Sensorimotor Mismatch Signals in Primary Visual Cortex of the Behaving Mouse

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INTRODUCTION

Responses of cells in visual cortex have generally been probed under conditions of passive viewing. Activity in visual cortex of anesthetized (Hubel and Wiesel, 1962, 1968; Niell and Stryker, 2008), awake paralyzed (Livingstone and Hubel, 1981), and awake fixating (Wurtz, 1969) animals has been shown to closely reflect the visual stimuli presented to the animal. However, visual input under natural conditions is largely self-generated visual feedback either in the form of saccades or in the form of visual flow during head, body, and slow eye movements. In freely viewing animals (Livingstone et al., 1996; Gallant et al., 1998; Fiser et al., 2004), the relationship between visual stimulus and activity in visual cortex is less clear, such that activity during natural vision has been hypothesized to be driven largely by ongoing cortical dynamics and only to a lesser extent by visual input (see also Tsodyks et al., 1999). More recent evidence in rodents has shown that locomotion influences visually driven responses in visual cortex (Niell and Stryker, 2010). One important function of the integration of sensory and motor signals in visual cortex could be the detection of feedback mismatch, i.e., changes in visual signals that cannot be predicted by motor output. Selective responses to feedback perturbations have been found in other modalities, for instance, in primary auditory areas of the zebra finch (Keller and Hahnloser, 2009) and the marmoset monkey (Eliades and Wang, 2008a), suggesting that already primary auditory areas are involved in feedback mismatch detection. In visual cortex, however, the role of motor-related signals in the processing of visual input, in particular in primary areas, remains elusive.

To investigate visual feedback processing in visual cortex, we used a visual-flow feedback paradigm in which the animal moves along a virtual corridor while head fixed on a spherical treadmill. With this setup, we could probe for visual feedback signals in a closed-loop configuration by coupling visual flow to the mouse’s locomotion, such that the speed of the moving grating was linearly related to the mouse’s locomotion on the ball (see Movie S1 available online). This approach also allowed for an open-loop configuration with the animal passively viewing visual flow. Finally, we also probed for responses to brief perturbations of the coupling between visual flow and locomotion (feedback mismatch) and for responses during locomotion in darkness. We found both a strong motor-related drive in visual cortex during running in darkness and clear responses to feedback mismatch.

RESULTS

We recorded neural activity in visual cortex of behaving mice using two-photon imaging of neurons expressing a genetically encoded calcium indicator (AAV2/1-hsyn1-GCaMP3; Tian et al., 2009; see Movie S2). Animals were head fixed on a spherical treadmill (Dombeck et al., 2007) flanked by two monitors that provided visual flow in the form of full-field vertical gratings coupled to the mouse’s movement on the ball (see Figure 1A). This setup allowed us to monitor calcium activity in visual cortex during both self-generated visual-flow feedback and playback of visual flow that was unrelated to its present locomotion but was generated by the mouse during a previous feedback session. In addition, we used brief (1 s) perturbations of visual flow in the form of flow halts to probe feedback mismatch responses. Each experiment consisted first of recording neural activity during 2 min of normal feedback activity (feedback session) with perturbations occurring at random times (on average four perturbations per minute, see Experimental Procedures), and then replaying the same visual flow three times (playback sessions), spaced by 2 min intervals of normal visual-flow activity.
feedback. The animal was free to run during the entire experiment, including playback sessions, and did so spontaneously (average fraction of time spent running was 0.22 ± 0.02 [mean ± SEM, n = 27 experiments in 7 mice], a value comparable to that reported by Dombeck et al., 2007). Feedback sessions were selected heuristically by the experimenter to be sessions of high running activity (average fraction of time spent running: 0.39 ± 0.03). During playback sessions, running activity levels remained stable (average fraction of time spent running: first playback session, 0.18 ± 0.04; second playback session, 0.16 ± 0.03; third playback session, 0.19 ± 0.05). We refer to phases of running coupled with visual flow as feedback, phases of running without visual flow as feedback mismatch, phases of sitting with visual flow as playback, and phases of sitting without visual flow as baseline. Note that feedback mismatch resulted both from brief feedback perturbations during feedback sessions and from running during playback sessions at times of no visual flow.

**Visual Input Alone Is a Poor Predictor of Activity in Primary Visual Cortex in the Behaving Mouse**

We recorded from a total of 1,598 layer 2/3 cells in monocular visual cortex of behaving mice. Roughly 73% of the cells (1,171 of 1,598) were active (see Experimental Procedures) at least once during the entire recording session (each lasting between 480 to 960 s, mean: 627 s). In interpreting these numbers, it should, however, be kept in mind that due to the fact that cells were selected also based on activity (see Experimental Procedures), our sampling of cells was biased toward active cells. We found that roughly half of the cells were active during running with visual-flow feedback (784 of 1,598 cells, see Experimental Procedures). In 269 of the cells, we also...
recorded the activity while the animal was spontaneously running in darkness. Note that in darkness the average fraction of time spent running was significantly higher at 0.70 ± 0.11 (mean ± SEM, n = 6 experiments in 3 mice).

To our surprise, we found the activity of only a relatively small fraction of neurons to be well explained by visual input alone. To quantify which fraction of variance of activity of each neuron could be explained by running or by visual flow, respectively, we calculated the correlation between activity and a binary running and a binary visual-flow vector for every neuron (Figure 1B). We found that the average fraction of activity explained by running ($R^2 = 0.041$) was significantly higher ($p < 10^{-10}$, Wilcoxon signed-rank test) than that explained by visual flow ($R^2 = 0.012$). We further compared responses during self-generated feedback to average responses to playback of the same visual flow and found that only about 22% (365 of 1,598 cells, an example depicted in Figure 1C) of the cells showed a significant positive correlation (Pearson’s correlation coefficient $> 0$, $p < 0.01$). This suggests that a large part of the feedback-related activity is not merely visually driven and might be motor related.

### Locomotion Drives Neural Responses in Primary Visual Cortex

As would be predicted from earlier results that showed increased activity to visual stimulation during running (Niell and Stryker, 2010), we found that average responses during feedback (average $\Delta F/F$: 5.6% ± 1.0%; Figures 1E and 1F) were significantly higher than average responses during playback (average $\Delta F/F$: 1.8% ± 0.5%; Figures 1E and 1F; all pairwise comparisons: $p < 10^{-10}$, Wilcoxon signed-rank test). To test whether motor-related signals are capable of driving visual responses completely without any visual input and to estimate the contributions of both motor-related input and visual input separately, we compared activity levels during feedback and during playback to activity during running in darkness. The responses we measured in darkness were often directly coupled to running activity (see Figure 1D for two example neurons that responded to running onset and offset, respectively). Surprisingly, we found that average activity during running in darkness, in absence of visual input (average $\Delta F/F$: 3.0% ± 0.6%; Figures 1E and 1F), was comparable in magnitude to the activity during playback, i.e., purely visually driven activity. This demonstrates that activity in visual cortex is not only modulated, as has been shown previously (Niell and Stryker, 2010), but is strongly driven by motor-related input. Furthermore, linear summation of average fluorescence during playback and running in the dark could account for most of the activity during feedback (4.8%; Figure 1F).

### Mismatch between Predicted and Actual Visual Feedback Strongly Drives Neural Responses in Primary Visual Cortex

To probe for signals that are potentially contingent on both motor-related and visual signals, we analyzed responses to perturbations of feedback during running on a single-cell basis. In agreement with the idea that there is motor-related activity in visual cortex, we found that many cells responded during running (Figures 2A and 2C, cell 1,049; see also Figure S1).

More interestingly, we found that a subset of cells responded predominantly during feedback mismatch ($n = 208$ or 13.0%, Figures 2A and 2B, cell number 677; see Experimental Procedures). We also found cells that responded predominantly during feedback ($n = 377$ or 23.6%, Figures 2A and 2C, cell number 452). Both of these latter signals require the integration of motor-related signals, potentially in the form of a prediction of visual feedback, with visual signals. We did not observe any indications for spatial clustering of different response types.

The feedback mismatch response was apparent not only in the responses of single cells, but also in the population average. Interestingly, the feedback mismatch onset response was much stronger than the average population response to running onset or to playback onset (Figure 3A; see also Figures S2 and S3). Averaged over the entire population and all feedback mismatch onsets, the peak $\Delta F/F$ change triggered on feedback mismatch onset was $3.3\%$ (1,598 cells, 266 feedback mismatch onsets, Figure 3A). Peak average running onset response (peak $\Delta F/F$ change: 1.5%) and playback onset response (peak $\Delta F/F$ change: 0.5%) were both significantly smaller ($p < 10^{-10}$, Wilcoxon signed-rank test). In agreement with this, we found that 334 of 1,598 cells showed significantly increased activity in a time window 0–1 s after feedback mismatch onset, as compared to average activity in the 1 s time window immediately preceding the feedback mismatch ($p < 0.01$, Student’s t test). The feedback mismatch-triggered response could not be explained by visual input alone, as there was no average population response to passive viewing of playback halts (Figure 3A). This shows that the feedback mismatch response was contingent on a coincidence of stopping of visual flow and running.

### Feedback Mismatch Responses Are Contingent on Previous Experience and Trigger a Behavioral Response

To test whether feedback mismatch responses are contingent on a learned correspondence between locomotion and visual feedback, we analyzed the time course of feedback mismatch signals in the open-loop condition (visual-flow feedback not driven by running). We found that feedback mismatch responses became smaller the longer the animal was exposed to an open-loop condition, which occurred during playback sessions. Feedback mismatch responses during the third playback session were significantly smaller than feedback mismatch responses during the first playback session (Figure 3B; $p < 10^{-10}$, Wilcoxon signed-rank test). This suggests that signals coding for expectations that link motor output to predicted sensory feedback are present in visual cortex and that these signals can be rapidly modified based on recent correlation of motor output and sensory feedback. Animals also showed a behavioral response to feedback mismatch. Average running speed triggered on feedback mismatch onset significantly decreased after feedback mismatch onset ($p < 10^{-4}$, Wilcoxon rank-sum test). This indicates that animals can not only detect feedback mismatch, but also that it is a behaviorally salient stimulus.

### Feedback Mismatch Responses Encode the Degree of Mismatch

Feedback mismatch signals would be expected to reflect the degree of mismatch. To test for this, we binned the feedback...
mismatch responses of the 2% of neurons with the strongest feedback mismatch response (31 of 1,598) by the animals’ running speed just prior to the feedback perturbation. If the animal runs faster, visual flow is faster and thus the perturbation-induced change in flow speed, and therefore mismatch, is larger. Feedback mismatch responses clearly increased with running speed just prior to perturbation onset (Figures 3C and 3D), indicating that a larger mismatch leads to a larger feedback mismatch response.

**Eye Movements Have Only Limited Influence on Average Population Activity**

It is well established that eye movements can modulate the responses of visual cortex (Wurtz, 1968; MacEvoy et al., 2008). To control for possible effects of eye movements, we recorded pupil position during all experiments. Average pupil position was independent of visual flow and feedback mismatch and only exhibited a small running-induced shift (2.1° nasal, 1.8° ventral; see Figure S4) that was considerably smaller than both the average size of receptive fields in mouse visual cortex (5°–15°; Niell and Stryker, 2008) and the field of view covered by the full-field gratings. The number of saccades during nonrunning phases was 0.13 ± 0.008 saccades per second (mean ± SEM, n = 27 experiments in 7 mice), comparable to previous reports (Sakatani and Isa, 2007). Passive viewing of playback had no effect on saccade frequency (0.12 ± 0.007 saccades per second). During running, however, average saccade frequency was significantly higher (0.30 ± 0.016 saccades per second). To test whether the increase of neural activity during running could be explained by the increased frequency of saccades, we calculated average saccade-triggered activity and found that the peak average saccade-triggered population response (peak ΔF/F change: 0.2%) was smaller even than the playback onset-triggered response (p < 10^-10, Wilcoxon rank-sum test). On average, saccades elicited surprisingly little activity in visual cortex. This could be explained by the fact that the visual stimulus we used was a full-field grating and thus resulted in similar visual input independent of exact eye position.

**DISCUSSION**

Our data demonstrate that visual cortex receives surprisingly strong and ubiquitous motor-related input in addition to visual input. Moreover, we found that visual input alone is a poor predictor of neural activity. Instead, certain combinations of visual input and locomotion, namely mismatch between running and visual feedback, proved to be much better predictors of neural activity.

To record neural activity in the behaving animal, we have employed functional two-photon imaging of the genetically encoded calcium indicator GCaMP3. As compared to more standard electrophysiological recording techniques, functional imaging offers two main advantages. One is the higher number of neurons that can be recorded simultaneously during an
The other advantage is that by imaging one gains information on the anatomical location of every recorded cell and is thus able to determine, e.g., cortical layer of origin, with high reliability and can detect patterns in the spatial arrangements of neurons having certain functional responses. However, the use of GCaMP3 as a functional indicator might lead to an underestimation of activity levels, as GCaMP3 only reports signals when firing rates are above a certain threshold (two to three spikes in a 500 ms window; Tian et al., 2009). In principle, it is conceivable that visual input causes more distributed firing than motor-related signals and is thus underrepresented in our data. Typical peak instantaneous firing rates in layer 2/3 neurons of mouse visual cortex in response to presentation of full-field gratings are above 10 Hz (Niell and Stryker, 2008) and should thus be well within the dynamic range of GCaMP3. Hence, while the use of GCaMP3 might prevent us from detecting weak activity, it is very unlikely that we are missing most of the visual signals.

Previous work has shown that locomotion can amplify responses of neurons in mouse visual cortex to the presentation of drifting gratings (Niell and Stryker, 2010). Our data go even further: they show that locomotion can drive neural responses in visual cortex in complete absence of visual input. This is evidence of a strong motor-related input to visual cortex capable of driving neural responses. A recent study by Szuts et al. (2011) using wireless recordings from the visual cortex of free-ranging rats came to similar conclusions. However, because there was no decoupling between visual input and locomotion, this study was not able to dissect the relative contributions from each source.

Motor-related input to visual cortex could come directly from motor cortex or indirectly via secondary visual areas. Direct interactions between sensory and motor cortices have been shown to play an important functional role in the mouse vibrissal system (Matyas et al., 2010; Mao et al., 2011). In the rat, primary visual cortex has been shown to be reciprocally connected with...
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cortical motor areas (Miller and Vogt, 1984). It is also possible that motor-related input is relayed to primary visual cortex via secondary visual areas that send strong projections back to motor cortex (Wang et al., 2011).

The fact that we also find feedback-mismatch signals in visual cortex provides a possible explanation for the presence of motor-related signals: theories of sensorimotor integration (Wolpert et al., 1995) postulate that an internal model is used to predict sensory feedback based on an efference copy signal of the motor command. Actual sensory feedback can then be compared to this predicted sensory feedback to detect mismatches between the two. These mismatch signals in turn could then be used for feedback control of motor output. Based on these theories, brain regions involved in sensorimotor integration should contain motor-related signals, sensory signals, and mismatch signals. The fact that our data show that all three signals exist in visual cortex of behaving mice seems to suggest that already primary visual cortex is involved in sensorimotor integration.

Auditory cortex of marmosets and auditory pallium of zebra finches have been shown to exhibit responses consistent with predictive coding (Elidades and Wang, 2008b; Keller and Hahn-loser, 2009), and even extraclaustral receptive-field effects in visual cortex can be well explained in a predictive coding framework (Rao and Ballard, 1999). Our data now show that mouse visual cortex uses predictive coding strategies during processing of self-generated visual feedback. It is important to note that this is not at odds with earlier studies performed in a non-behaving context, as motor-related signals would not be apparent in such experiments, and responses would therefore largely reflect sensory input.

Thus, our data add to the accumulating evidence for the idea that cortical sensory processing—even at the earliest stages—involves predictions and the calculation of mismatch between predicted and actual sensory feedback and therefore goes beyond pure feedforward processing schemes.

EXPERIMENTAL PROCEDURES

Subjects and Imaging
All experimental procedures were carried out in accordance with the institutional guidelines of the Max Planck Society and the local government (Regierung von Oberbayern). Data were collected from seven adult (postnatal days 67–234 [P67–P234]) C57/BL6 mice. Mice were injected with AAV2/1-hsyn1-GCaMP3 between P39 and P55. At the time of virus injection, 5 mm circular glass coverslips were implanted flush with the skull. This resulted in a slight compression between P39 and P55. At the time of virus injection, 5 mm circular glass coverslips were implanted flush with the skull. This resulted in a slight compression of the brain in the center of the cranial window but had the advantage of preventing bone growth and dramatically reducing movement artifacts during awake imaging. Experiments were carried out 2–26 weeks postinfection.

Functional calcium imaging was performed with a custom-built two-photon microscope. Illumination source was a Spectra Physics MaiTai eHP Laser with a DeepSee prechirp unit (<70 fs pulse width, 80 MHz repetition rate). We used an excitation wavelength of 910 nm and a 525/50 emission filter (BrightLine HC 525/50). The scanhead was based on a 4 kHz Cambridge Technology resonant scanner, used in bidirectional mode. This enabled frame rates of 18.5 Hz at 400 × 600 pixels. We used a Nikon 16×, 0.8 NA and an Olympus 40×, 0.8 NA objective. Data were acquired with a 10 MHz data acquisition card (National Instruments, PCI-6115).

Trackball and Visual Stimulation
Animals were head fixed and free to run on a spherical treadmill based on the design of Dombeck et al. (2007) (air-supported polystyrene foam ball).

Rotation of the ball around the vertical axis was restricted with a pin. This significantly reduced the time required for the animals to exhibit normal spontaneous running behavior on the ball. Mice were prevented from seeing the ball using a black cover screen. Visual stimuli were presented on two screens arranged at an angle of 60° relative to each other in front of the mouse, covering 180° in the horizontal axis and 50°–65° in the vertical axis of visual space (see Figure 1A). This arrangement of screens simulated visual flow similar to that experienced when running between two walls. Visual stimuli presented on the screen were full-field vertical gratings. Motion of full-field gratings either was controlled by the mouse’s movement on the ball (closed-loop configuration, forward running induced movement of the gratings in the opposite direction) or was playback of visual flow generated by the mouse in a previous session (open-loop configuration). Note that mice were free to run during playback sessions and even showed a tendency to match running to playback of visual flow—that is, in some experiments, onset of visual flow induced the animal to run. In addition, in the closed-loop configuration, visual feedback was perturbed by briefly halting visual flow for 1 s at random times (Poisson distribution, with a probability of 0.25% of perturbation every 30 ms time bin, with a refractory period of 3 s).

Experimental Design
Animals were briefly (approximately 10 s) anesthetized with isoflurane for head fixation on the ball. Animals were then allowed to get used to the head fixation and the setup and were exposed to normal visual feedback for 10–30 min. Experiments consisted of a 2 min feedback session with 1 s perturbations of visual flow at random times and a series of three to four playback sessions, each typically consisting of 2 min feedback without perturbations and 2 min of replay of the visual flow generated by the mouse during the first 2 min feedback session (including perturbations). In initial experiments, data were not recorded during the 2 min feedback without perturbations between playback sessions. Thus, each experiment consisted of 8–16 min of recording.

Eye Tracking
During all experiments, the animal’s left eye was filmed with a video camera (The Imaging Source, 30 fps). Pupil position and pupil diameter were extracted online with custom-programmed software based on the design of Sakatani and Isa (2007).

Data Analysis
Two-photon images were full-frame registered using a custom-written registration algorithm. The standard deviation of brain displacement parallel to the imaging plane was 2.4 ± 0.3 μm (mean ± SEM). All data in which cells visibly moved perpendicular to the imaging plane were discarded. Cells were selected based on mean and maximum projections of the data by hand (typically the nucleus was excluded from the selection). Use of the maximum projection ensured the inclusion of all active cells, even ones that were not visible in the mean projection. This slightly biased our cell selection toward active cells. Fluorescence traces were calculated as average fluorescence of pixels lying within the cell in each frame. To remove slow signal changes in raw fluorescence traces, we subtracted the 8th percentile value of the fluorescence distribution in a ±15 s window from the raw fluorescence signal (Dombeck et al., 2007). In addition, signals were low-pass filtered at 10 Hz. ΔF/F signals were calculated by dividing raw fluorescence signal by the median calculated over the entire fluorescence distribution of each cell and then subtracting 1 from this value.

To minimize the influence of movement artifacts on average fluorescence measurements, we estimated the movement-related signal noise by calculating the standard deviation ΔMN of the lower half of the fluorescence distribution [ΔF/F < median(ΔF/F)] for each cell individually. For the calculation of time averages of ΔF/F (Figures 1F and 2B), all ΔF/F values < 3.72 × ΔMN were set to 0 (3.72 is the approximate 2 score value of p = 0.9999, i.e., only 1 in 10,000 values will be above this threshold by chance). The median ΔMN value was 0.078, and 95% of ΔMN values were below 0.2.

Cells were considered active if they crossed the 3.72 × ΔMN threshold for at least 500 ms (nine frames). Cells were considered predominantly activated by a stimulus condition if average response during one condition was at least twice as high as average response during any of the other conditions.
SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures and two movies and can be found with this article online at doi:10.1016/j.neuron.2012.03.040.

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Figure S1. Shown are activity traces (ΔF/F) of 10 cells recorded simultaneously during one experiment, all classified as active at least once during the recording session. Note that the different noise levels arise due to different values of the baseline fluorescence (F) in the ΔF/F calculation.
Figure S2. Feedback mismatch can arise in two ways: a) the animal is running and visual flow stops (induced by a perturbation of visual flow, referred to as type 1), and b) the animal starts running in a playback session during a period of no visual flow (type 2). Feedback mismatch neurons respond to both types of mismatch. (A) Sample fluorescence trace of a feedback mismatch selective neuron during a playback session. Feedback mismatch type 1 and type 2 are marked by red and blue arrows. Note that the neuron responds to both type 1 and type 2 onsets. (B) Average response to feedback mismatch type 1 (red) and type 2 (blue) of the 2% strongest feedback mismatch neurons (31 of 1598 cells).
Figure S3. Shown on the left are average population feedback mismatch responses (as in Figure 3A, orange curve) for all of the experiments with at least 50 neurons (15 of 23), and on the right, average population feedback mismatch responses for all animals with more than 2 experiments (5 of 7).
Figure S4. Influence of running, playback and feedback mismatch on eye position. Eye tracking data was recorded during all experiments (the data from one experiment was excluded from analysis as recording quality did not permit automatic pupil tracking). The total dataset used for analysis comprised 4.5 hours of eye tracking data recorded in 7 animals. (A) Comparison of pupil position during times when the animal is running (red) and during times when the animal is not running (blue). Left: Distribution of pupil center position. White (black) indicates frequent (infrequent) pupil position. Number in the top left corner indicates fraction of total time spent in the respective condition. Right: Marginal distributions of pupil center position. Average pupil position is shifted 1.8 degrees ventrally and 2.1 degrees nasally during running. (B) Comparison of pupil position during feedback mismatch and during feedback conditions. (C) Comparison of pupil position during playback and during baseline conditions. (D) Sample image of the eye tracking recording.