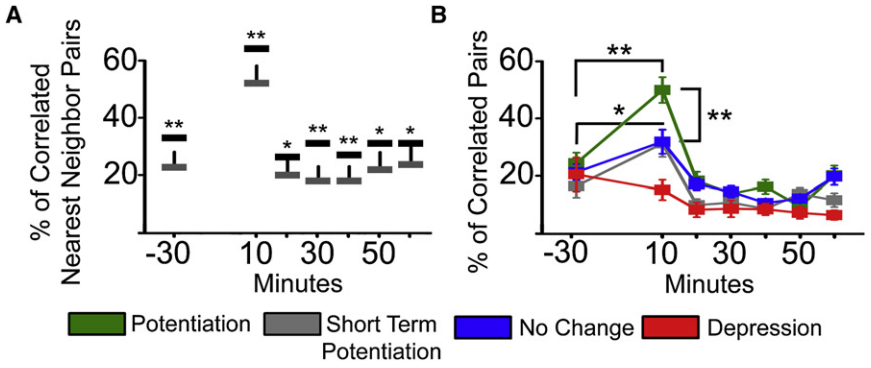

Figure 8. Network Analysis Reveals Increased Correlated Activity in Neurons Exhibiting Potentiation

(A) Correlated activity between neurons is significantly more common among nearest neighbors. Black bars denote fraction of nearest neighbor pairs with significant (p < 0.05) correlations; gray bars and tails denote bootstrapped means and 95% confidence intervals for fraction of nearest neighbor pairs with significant correlations under random reassignment of neuronal activity patterns (*p < 0.05, **p < 0.01, one-tailed t test).

(B) Correlated activity increases after spaced training. Average (±SEM) fraction of significant (p < 0.01) pairwise correlations between all cells for each plasticity group (* p < 0.05, ** < 0.01, t test). (A) and (B) data, pairwise correlations calculated over 10 min epochs; n for all data sets in Figure 8 are the same as in Figure 4.

properties toward potentiation of the trained OFF responses. The absence of change in ON RFs following OFF spaced training demonstrated clear specificity of RF plasticity to characteristics of the input training stimulus. Improved long-term neuronal performance restricted to characteristics of specific training stimuli has been demonstrated in primary visual and auditory cortex (Pantev et al., 1998; Schoups et al., 2001; Sengpiel et al., 1999; Zhang et al., 2001), though such acute effects at the embryonic stage where RFs are generally broad and unrefined are striking.

Here, we find that spaced training is also associated with an increase in correlated spontaneous firing between potentiated neurons. Although enhancement of correlated circuit activity induced by spaced training is transient, these correlations may play a significant role in functional plasticity among neurons within select subnetworks by promoting transition to long-lasting forms of potentiation (Voigt et al., 2005).

In contrast to spaced training, invariant light stimulation to the immobilized eye shifted the majority of ensemble response properties toward depression. Binocular deprivation in early postnatal development leads to similar effects, depressing synaptic transmission and rendering visual cortical neurons unresponsive to subsequent visual stimulation (Bear et al., 1987; Freeman et al., 1981; Prusky et al., 2000; Rittenhouse et al., 1999). In both invariant training and binocular deprivation, BCM theory predicts induction of depression due to decreased presynaptic activity which is insufficient to reach the threshold required for potentiation.

Variable long-lasting functional plasticity outcomes induced by both spaced training and invariant training were accurately predicted by measuring the pretraining activity of individual cells. Neurons with high spontaneous firing rates during pretraining periods exhibited predisposition for training-induced depression, while neurons with low spontaneous firing rates demonstrated predisposition to potentiation. This metaplastic result follows the BCM learning rule, where the plasticity modification threshold depends on average pretraining activity and will increase or decrease with higher or lower firing rates.

We find strong support for BCM theory by increasing pretraining activity using white noise visual stimulation. We demonstrate that white noise dramatically shifts tectal neuronal plasticity responses to spaced training toward depression. One explanation for these results is that the sliding threshold of BCM theory acts as a homeostatic mechanism to maintain synapses, dendritic integration, and resulting afferent-evoked neuronal activity within useful dynamic ranges (Abraham et al., 2001). Hence, this rule makes it more difficult for highly active neurons to potentiate further and easier for them to depress (Abbott and Nelson, 2000; Abraham, 2008; Abraham and Tate, 1997). Our findings that APV blocks the ability of white noise priming to shift plasticity outcomes implicate NMDA-R-mediated metaplasticity (Huang et al., 1992) as an underlying mechanism of experience dependent metaplasticity in the awake developing brain.

The demonstration that brief sensory experience induces variable functional plasticity throughout nascent developing neuronal ensembles as a function of each individual neuron's recent past activity and intrinsic RF properties has major implications for the functional development of neural networks. Neurons exhibit bias to refine their existing RF responses by strengthening if weak and weakening if strong, thereby maintaining responses within a functional dynamic range. Metaplasticity to restrict significant alteration in RF properties and training specificity to limit plasticity to distinct stimuli may elucidate evidence for stability in RF responses throughout maturation (Niell and Smith, 2005), as well as the ability for brief sensory stimuli to elicit substantial input specific plasticity. In this manner, environmental experience may drive discrete modification of developing central circuits to optimize performance within a relevant range.

EXPERIMENTAL PROCEDURES

Animal Rearing Conditions

Freely swimming albino *Xenopus laevis* tadpoles were reared and maintained in 10% Steinberg's solution (1 × Steinberg's in mM: 10 HEPES, 58 NaCl, 0.67 KCl, 0.34 $Ca(NO_3)_2$, 0.83 $MgSO_4$ [pH 7.4]) and housed at 22°C on a 12 hr light/dark cycle. All experimental procedures were conducted on stage 50 tadpoles (Nieuwkoop and Faber, 1967) according to the guidelines of the Canadian Council on Animal Care and were approved by the Animal Care Committee of the University of British Columbia's Faculty of Medicine.

Calcium Indicator Loading

The calcium-sensitive fluorescent indicator Oregon green 488 BAPTA-1, AM (OGB1-AM, Molecular Probes, Eugene, OR) was bulk loaded into neurons within the tadpole brain (Brustein et al., 2003; Niell and Smith, 2005; Stosiek et al., 2003). OGB1-AM was prepared at a concentration of 10 mM in DMSO with 20% pluronic acid (Molecular Probes) and further diluted 10:1 in Ca^{2+}-free Amphibian Ringers solution (in mM): 116 NaCl, 1.2 KCl, 2.7 $NaHCO_3$. Under visual guidance using an upright stereomicroscope, a sharp glass pipette loaded with OGB1-AM solution was inserted into the optic tectum of tadpoles anesthetized with 0.01% 3-aminobenzoic acid ethyl ester (MS222, Sigma-Aldrich, St. Louis, MO). Dye solution was slowly perfused into the brain using low-pressure (<10 psi) on a Picospritzer III (General Valve Corporation, Fairfield, NJ). Tadpoles were subsequently returned to normal bath solution and allowed to recover from anesthesia under dim light conditions (Brustein et al., 2003; Niell and Smith, 2005). One hour following OGB1-AM loading, tadpoles were immobilized with 5 min bath application of 2 mM pancuronium dibromide (PCD; Tocris, Ellisville, MO), embedded in 1% agarose, and placed in an imaging chamber continuously perfused with oxygenated 10% Steinberg's solution. PCD is a reversible paralytic and typically wears off 2 hr post-application at this dosage.

In Vivo Two-Photon Calcium Imaging of Neuronal Dynamics

The imaging chamber was mounted on the stage of a custom-built two-photon laser-scanning microscope, constructed from an Olympus FV300 confocal microscope (Olympus, Center Valley, PA) and a Chameleon XR laser light source (Coherent, Santa Clara, CA). Optical sections through the optic tectum were captured using a 60×, 1.1 NA, water immersion objective (Olympus), and images were recorded and processed using Fluoview software (Olympus). The optic tectum was imaged at a resolution of 640 × 480 pixels and zoom factor of 1.5×, encompassing an area of 177 × 133 μm, allowing simultaneous imaging of approximately 100–200 neurons in a single X-Y scan. Repeated X-Y scans of a single optical section were taken at a rate of 1.2 s per frame using a wavelength of 910 nm to excite OGB1-AM dye. The unique dye-loading pattern allows morphological corrections for drift over time without the need for a secondary morphological marker (confirmed by dual labeling with OGB1-AM and Red-fluorescent CellTracker Red CMTPX; Molecular Probes, Eugene, OR). Drift corrections (if necessary) were made every four minutes.

Visual Stimulation

To apply light stimuli, a diode (590 nm) was projected through the camera port of a trinocular eyepiece for whole-field illumination. A colored Wratten Filter 32 (Kodak, Rochester, NY) assured no bleed-through into the imaging channel. Illumination intensity, timing, and duration of light stimuli was varied with

custom written software (Matlab, The Mathworks Inc., Natick, MA) synched to the microscope's 'ttl' output for the onset of frame scanning. Step changes in whole-field light intensity from the background illumination were used as visual stimuli.

Single-Cell Excitability Probing (SCEP)

Evoked responses to visual stimuli were probed every 60 s during 20 min pretraining and 60 min posttraining (Figure S3A). Two visual stimuli were used for SCEP probing: a 50 ms OFF stimulus (Figure S3A) and a 60 s OFF stimulus (Figure S3B). For control stimulation, 60 s probing was continued throughout the training window (Figures S3A and S3C). Evoked OFF responses were recorded at each 50 ms stimulus and at the beginning of each 60 s stimulus. Evoked ON responses were recorded at the end of each 60 s stimulus.

Visual Training

To test RF plasticity after visual training, we utilized two separate training stimuli, each 25 min in duration (Figures S3A and S3C). Invariant training consisted of 25 min ON light stimulation to the paralyzed eye. Spaced training consisted of three sets of 90 50 ms OFF stimuli at 0.3 Hz, spaced by 5 min intervals of ON stimulation. Previous studies have demonstrated persistent 5 min spaced training with a moving bar stimulus can induce long-term synaptic plasticity in the presence of endogenous spontaneous neuronal activity (Zhou et al., 2003).

White Noise

Wide field white noise stimulation was accomplished by random variation of the diode voltage at 5 Hz between empirically determined maximum and minimum intensity values within the diode's linear range. White noise was presented to the paralyzed eye for one hour prior to pretraining SCEP probing.

Blockade of NMDA-R

For NMDA-R blockade of spaced training and invariant training induced plasticity, tadpoles were injected with 50 μM D-APV (Sigma Aldrich, St. Louis, MO) immediately before training. For NMDA-R blockade during the metaplasticity phase, 50 μM D-APV was injected one hour prior to pretraining imaging, during which time white noise visual stimulation was presented to the tadpole.

Analysis of Imaging Data

Fluorescence data stacks were initially x-y aligned using Turboreg (Thévenaz et al., 1998; ImageJ, NIH). Experiments that showed z-drift after alignment were discarded (approximately 1 in 4 cases). For each experiment, individual regions of interest (ROIs) were manually drawn over neuronal cell bodies in each optical section and analyzed using custom written software. For each neuron ROI the change in fluorescence intensity was calculated as $\Delta F/F_0 = (F - F(t)_{base})/ F(t)_{base}$, where F is the average intensity of the ROI in an image frame and F_{base} is a simple linear regression fit to image frames with florescence values one standard deviation from the minimum-recorded fluorescence intensity. This fitting served to eliminate the amplitudes of spontaneous spiking events from weighting the baseline fluorescence trace. Images frames within 36 s (30 frames) of an evoked response were not included in the linear regression.

Evoked Responses

SCEP records the evoked responses of single neurons over time. Response amplitudes were taken to be peak $\Delta F/F_0$ within three image frames after the visual stimulus. Only evoked responses with ΔF/F peak values >1.5 standard deviations (STD) above the mean baseline fluorescence trace were included in analysis (standard deviation of average baseline fluorescence trace, $\Delta F/F_0 = 0.09$). Somatic Ca^{2+} spikes elicited by visual OFF stimuli are rapid (<100 ms), long-lasting (peak amplitudes of approximately 2 s), and reproducible (Figure S1). ON stimuli evoke similarly long duration and consistent amplitude somatic calcium events, yet spike initiations are slower (approximately 900 ms to peak) than OFF responses.

Spontaneous Spiking

Fluorescence values were considered spikes if their $\Delta F/F_0$ > STD above average baseline fluorescence trace and they showed characteristic fast onset. Coincident peak values >STD above the mean were taken as new spikes if $\Delta F/F_0$ values between events fell below the initial peak minus STD. To assure spontaneous spiking was not influenced by evoked responses, image frames within 48 s (40 frames) of an evoked response were not included in spontaneous activity analysis.

Inclusion Criteria

For all analysis, only cells with >70% of probed evoked responses with $\Delta F/F_0$ peak values >1.5 STD above the mean were included. In addition, cells were required to have consistent pretraining responses, where the mean evoked responses of three out of four 5 min epochs of SCEP pretraining (0–5, 5–10, 10–15, 15–20) could not differ significantly from the total mean evoked pretraining responses (0–20 min; unpaired heteroscedastic two-tailed t test). Cells with constantly drifting evoked responses were also excluded. These were determined by fitting pretraining responses with a linear regression and excluding all cells with slope values not equal to 0 within the 95% confidence interval. 92% of responding cells fit these criteria.

Plasticity Criteria

For long-term changes, response properties were assayed by comparing responses during the 20 min of pretraining to both the responses during the first 20 min of posttraining and the responses between 40 and 60 min posttraining. Cells undergoing long-lasting potentiation and long-lasting depression showed significant changes both immediately and persistently after training (t test, $p < 0.05$). Cells undergoing short-term potentiation showed significant increase in responses during the first 20 min posttraining but no significant change in responses 40–60 min posttraining. Cells that showed no significant change in responses were categorized as "no change."

Spontaneous Correlations

Pairwise correlations of spontaneous activity were calculated with the Pearson product-moment correlation coefficient (Rodgers and Nicewander, 1988). Correlations were calculated over 10 min epochs.

Clustering

Numerical simulation was used to estimate the probability that observed levels of anatomical clustering of receptive fields and correlated activity arose under a null hypothesis of no spatial organization. To do this, the observed receptive field values or activity patterns were randomly reassigned among neurons within a given tadpole and clustering measures were calculated. This process was repeated 100,000 times. Reported p values are the fraction of simulations that showed more clustering than was measured in the original data.

SUPPLEMENTAL DATA

Supplemental Data include nine figures and can be found with this article online at http://www.cell.com/neuron/supplemental/S0896-6273(09)00672-2.

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