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State-dependent performance of optic-flow processing interneurons

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interneurons

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Abstract

Active locomotive states are metabolically expensive and require efficient sensory processing both to avoid wasteful movements and to cope with an extended bandwidth of sensory stimuli. This is particularly true for flying animals, because flight, as opposed to walking or resting, imposes a step-like increase in metabolism for the precise execution and control of movements. Sensory processing itself carries a significant metabolic cost, but the principles governing the adjustment of sensory processing to different locomotor states are not well understood. We use the blowfly as a model system to study the impact on visual processing of a neuromodulator, octopamine, which is known to be involved in the regulation of flight physiology. We applied an octopamine agonist and recorded the directional motion responses of identified visual interneurons known to process self-motion-induced optic flow to directional motion stimuli. The neural response range of these neurons is increased, and the response latency is reduced. We also found that, due to an elevated spontaneous spike rate, the cells’ negative signalling range is increased. Meanwhile,
the preferred self-motion parameters the cells encode were state-independent. Our results indicate that in the blowfly energetically expensive sensory coding strategies, such as rapid, large responses, and high spontaneous spike activity, could be adjusted by the neuromodulator octopamine, likely to save energy during quiet locomotor states.
Introduction

Locomotion increases the dynamic ranges of sensory stimuli from the values at rest. As a consequence, cells involved in sensorimotor control may need to adjust their dynamic response range to maintain efficient movements. This is particularly true for visually oriented animals, as the retinal image flow induced by self-motion varies considerably with locomotor state. Locomotion also involves body-wide physiological changes that help meet the energetic demands. We were interested to understand how these physiological changes affect sensory processing.

Neural coding itself comes at a significant energy cost, with the central nervous system in humans using 20% of the resting energy budget (Attwell and Laughlin 2001; Lennie 2003), and the retina of the blowfly using an estimated 8% of the resting metabolic rate (Howard et al. 1987). However, locomotion can increase the maximal metabolic rate by a factor of 40 from the basal level in mammals (Weibel 2002), and by greater factors in insects during flight (Dudley 2000). It is not clear how the nervous system adjusts to an increased sensory dynamic range caused by locomotion, when the metabolic demands due to muscular activity are also significantly elevated. We have investigated whether the coding properties of fly visual interneurons differ between resting and a pharmacologically-induced physiological state change.

We used systemic application of an octopamine agonist, chlordimeform (CDM), to induce a physiological state-change in the blowfly. We chose the blowfly as a model system, because the cells and circuits that process visual motion information, and contribute to motor control, are exceptionally well studied (rev.: Krapp and Wicklein 2008). Furthermore, the blowfly’s head movements dictate the optic flow.
experienced, and these movements have been well studied in different locomotor
states. For instance, during flight the amplitude of head yaw rotations and the velocity
of head rolls may be double those while walking (Hateren and Schilstra 1999; Blaj
and van Hateren 2004).

Octopamine is an invertebrate neurotransmitter, neuromodulator and neurohormone
associated with “fight-or-flight” responses in many species (Roeder 2005).
Octopamine agonists switch the neural state from resting to active in many insects
(locusts: Sombati and Hoyle 1984; hawk moths: Kinnamon et al. 1984), and alter the
metabolism for flight (Wegener 1996). In crickets and locusts, octopamine levels in
the haemolymph increase upon take-off (Goosey and Candy 1980; Adamo et al.
1995), and in Drosophila it plays a significant role in flight initiation and maintenance
(Brembs et al. 2007). The highest density of octopamine receptors is in the optic lobes
(Roeder and Nathanson 1993), and octopaminergic neurons specifically innervate the
optic lobes and flight muscles in the blowfly, suggesting their role in vision and flight
motor activity (Schlurmann and Hausen 2003; Sinakevitch and Strausfeld 2006).

Key elements along the blowfly visuomotor pathway are the lobula plate tangential
cells (LPTCs). They are individually identifiable and their visual response properties
have been described in great detail (rev.: Hausen 1984, 1993; Egelhaaf et al. 2002;
Borst and Haag 2002). LPTCs are connected to descending interneurons and motor
neurons, providing signals to the various motor systems (Strausfeld and Bassemir
1985; Strausfeld and Seyan 1985; Haag et al. 2007; Wertz et al. 2008; Huston and
Krapp 2008). The receptive field organization of two spiking LPTCs, V1 and V2,
suggests they analyse optic flow generated during banked turns and roll rotations,
respectively (Hausen 1984, 1993; Krapp 1995; Krapp et al. 2001; Karmeier et al.
We recorded and analysed the responses of the V1 and V2 cells, to local visual motion stimuli, before and after the application of CDM.

**Materials and Methods**

**Preparation**

Experiments were performed on female blowflies, *Calliphora vicina*, either taken from cultures in the Department of Zoology, Cambridge, U.K., or wild specimens, caught next to East Reservoir, London, U.K. Because the majority (36 out of 38) were from the culture stock, we could check for age effects in the results. All culture flies were between 2 and 20 days old. The results in the two wild specimens were not different from those observed in culture stock flies. The flies were briefly immobilized using ice before their legs, wings and mouthparts were removed and the wounds covered with wax. The antennae were left intact. The head and body were held in position on a custom holder using wax. The orientation of the head was aligned with the stimulus apparatus by using the deep pseudopupil (Franceschini 1975). Portions of the cuticle covering the rear head capsule were removed and the fatty tissue and air sacs were removed to expose the lobula plate on both sides of the head. The tissue was kept moist using fly Ringer solution (recipe as described in Karmeier et al. 2001).

**Electrophysiology**

We recorded extracellularly the heterolateral LPTCs V1 and V2 in the right-hand lobula plate using 3 MΩ tungsten electrodes (FHC Inc., U.S.A.). The indifferent electrode was a tungsten electrode placed in the haemolymph through a small hole in the left head capsule. The signal was bandpassed filtered (0.5-10 kHz), amplified
using a custom built EPV 88 amplifier (Workshop, Max Planck Institute for Biological Cybernetics, Tübingen, Germany), and sampled at 10 or 20 kHz using a NI PCI-6025E data acquisition card (National Instruments Corp., U.S.A.). Visual stimulation (see below) and data acquisition were controlled by interface software written in MATLAB (MathWorks Inc., U.S.A) called MATDAQ (© H.P.C. Robinson, 1997-2001). We used custom-written MATLAB software to sort spikes by peak-to-trough amplitude and shape. The spikes of individual units were well isolated and the signal-to-noise ratio was always sufficiently large so that the V1 and V2 cells could be unambiguously identified. Recordings were excluded from the data analysis when they contained the distinctive bursting activity that coincides with antenna movements. An example of a recorded V1 cell response to a moving grating stimulus is shown in Fig. 1b. The recordings were performed at 24-26 °C with the dot stimuli (see below) and at 20-23 °C with the grating stimuli (see below).

The V1 cell was identified by its sensitivity to downwards motion presented to the frontolateral eye equator of the contralateral visual field (e.g. Hausen 1984; Krapp and Hengstenberg 1997; Karmeier et al. 2003). The V2 cell is sensitive to upwards motion in the lateral visual field. V2 spikes can be recorded from either the dendritic field or from the telodendritic field. The V2 telodendritic field is the axonal field in the contralateral hemisphere, distinct from the local axonal field in the ipsilateral hemisphere. The identities of both cell types were confirmed after off-line data analysis by comparing the distribution of local preferred directions and motion sensitivities throughout their receptive fields with results from previous studies (Krapp 1995; Karmeier et al. 2003).
Application of CDM

The tissue-permeable octopamine agonist chlordimeform-HCL (CDM; chlorodimeform-HCL, Sigma-Aldrich) was dissolved in fly Ringer solution into 0.26 μM and 2.6 μM solutions, and stored at 4-8 °C. We reliably found a significant increase in the spontaneous spike rate of both the V1 cell and the V2 cell six minutes after application of 10 μl of 2.6 μM CDM to the head capsule, which lasted for the duration of the stimulus presentations, 40 ±11 minutes. At this concentration, CDM binds to 50% of the octopamine receptors in samples of locust brain tissue (Hiripi et al. 1999). The amount of CDM per gram wet weight is 1.4 ×10⁻⁹ mol/g calculated for the head and 2.4 ×10⁻¹⁰ mol/g calculated for the body, given the head weighs 8 mg, and the body 100 mg (Hateren and Schilstra 1999). For comparison, in other behavioural studies in which CDM was injected into the animal, 10⁻⁹ - 10⁻⁷ mol/g has been used in the hawk moth Manduca sexta (Kinnamon et al. 1984), and 10⁻⁷ mol/g in the cricket Gryllus bimaculatus and the cockroach Periplaneta americanus (Stevenson et al. 2005; Rosenberg et al. 2007). In electrophysiology studies, bath solutions of 10 – 100 μM CDM have been used in the locust Schistocerca gregaria, and in Manduca sexta (Duch and Püflger 1999; Vierk et al. 2009). We therefore considered that our concentration of 2.6 μM to be at the lower end of a range of physiologically relevant concentrations for CDM, and we used this concentration in all subsequent experiments.

CDM was applied using a micropipette (n=30), or a 1 ml syringe (n=8). There was no significant effect of the application method on the V1 or V2 cells’ spontaneous spike rate increases (p>0.05; two-sample t-tests, n=4).
The V1 and V2 cells are heterolateral LPTCs. Each cell has a dendritic field in one hemisphere, and an axonal field in the contralateral hemisphere. To test whether the location of CDM application affected the change in spontaneous spike rate, we applied CDM to either the left or the right lobula plate. We found no significant difference between the results at each location after the application of CDM (Fig. 2e, f). Therefore the results of these experiments were pooled and, in subsequent experiments, CDM was always applied to the right-hand lobula plate.

**Visual stimulation**

*Dot stimulus.* A moving dot stimulus was used to determine the local preferred direction and motion sensitivities in the binocular receptive fields of the V1 and V2 cells (Krapp and Hengstenberg 1997). We used a phosphor CRT display (Tektronix, UK) with a refresh rate of 182 Hz mounted on a meridian frame that could be moved to different azimuth positions relative to the fly (Fig. 1a). A 7.6° diameter dark dot in front of a bright background (Michelson contrast of 60%) was moved clockwise for 3 seconds on a 10.4° diameter circular path at a speed of 2 cycles/second. The background alone was then presented for ≥2 s, before the dot moved counterclockwise for another 3 seconds. The CRT images were generated using a Picasso Image Synthesizer (Innisfree, USA), and custom electronics (Dept. Zoology, University of Cambridge, UK). The spontaneous spike rate was recorded for 500 ms before each clockwise and counterclockwise stimulus presentation. The stimulus was displayed at 66 different positions in the range from -70° to 75° elevation and ±120° azimuth, chosen in a pseudorandom order to avoid consecutive stimulation of neighbouring areas.
Grating stimulus. A moving square-wave grating was used to characterise the information in the motion tuning curve at positions of high motion sensitivity. The azimuth and elevation of these positions were -30°, 15° for the V1 cell and 120°, 15° for the V2 cell. The gratings were displayed behind a circular, 21° diameter aperture of the CRT, and had a spatial wavelength of 10°, a Michelson contrast of 60%, and a temporal frequency of 2.6 Hz. Grating motion was presented for 500 ms in 16 different directions equally spaced at 22.5°. The spontaneous spike rate was recorded for 500 ms before and after each stimulus presentation in response to a zero-contrast screen. The grating stimulus for each motion direction was repeated 100 times.

Data analysis

Information calculations. The mutual information between a stimulus, $S$, and the neural response, $R$, quantifies how much information the response expresses about the stimulus (Cover and Thomas 1991). We calculated the mutual information, $I$, as the difference between the entropy of the responses independent of the stimulus, $H(R)$, and the responses conditioned on the stimulus, $H(R|S)$, in the time window, $\Delta t$:

$$I(\Delta t) = H(R(\Delta t)) - H(R(\Delta t) | S).$$

We characterized the response to the stimulus as the spike count in the time window, $\Delta t$. To minimise the bias in our estimates that results from our limited sampling of the entropy distributions, we calculated the entropies using the method of Nemenman et al. (2004). We applied various time window lengths between $\Delta t = 25$ ms and $\Delta t = 475$ ms, starting after a latency of 25 ms from the stimulus onset. The behavioural latencies of course changes during chasing flights in the housefly, *Fannia canicularis,*
can be as low as 25 ms (Land and Collett 1974), and a steady-state response to our
stimuli was reached after 475 ms.

To assess the effects an increase in spontaneous spike rate on its own has on the
direction information, we modelled the cell’s spike generation as a Poisson process.
For each cell type at each stimulus direction and in each time interval, we modelled
100 responses from a Poisson distribution with a mean value corresponding to the
mean spike rate of the cell’s tuning curve that was measured before the application of
CDM. This modelled tuning curve was then offset by the increase of the mean
spontaneous spike rate observed after the application of CDM. This process was
repeated using the new mean values. From these spike counts, the information could
be calculated as before.

The information per second, \( I_{\text{Time}} \), was calculated as the mutual information divided
by the duration of the time window, \( \Delta t \):

\[
I_{\text{Time}}(\Delta t) = \frac{1}{\Delta t} \left( H\left( R(\Delta t) \right) - H\left( R(\Delta t) \mid S \right) \right).
\]

Using this measure, we assessed the speed of information communication by the cell
in a given time window, and compared the value for the neurons before and after the
application of CDM. To infer the rate of information communicated independent of
the time window duration, a quantity known as the information rate (Borst and
Theunissen 1999), we would need to use a dynamic stimulus and extrapolate the
temporal changes in the entropy components of the mutual information to infinity
(e.g. Strong et al. 1998).
The information per spike, $I_{\text{Spike}}$, was calculated as the mutual information normalised by the mean of the spike count, $N_{\text{Spikes}}$, in the time window $\Delta t$:

$$I_{\text{Spike}}(\Delta t) = \frac{1}{N_{\text{spikes}}(\Delta t)} \left( H(R(\Delta t)) - H(R(\Delta t) \mid S) \right).$$

To calculate the mean spike count, the total number of spikes contributing to the tuning curve during the time window was normalised by the total number of trials. We used $I_{\text{Spike}}$ to measure how efficiently spikes are used to encode information about the direction of motion.

**Stimulus response latency.** The latency of the response to the rotating dot stimulus at a specific location was calculated by appropriately comparing the clockwise and anticlockwise motion tuning curves (Krapp and Hengstenberg 1997). The counterclockwise tuning curve was inverted and phase shifted by 180°, so that the two tuning curves were in the same motion coordinate system. The phase difference between the two tuning curves was then equal to twice the phase delay between stimulus and response. The phase delay was converted to the latency in the time domain by dividing by $2\pi$ times the speed of the dot, which was 2 cycles/second. For each cell, we calculated the mean latency of a number of positions where the response was strong, to reduce the impact of noise on our latency estimation. For the V1 cells, these positions were in the range of $-90^\circ \leq \text{azimuth} \leq 15^\circ$, and $-30^\circ \leq \text{elevation} \leq 75^\circ$, and for the V2 cells, they were $30^\circ \leq \text{azimuth} \leq 120^\circ$, and $-30^\circ \leq \text{elevation} \leq 75^\circ$.

To calculate the latency of the response to the grating stimulus, we applied the method described by Warzecha and Egelhaaf (2000) so that the latencies of the V1 and V2 cells could be directly compared to those of the well studied H1 LPTC. We
briefly summarise the method here (for a discussion of the method, see Warzecha and Egelhaaf 2000). First, we generated a smooth estimate of the spike rate by calculating the mean across all responses to a stimulus in the preferred direction and across all cells of the same type. The resulting data were further smoothed by a sliding window square-wave filter of 3-15 ms length. We then calculated a threshold value equal to the mean plus twice the standard deviation of the spontaneous spike rate as estimated within the time interval 375 ms period prior to stimulus onset. The latency was defined as the point in time when the smoothed mean spike rate after stimulus onset exceeded the threshold value for ≥6 ms. This criterion was not fulfilled before the stimulus onset for any cell type before or after CDM application.

Receptive fields. We characterised the cell’s local preferred direction (LPD) and local motion sensitivity (LMS) from the motion tuning curves obtained with the dot stimuli using the method described by Krapp and Hengstenberg (1997). The local preferred direction (LPD) corresponds to the stimulus direction of the circular mean of the response. The local motion sensitivity (LMS) is the difference between the mean response over the intervals LPD ±45° and LPD +180° ±45° (Fig. 1c). For the responses to the grating stimuli, where the stimulus directions are fixed and binned, the LMS is the modulation depth of the tuning curve between the maximum and minimum responses. These two response parameters are represented in the panels of Fig. 8 by arrows whose length and directions indicate the LMS and LPD, respectively.

Comparison of receptive fields with self-motion-induced rotation and translation optic flow fields. To quantify the similarity of a given receptive field, $RF$, to a rotational optic flow field, $ROF$, we calculated a similarity index, $SI$. The $SI$ is a normalised sum of the local projections of the $RF$ into the $ROF$, and defined as
where $RF_{ij}$ and $ROF_{ij}$ are the local vectors at azimuth $i$ and elevation $j$. To correct for bias in the sampling density of spherical data in a cylindrical projection, the $RF$ were interpolated to values at 15° intervals, using linear interpolation, and the values at each elevation for both the $RF$ and $ROF$ were weighted by the cosine of the elevation, $\cos(\theta)$. The exception to the 15° interval spacing is the 10° interval between -70° and -60° elevation. The $RF$ and $ROF$ were normalised to the maximum LMS of each map so that, in conjunction with the denominator term, the $SI$ returns a value between +1 for a perfect match and -1 for an exactly inverse match. At an inverse match, the LMSs match and the LPDs are in the antipreferred direction. The mean value of the $ROF_{ij}$ elements varied by $\leq 1\%$ using this method, so that matches with different $ROF$ are comparable. We then searched for the axis of the $ROF$ that maximised $SI$, at a step width of 1° along azimuth and elevation. The $SI$ surfaces were smooth and unimodal, so we could be certain of avoiding local minima.

**Results**

The most obvious effect the application of CDM had on the spiking V1 and V2 cells was an increase in the spontaneous spike rate, indicating a change in the neurons' states. We identified the concentration of CDM sufficient to induce a reproducible effect that was stable throughout our experiments. We then studied changes in the
local response properties, before we characterized the global receptive field organization of the cells after CDM application.

**Spontaneous spike rates**

The spontaneous spike rates of both cells, V1 and V2, were increased after application of 10 μl of 2.6 μM CDM to the optic lobe (Fig. 2a, b; p<0.005, Wilcoxon signed rank tests, n=12). The mean spontaneous spike rate of the V2 cell increased by 290% from 7.7 Hz to 21.9 Hz, and that of the V1 cell by 230% from 18.5 Hz to 40.2 Hz. A lower concentration of 0.26 μM CDM tested on an experimental group of four animals did not significantly change the spontaneous spike rate of both cell types (Fig 2a, b; V1, p<0.05, V2, p>0.05, paired t-tests). In all subsequent experiments, therefore, we applied 2.6 μM CDM which elevated the mean spontaneous spike rates of all the cells for the entire duration of the experiments (Fig. 2c, d).

The V1 and V2 cells have a dendritic field in one hemisphere of the brain, which is connected by a thin axon to a telodendritic output arborisation in the contralateral optic lobe. We tested whether the CDM application site affects the degree of the spontaneous spike rate increase. We found no significant differences between the mean spike rates measured when CDM was applied to the haemolymph of the site of the brain that contains the dendritic arborisation, and the one containing the telodendritic arborisation (Fig 2e, f; p>0.05, two-sample t-test, n=4). For further analysis, therefore, we pooled the results obtained from both CDM application sites.

**Motion tuning curves**

The application of CDM increases the responses to gratings moving in the preferred direction as well as the spontaneous spike rates. The motion tuning curves measured
at locations near the cells’ maximum motion sensitivity are shown in Figure 3a and b. To emphasise the contributions of excitatory and inhibitory inputs in the panels we have subtracted the mean spontaneous spike rate. Thus, negative values indicate a reduction of the spike rate below the spontaneous level.

The application of CDM increased the depth of the tuning curve for both cells (Fig. 3c; p<0.01, paired t-tests, n=4), by 33 ±3.5 Hz for the V1 cells and 17 ±2.6 Hz for the V2 cells. These are proportionally similar increases, at 43% for the V1 cells, and 48% for the V2 cells. The tuning curve depth was particularly amplified in the region of the inhibitory responses. The mean spontaneous spike rates increased, by 16.6 Hz for the V1 cells, and 9.1 Hz for the V2 cells, which resulted in an extension of the cells’ negative response range. The mean inhibitory signalling range increased by 13.9 Hz for the V1 cells, and by 6.3 Hz for the V2 cells, relative to the spontaneous rate (Fig. 3a, b). These are large proportional increases of 152% and 114%, respectively. By comparison, the response to gratings moving in the preferred direction increased by 29% for the V1 cells and 36% for the V2 cells.

The variability of the responses also increased after the application of CDM (Fig. 3a, b). The mean standard deviation of the response across all grating directions was significantly increased for both cells (Fig. 3d; p<0.05, paired t-test, n=4), rising from 5.5 Hz to 6.9 Hz for the V1 cells, and from 4.1 to 6.3 Hz for the V2 cells. These absolute changes were comparable between the cells (Fig. 3d), and therefore proportionally higher for the V2 cells than the V1 cells.

For all tuning curves obtained, the proportional gains in the depth of the tuning curve were larger than the increases in variability. This can be understood by evaluating the signal-to-noise ratio (SNR), the ratio between the square of the mean response and the
For the V1 cells, the SNR increased across the whole tuning curve after the application of CDM, from a mean value of 20.0 to 33.1 (Fig 3e). For the V2 cells, the SNR decreased in the preferred directions, but increased in the antipreferred directions (Fig. 3f). These changes effectively balance, resulting in only a small increase in the mean SNR from 4.8 to 5.0.

### Direction information

To quantify the overall gain in direction information after the application of CDM, we calculated the mutual information of the stimulus direction and the neuronal response. The direction information was elevated for both cells (Fig. 4a). The gain in direction information was significant for both the V1 cell (p<0.005) and the V2 cell (p<0.05, paired t-tests, n=4), for time windows up to 50ms. In these time windows, the mean information gains were similar. For example, over 50 ms the gain was 55% and 39% for the V1 cell and the V2 cell, respectively (Fig. 4d, e).

In longer time windows, the increase in the variability of the V2 response reduces the gain in the direction information after the application of CDM. Over a time window of 475 ms the entropy quantifying the stimulus variability, $H(R|S)$, increases by 0.62 bits for the V2 cell, and only 0.52 bits for the V1 cell (Fig. 4c). In comparison, the entropy quantifying the tuning curve depth, $H(R)$, increased by comparable values of 0.72 bits for the V1 cell and 0.68 bits for the V2 cell (Fig. 4b). The results suggest that it is the response variability, rather than its low spike rate, which keeps the direction information encoded by the V2 cell lower than that encoded by the V1 cell.

The increases in the spontaneous spike rate alone cannot account for the increases in direction information. To illustrate this point, we modelled the tuning curve responses
as Poisson processes. This approximation was justified because, under our stimulus conditions, the variance of the responses of both cells scaled nearly linearly with the mean response spike rate (Fig. 5a). We calculated the predicted information for V1 and V2 cells from the mean values of the tuning curves measured before the application of CDM. We then compared these values to the direction information calculated from responses which were offset by the increase of the spontaneous spike rate, and had a proportionally increased variance (Fig. 5b). The increase in the spontaneous activity decreased the information. A simple increase in the spiking response due to an offset will only decrease the information, because the depth of the tuning curve is not increased but the noise is.

Information per second and information per spike

To investigate the communication speed, we calculated the information per second, $I_{\text{Time}}$, which is the information normalised by the duration of the time window (Fig. 6a). As expected, the application of CDM increases $I_{\text{Time}}$ for both cells, and the significance levels are the same as for the increased direction information. Over a time window of 50 ms, the information per second increases from a mean of 7.4 bits/s to 11.2 bits/s for the V1 cell, and from 2.9 bits/s to 3.6 bits/s for the V2 cell.

If more direction information is communicated within the same time interval, how energy-efficient is such a change? As a consequence of the mechanisms of action potential propagation, the majority of the energy available for signalling scales approximately linearly with the average action potential firing rate (Attwell and Laughlin 2001; Attwell and Gibb 2005). We could therefore address this question indirectly, by calculating the information per spike, $I_{\text{Spike}}$, the information normalised by the mean spike count per trial.
The application of CDM reduced $I_{Spike}$ for both cells, significantly for all time windows $\geq 100$ ms (p<0.05, paired t-tests, n=4; Fig. 6c,d). Over the duration of the stimulus, the increases in the mean spike counts, at 74% for the V1 cell and by 79% for the V2 cell, were much greater than the increases in the direction information (Fig. 6b). This suggests that the application of CDM increases the direction information at the expense of energy efficiency, as assessed by the information per spike, because more spikes are being used to encode the same number of different directional stimuli.

**Stimulus response latency**

If CDM increases the information per second, does octopamine receptor activity speed up responses to visual stimuli? This would help the animal to maintain stability even if it encounters more rapidly changing visual inputs.

The application of CDM reduced the latencies of the responses to the grating stimulus for both cells (Fig. 7d). The mean response of the V1 cells to motion stimuli in its preferred direction is shown in Figure 7c, to illustrate the method used. The absolute value of our response latency estimation depends on the duration of the smoothing filter. We found, however, that for all filter settings tested the response latency was reduced for the V1 and the V2 cell (Fig. 7d). CDM reduced the latency of the V1 cell response by 6.0% from 23.4 ms to 22 ms with a 4 ms filter, and reduced the latency of the V2 cell response by 4.4% from 21.4 ms to 20.5 ms. Over the range of filter durations from 5 – 15 ms, the latency of the V1 cell and the V2 cell were reduced by 6.3 - 12.7% (1.3 – 2.5 ms) and 2.2 – 8.2% (0.4 – 1.45 ms) respectively (Fig. 7d).

CDM also significantly decreased the latency of the response to the dot stimulus, for the V1 cells (p<0.05) and the V2 cells (Fig. 7a, b; p<0.005, Wilcoxon signed rank
tests, n=12), when it was presented at a location near the cells’ sensitivity maximum. The mean V1 cell latencies were reduced by 11% from 46.8 ms to 41.8 ms and the V2 cell latencies by 13% from 54.1 ms to 46.9 ms. These latencies include a stimulus-dependent component, because they depend on the velocity of the dot-stimulus (Krapp 1995), which accounts for their high values compared to the grating-induced response latencies.

There were no significant changes in the response latencies when we grouped the data by spontaneous spike rate into two equally sized groups, one with low spontaneous spike rates, and one with high spontaneous spike rates (p>0.05, two-sample Wilcoxon signed rank tests, n=12). This finding indicates that the reduction in response latencies was a result of the action of CDM, rather than the cells’ elevated spontaneous spike rates.

**Binocular receptive field organization**

It is striking that the receptive fields of both cells appear to match the optic-flow that results from specific rotations or translations (Fig. 8a, b). The patterns of optic flow flies experience depend on the locomotor state, which in turn defines the bandwidth of the visual motion stimuli the fly encounters. We therefore investigated how the receptive field organization changed with the application of CDM.

Figure 8b shows for the first time the binocular receptive field of the V2 cell. The cell responds vigorously at azimuth 120° elevation 15° to vertical upward motion, in agreement with previous results obtained from measurements in only one visual hemisphere (Hausen 1984; Krapp 1995). From this caudal position, the local preferred directions (LPDs) change continuously to horizontal in the frontal dorsal visual field,
and to vertically downward in the contralateral visual field. The V1 binocular receptive field is consistent with previously presented data (Fig. 8a; Hausen 1984; Krapp and Hengstenberg 1997; Karmeier et al. 2003).

The application of CDM had a clear effect on the mean local motion sensitivities (LMSs) of the V2 cells, increasing the values at most locations (Fig. 8d, f). In particular, the increase was significant at the locations where the grating stimulus was previously presented in the regions of maximum motion sensitivity (p<0.05, Wilcoxon signed rank test, n=12), the encircled position in Fig 8d. By contrast, the mean LMS across the V1 cells was not significantly altered at any location (Fig. 8c, e). It is important to note that for both cells, none of the local preferred directions were significantly affected by CDM application (Fig. 8c, d). Therefore, the cells encode the same self-motion parameters independent of the octopamine-induced change of their activity state.

A threefold increase of the inhibitory signalling range from 4.7 Hz to 15.3 Hz entirely accounts for the increase in the LMS of the V2 cell, while the excitatory signalling range was unchanged (Fig. 9b). An increase in the inhibitory signalling range also accounts for the lack of change in the LMS of the V1 cell (Fig. 9a). The inhibitory signalling range nearly doubled from 13.9 Hz to 25.2 Hz, which matched a decrease in the excitatory signalling range from 49.0 Hz to 37.7 Hz, leaving the overall dynamic response range unchanged.
Comparison of receptive fields with self-motion-induced rotation and translation optic flow fields

What are the functional consequences of the CDM-induced activity changes for spiking LPTCs? As the V1 cell and the V2 cell encode specific self-motion parameters, such as rotations around and translation along particular body axes, changes in the receptive fields of these cells should affect their coding properties.

We calculated a similarity index, $SI$, to quantify how well each cell's receptive field matches an optic flow field that results from a specific rotation or translation, before and after the application of CDM. The value of $SI$ can range from +1 for a perfect match to -1 for an exactly inverse match. For each cell, we searched for the preferred rotation and translation optic flow fields resulting in the highest $SI$. The preferred rotation axis indicates around which axis the fly has to turn so that the cell is most strongly activated, while the preferred translation axis predicts the direction the animal has to head in so that the cell strongly responds.

The $SI$ for rotation optic flow is greater than the $SI$ for translation optic flow for both the V1 and the V2 cells (Fig. 10a, b; $p<0.01$, Wilcoxon signed rank test, $n=12$; Krapp et al. 2001). The preference for rotations is also reflected in the smaller variance of the fitted rotation axes compared to the fitted translation axes (Fig. 10c, d), indicating that the cells are able to code the axis of their preferred rotation more accurately than the direction of a translation.

The application of CDM increased the $SI$ of the V2 cell receptive field for rotation optic flow, (Fig. 10a; $p<0.05$, Wilcoxon signed rank test, $n=12$) and to a smaller degree the $SI$ for translation optic flow (Fig. 10b; $p<0.05$, Wilcoxon signed rank test,
n=12). In contrast, CDM did not significantly change the $SI$ for the V1 cells (Fig. 10a, b), nor the axes of the matched rotations and translations for either cell (Fig. 10c, d; $p>0.05$, paired t-test, $n=12$). So CDM increases the V2 cell signalling response to components of self-motion, without affecting the axes of self-motion that both cells are tuned to.

Discussion

We have investigated how the application of an agonist of octopamine, a neuromodulator involved in the regulation of flight physiology, changes the responses of individually identified LPTCs in the blowfly to directional motion stimuli. Application of the agonist, CDM, increased the response ranges of both cells by $>40\%$ over the duration of the stimulus (Fig. 3c). The dynamic response range was increased in two ways: the spontaneous spike rate was elevated (Fig. 2a, b), which more than doubled the inhibitory signalling range of both cells; and the response to stimuli in the preferred direction increased, in addition to the change in the spontaneous spike rate (Fig. 3a, b);

The expanded response range increased the amount of information the cells encoded about the direction of motion (Fig. 4). At the same time, the latencies of the responses decreased for both cells, by between 4.7% and 13% (Fig. 7). However, the amount of information per spike decreased, because the mean spike rate increased more than the direction information (Fig. 6). The application of CDM increased the speed of the responses and the amount of direction information encoded in a given time interval, but at a reduced efficiency of each spike.
To assess the functional consequences of these changes, we examined the effect of CDM application on the cells’ binocular receptive field properties (Fig. 8). CDM did not alter the cells’ distributions of local preferred directions nor did it change their specific preferences for certain optic flow fields (Fig. 10). For the V2 cell, CDM did increase the magnitude of the match of the receptive field to translational and, in particular, to rotational optic flow (Fig. 10), which, overall, increased the cell’s rotation-selectivity. The fact that the distribution of local preferred directions is independent of the cells’ activity state further supports the hypothesis that LPTCs act as matched filters for optic flow processing (Franz and Krapp 2000).

The application of CDM had different effects on the excitatory responses to the dot and the grating stimuli. This result may be due to the sized-dependent mechanisms underlying dendritic gain control in LPTCs (Borst et al. 1995), which would be differently affected by the dot motion, which subtended 7.6°, and the grating stimulus, which subtended 21°. This explanation, however, would require further experiments to test. We predict that CDM would increase the magnitude of the matches of V1 receptive fields to rotation and translation optic flow, if the receptive fields were mapped using the grating stimulus, because CDM modulated the depth of the directional tuning curve when the grating stimulus was used.

Octopamine haemolymph levels can be elevated by stress as well as flying (Davenport and Evans 1984; Adamo et al. 1995). It is likely that handling the animals increased the octopamine haemolymph levels in an unpredictable way, and this may be one contributing factor to the variability in spontaneous spike rate before the application of CDM. However, we used pairwise tests in our statistical analysis, so the
significant effects of CDM could be observed independently of different initial levels of octopamine.

**Comparisons with other studies**

Our results are consistent with previous studies investigating the effects of octopamine on visual processing in insects. The result that the rotation tuning of the V1 cell and V2 cell are differently affected by CDM is consistent with studies in the honeybee, *Apis mellifera*. Erber and Kloppenburg (1995) found that injections of octopamine in the optic lobe of the honeybee increased the magnitude of a behavioural reflex response to visual roll stimuli. A concurrent study on the neural circuits controlling the reflex showed that octopamine affected unidentified spiking cells in the lobula complex that were motion-sensitive and direction-selective (Kloppenburg and Erber 1995). Similar to our results obtained with the dot stimulus, octopamine increased the sensitivity of only some of the neurons sensitive to binocular vertical motion, but not of other neurons.

In the locust, octopamine increases the sensitivity of two neurons that mediate escape behaviour and collision avoidance in response to visual looming stimuli, the lobula giant movement detector (LGMD) and the descending contralateral movement detector (DCMD) (Bacon et al. 1995; Rind et al. 2008; revs.: Gabbiani et al. 2004, Krapp and Wicklein 2008). Similar to our findings, octopamine increases the spike rates of the responses to looming stimuli of LGMD and DCMD. However, it does not increase the spontaneous spike rates in either of those cells (Bacon et al. 1995). An elevated spontaneous spike rate may not be useful to the LGMD and the DCMD, because during object approach spiking in these neurons increases rather than exploiting the neurons’ negative signalling range (e.g. Gabbiani et al. 2004).
Comparison with norepinephrine studies

The roles of octopamine in regulating the metabolism and neural control of “fight-or-flight” states in invertebrates are similar to the roles of norepinephrine in such states in vertebrates (Evans 1985; Charmandari et al. 2005; Sara 2009). For instance, exercise and stress elevate cortical levels of norepinephrine and increase the cerebral metabolism (Pagliari and Peyrin 1995; Feenstra et al. 2001; Dalsgaard 2006). In general, norepinephrine facilitates sensory coding (Berridge and Waterhouse 2003, Hurley et al. 2004). Norepinephrine can increase response amplitudes for neurons across the sensory modalities, (Waterhouse et al. 1990; Waterhouse et al. 1998; Bouret and Sara 2002; Mulders and Robertson 2005), and decrease the response latency (George 1992; Devilbiss and Waterhouse 2004). Our results are in agreement with these effects, in that more energy is invested by generating a greater number of spikes to increase the signalling range.

Norepinephrine can also facilitate sensory coding by significantly reducing the spontaneous spike rate levels and marginally reducing the response amplitude (Foote et al. 1975; Kasamatsu and Heggelund 1982; McLean and Waterhouse 1994; Hasselmo et al. 1997). This is contrary to our results in that less energy and spikes are used to code the sensory stimuli. However, exercise or stress raises the cerebral metabolism with significant regional differences (Kemppainen et al. 2005; Dalsgaard 2006). It appears that the metabolism of task-relevant cortical areas is upregulated at the expense of less relevant areas, although this has not been proven unequivocally.

The effects of norepinephrine on a given system can be diverse. Both excitatory and inhibitory signals are typically affected, leading to dose-dependent increases or decreases in signal quality, and different cell types within the population studied can...
respond quite differently due differences in receptors and cellular mechanisms (Kasamatsu and Heggelund 1982; McLean and Waterhouse 1994; Devilbiss and Waterhouse 2000; Ego-Stengel et al. 2002). Partly as a result of this complexity, it has been difficult to explain the actions of norepinephrine on sensory processing in a simple explanatory framework (Berridge and Waterhouse 2003; Hurley et al. 2004; Aston-Jones and Cohen 2005; Bouret and Sara 2005; Yu and Dayan 2005). In the blowfly, where the circuitry and behaviour are well-defined, the principles governing the impact of octopamine on sensory information processing may be easier to identify.

**Biophysical mechanisms**

Octopamine receptors and octopaminergic processes are found throughout the optic lobes (Roeder and Nathanson 1993; Sinakevitch and Strausfeld 2006). It is likely, therefore, that octopamine in the haemolymph and systemically applied CDM will affect visual processing at multiple locations. In *Drosophila*, the bath application of octopamine increases the effective sensitivity of photoreceptors by increasing the latency and duration of responses to flashes of light (Chyb et al. 1999). Synaptic transmission between interneurons can also be enhanced by octopamine, both pre- and postsynaptically (Johnson and Harris-Warwick 1990; Casagrand and Ritzmann 1992; Matheson 1997; Hill and Blagburn 2001). In addition, octopamine can affect the sensitivity and intrinsic properties of interneurons, such as bursting and plateau potentials (Harris-Warwick and Flamm 1987; Ramirez and Pearson 1991a, 1991b).

The cellular basis for the octopamine-mediated change in sensitivity can include the Na⁺/K⁺ pump (Zhang et al. 1992), activating voltage-gated Ca²⁺ and Ca²⁺-activated...
K⁺ channels (Achenbach et al. 1997), Na⁺ channels (Harris-Warick and Flamm 1987), and PKA signalling (Hildebrandt and Muller 1995; Widmer et al. 2005).

The increase in spontaneous spike rate of the V1 and V2 cells after the application of CDM could be accounted for by a membrane potential depolarisation. This is an interesting hypothesis because in vertebrates sensory information processing is facilitated during arousal by up-states, in which neocortical pyramidal cells are depolarised (Steriade et al. 2001; Castro-Alamancos 2004). However, it is unlikely that a membrane depolarisation alone accounts for all the observed changes in sensitivity. This is partly because we expect that photoreceptor activity and neural signalling presynaptic to the V1 and V2 cells to be affected by octopamine receptor activity, as in Drosophila discussed above. We confirmed in our analysis that the decrease in response latencies to the dot stimulus did not depend on the spontaneous spike rate, but on the application of CDM. In addition, the increased depth of the tuning curves cannot be accounted for by a simple increase in the cells’ gain because the inhibitory signalling range was increased by a much greater factor than the excitatory signalling range. Octopamine can increase the magnitude of inhibitory inputs presynaptically as well as increase the excitability of the cell, as has been shown for the locust chordotonal organ of the locust (Matheson 1997). In other systems, the application of octopamine to interneurons has resulted in membrane potential depolarisations, but this effect was not sufficient to explain changes in the sensitivity of the interneurons which can even decrease (lobster: Johnson and Harris-Warick 1990; locust: Leitch et al. 2003).

In the locust it has been possible to identify and stimulate octopaminergic neurons to observe the local effects of octopamine on movement sensitive neurons in the lobula.
complex (Bacon et al. 1995; Stern 2009). Specific octopaminergic neurons also innervate the optic lobes but they have not yet been characterised electrophysiologically (Sinakevitch and Strausfeld 2006). Now that we have established a role for haemolymph octopamine levels in modulating visual responses in the blowfly, injections of octopamine into the lobula plate can be used in future studies to investigate the local octopaminergic control of visual processing, and to identify the biophysical mechanisms responsible.

**Implications for behaviour**

Systemic changes in octopamine levels are associated with the flight state (Adamo et al. 1995). Our results suggest that blowflies could use the levels of octopamine in the haemolymph to set the dynamic response ranges and latencies of spiking visual interneurons that contribute to visuomotor control. Flies encounter only a reduced dynamic stimulus range on the ground, which they should be able to encode with a limited neuronal response range. When airborne, however, flies cope with a markedly increased dynamic stimulus range, and the response range and latencies need to be adjusted to maintain rapid and efficient behavioural control.

This strategy also potentially allows the blowfly to save energy when not flying, by reducing the number of action potentials and increasing the spike coding efficiency. Action potentials carry a significant metabolic cost (Laughlin 2001), and therefore, spikes may only be generated if actually needed. The animal cannot avoid increasing its energy budget when flying, but it can decrease it when resting or walking on the ground by regulating its neural processes. At rest, the blowfly retina accounts for an estimated 8% of the resting metabolic rate (Howard et al. 1987), and so maintaining
energy efficiency in the visual system is relevant to the animal’s survival and fitness (Niven and Laughlin 2008).

Sensory neurons not required during flight may also be down-regulated to save energy by octopamine. Octopamine typically increases the sensitivity of sensory neurons, but can decrease the sensitivity of particular mechanoreceptors or reduce synaptic transmission (Pasztor and Bush 1987; Zhang et al. 1992; Leitch et al. 2003). Moreover, the activity of individual octopaminergic neurons may be up- or down-regulated during flight (Morris et al. 1999). A candidate for sensory down-regulation during flight is the leg proprioceptor system. Octopamine regulates the tension and proprioceptors in locust leg muscles, so that the muscles can be tense in a manner suitable for the fixed postures of flight, or less tense and responsive to movement as required during walking (Evans and Siegler 1982; Matheson 1997). It is possible that in the blowfly, octopaminergic signalling could be used during flight to set leg muscle tone and save energy in coding of leg position by proprioceptors, but this will require further investigation.

What are the functions of the V1 and the V2 LPTCs? Both neurons are heterolateral elements that convey self-motion information to the contralateral lobula plate (Hausen 1984). Their receptive fields are well matched to combinations of rotation and translation optic flow, and both cells synapse with LPTCs that communicate information about optic flow to the neck motor neurons and descending neurons. If the cells are involved in gaze-stabilization, then their spiking responses support postural changes to wide-field optic flow stimuli mimicking roll rotations and banked turns (Huston and Krapp 2008). Our results suggest that these behavioural responses will be increased by the change in octopamine concentration associated with the
animal’s switch to the flight state. This can be tested experimentally by applying octopamine antagonists such as epinastine to blowflies in tethered flight, and observing the effects on the well-established, compensatory head movements that stabilise the gaze in response to visual stimuli (rev.: Hengstenberg 1993).

The V1 cell also provides an excitatory input to VCH, a centrifugal LPTC (Hausen 1993). The VCH cell, in turn, mediates wide-field inhibition to the figure detection cell FD1 (Warzecha et al. 1993) which is sensitive to small-field objects moving relative to the background (Egelhaaf 1985). Therefore, we would expect that in the fly octopamine enhances the FD1 cell’s small-field selectivity and thus facilitates object detection during flight.

**Conclusion**

Our results indicate that blowflies may regulate the response latency and dynamic response range of their sensory neurons through the systemic levels of the neuromodulator octopamine. By using octopamine levels to down-regulate visual processing when on the ground, the animals will reduce the energetic costs of visual system at the expense of performance. For animals engaged in fast and energetically demanding activity, however, rapid responses and increased sensory sensitivity may be sufficiently important to warrant the energy costs which come with an increased neural signalling range. These additional costs may prove energy-efficient overall, because improved sensory coding may allow the animal to perform behavioural tasks more effectively.
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**Figure Legends**

**Figure 1**

Determining the local motion tuning curves. **a)** The stimulus is presented on a CRT that is mounted on a meridian frame at a fixed distance from the fly’s head, and which can be moved to different elevations, $\Theta$, and azimuths, $\Psi$. The grating stimulus illustrated is moving in the direction indicated by the arrow. **b)** Example of an amplified, extracellular recording trace from a V1 cell, responding to a grating moving in the cell’s local preferred direction. Stimulus timing is indicated by the black horizontal bar. **c)** An example V1 tuning curve in response to the grating stimulus. The mean and standard deviation of the responses are shown (grey line and error bars), and the local preferred direction (LPD) and local motion sensitivity (LMS) are labelled with black arrows.

**Figure 2**

Effects of CDM on the spontaneous spike rate. **a)** & **b)** The spontaneous spike rates are shown for the different concentrations of CDM solutions applied, for the V1 cells (a) and the V2 cells (b). Mean and standard error (se) values are plotted. Asterisks denote the significance of the changes: * $\equiv$ p<0.05, ** $\equiv$ p<0.01, *** $\equiv$ p<0.005.

Wilcoxon signed rank tests (n=12) were used to test the significance of the changes between 0 and 2.6 $\mu$M CDM, and paired t-tests (n=4) were used for the changes between 0 and 0.26 $\mu$M CDM. **c)** & **d)** The variability in the spontaneous spike rate before and after the application of 2.6 $\mu$M CDM, for the V1 cells (c) and the V2 cells (d). Mean ±se values shown (n=12), calculated over blocks of 12 trials. **e)** & **f)** 2.6 $\mu$M CDM was applied either to the hemisphere containing the dendrites (Dend) or
axons (Axon) of the V1 cells (e) and the V2 cells (f). There were no significant
differences between the Dend and Axon conditions after the application of CDM.
Mean ±se values are shown (n=4), and significance was tested using paired t-tests: * =
p<0.05.

Figure 3
Local motion tuning curves obtained with the grating stimulus. a) Tuning curves of
the V1 cells before (grey line) and after (black line) the application of CDM obtained
at azimuth -30°, elevation 15°. The mean responses are plotted relative to the mean
spontaneous spike rate, <SSR>. Error bars denote the standard deviation of responses
(n=4). b) Tuning curves of the V2 cells, obtained at azimuth 120°, elevation 15°, and
displayed as in a). c) Absolute changes in the LMS after the application of CDM,
ΔLMS, for both cell types, over different time windows. The changes are significant
over all time windows (p<0.01, paired t-tests, n=4). Mean ±se values are shown. d)
The difference in the mean standard deviation of the responses across all stimuli,
Δ<σ(R)>, before and after the application of CDM. The last data points correspond to
the change in the mean lengths of the error bars in a) and b). The changes are
significant over all time windows (p<0.05, paired t-tests, n=4). Mean ±se values are
shown. e) & f) Signal-to-noise ratio (SNR) plots of the tuning curves before and after
the application of CDM for the V1 cells e), and the V2 cells f). The SNR is calculated
as the ratio of the square of the mean of the response and the variance.

Figure 4
Mutual information of the responses and the stimulus, the direction of the grating. a)
The mutual information (I), before and after the application of CDM, calculated over
different time windows. Mean ±se values shown (n=4). b) & c) The stimulus-independent entropy of the response, $H(R)$, (b) and the stimulus-conditional entropy, $H(R|S)$, (c). Figure legends as in a). Mean ±se values shown. d) & e) The multiplicative gain in the mean information of the V1 cells (d) and the V2 cells (e), after the addition of CDM. Asterisks denote significant increases in the information when calculated over the corresponding time window: * $\equiv p<0.05$, ** $\equiv p<0.01$, *** $\equiv p<0.005$; paired t-tests, n=4.

**Figure 5**

An increase in the spontaneous activity of the cells is predicted to decrease the direction information, when the cells are modelled as Poisson processes. a) The variances of the pre-CDM V1 and V2 responses to the grating stimulus at each stimulus direction are plotted as functions of the mean responses. The relationship is nearly linear, indicated by the black line. b) The cells were modelled as Poisson processes by generating Poisson distributed spike count distributions with means matching the pre-CDM mean tuning curves. The direction information was calculated from this modelled response (grey lines labelled “Poisson”). This modelled response underestimates the true measured information (dashed grey lines). The increases in spontaneous spike rate ($\Delta SSR$) observed after the application of CDM were then added to the mean values and new Poisson distributed data generated, and the information calculated again (black lines labelled “Poisson + $\Delta SSR$”).

**Figure 6**

Information per second and Information per spike. Mean ±se values shown in all panels. a) Information per second, $I_{Time}$. The significant increases were the same as
those for the direction information, indicated in Fig. 4d, e. b) The mean spike count per trial was significantly increased by the application of CDM for both cell types over all time windows (p<0.025, paired t-tests, n=4). Figure legend as in a). c) & d) Information per spike, $I_{\text{spikes}}$, for the V1 cells (c) and the V2 cells (d). The decreases in $I_{\text{spike}}$ are significant for both cell types, for all time windows ≥100 ms (p<0.05, paired t-tests, n=4).

**Figure 7**

Response latencies. a) Latencies of the responses to the dot stimulus, before and after the application of CDM. Mean ±se values shown. Asterisks indicate the significance of the changes: * ≡ p<0.05, ** ≡ p<0.01, Wilcoxon signed rank tests, n=12. b) Changes in individual dot stimulus response latencies. c) Examples of the mean V1 cell response to the grating stimulus in the preferred direction (n=4; 4 ms smoothing filter). The mean spontaneous spike rate, <SSR>, has been subtracted from the response rate. d) The grating stimulus response latencies for the V1 and V2 cells over the range of smoothing filter durations, before and after the application of CDM.

**Figure 8**

Binocular receptive field organization. The orientation and length of each arrow indicate the local preferred direction, LPD, and local motion sensitivity, LMS, respectively. The LMS is scaled by the same scaling factor for both cell types. Black arrows denote experimental data, and the grey arrows give interpolated results. The V1 and V2 cell responses to the grating stimulus were studied at the encircled positions. a) & b) The mean response fields (n=12) of the V1 cells (a) and the V2
cells (b) before the application of CDM. c) & d) The mean response fields (n=12) of
the V1 cells (c) and the V2 cells (d) after the application of CDM. e) & f) The
differences between the pre- and post-CDM response fields, scaled by a factor of 3.

Figure 9
Local motion tuning curves obtained with the dot stimulus. a) & b) Tuning curves of
the V1 cells (a) and the V2 cells (b), showing the mean spike rate in response to the
dot stimulus, before and after the application of CDM. Error bars denote standard
error (n=12). Responses are plotted relative to the cells’ mean spontaneous spike
rates, <SSR>. The responses were recorded when the dot stimulus was displayed at
the encircled locations in Fig. 8 for the respective cells.

Figure 10
Similarity index (SI) of response fields to self-motion rotation and translation optic
flow fields. a) & b) SI for rotation optic flow (a) and translation optic flow (b) for the
V1 and V2 cells, before and after the application of CDM. Mean ±se values are
shown (n=12). Significance was tested using Wilcoxon signed rank tests: * ≡ p<0.05.
c) & d) The axes of the optimal matched rotation (c) and translation (d) optic flow
fields, for the V1 and V2 cells, before and after the application of CDM. Mean
±standard deviation values shown.
<table>
<thead>
<tr>
<th>Stimulus presentation no.</th>
<th>Rate / Hz</th>
<th>CDM concentration / µM</th>
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<tbody>
<tr>
<td></td>
<td></td>
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**Spontaneous spike rate / Hz**

**CDM concentration / µM**

**V1**

- Stimulus presentation no. 1
  - Rate / Hz: 10
  - CDM concentration: 0 µM

**V2**

- Stimulus presentation no. 2
  - Rate / Hz: 20
  - CDM concentration: 0 µM

**Stimulus presentation no. 3**

- Rate / Hz: 30
  - CDM concentration: 0 µM
a) V1

b) V2

c) Change in LMS

d) Change in variability

e) V1 signal-to-noise ratio

f) V2 signal-to-noise ratio
a) Mutual information

b) $H(R)$

c) $H(R|S)$

d) V1 information gain

e) V2 information gain
a) Variance / Hz² vs. pre-CDM Rate / Hz

b) Pre-CDM, Poisson, Poisson + ΔSSR vs. Time window / ms
a) Information per second

b) Mean spike count per trial

c) V1 information per spike

d) V2 information per spike
a) Dot stimulus response latencies

- **pre-CDM**
- **post-CDM**

b) Dot stimulus response latencies

- V1
- V2

c) V1 grating stimulus responses

- pre-CDM threshold
- post-CDM threshold
- pre-CDM response
- post-CDM response

d) Grating stimulus response latencies
Elevation

\( \Delta V1 \)

\( \Delta V2 \)

a) V1 pre-CDM

b) V2 pre-CDM
c) V1 post-CDM
d) V2 post-CDM
e) 3 \times \Delta V1

f) 3 \times \Delta V2
a) V1

b) V2
a) Rotation SI
- pre-CDM
- post-CDM

b) Translation SI
- pre-CDM
- post-CDM

c) Matched rotation axes
- V1 pre-CDM
- V1 post-CDM
- V2 pre-CDM
- V2 post-CDM

d) Matched translation axes
- V1 pre-CDM
- V1 post-CDM
- V2 pre-CDM
- V2 post-CDM