Current Biology

Persistent Firing and Adaptation in Optic-Flow-Sensitive Descending Neurons

Graphical Abstract

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In Brief

Nicholas and Nordström show that descending neurons that connect the fly optic lobes with the motor command centers display persistent firing following visual stimulation. This is strikingly different to their presynaptic counterparts. However, if this after-effect is taken into account, optic-flow-sensitive descending neurons show signs of adaptation.

Highlights

- Optic-flow-sensitive descending neurons give sustained responses to widefield motion
- The neurons display persistent firing following the offset of stimulation
- The persistent firing depends on stimulus contrast but not temporal frequency
- A test-adapt-test paradigm reveals adaptation to visual motion

Article

Persistent Firing and Adaptation in Optic-Flow-Sensitive Descending Neurons

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<https://doi.org/10.1016/j.cub.2020.05.019>

SUMMARY

A general principle of sensory systems is that they adapt to prolonged stimulation by reducing their response over time. Indeed, in many visual systems, including higher-order motion sensitive neurons in the fly optic lobes and the mammalian visual cortex, a reduction in neural activity following prolonged stimulation occurs. In contrast to this phenomenon, the response of the motor system controlling flight maneuvers persists following the offset of visual motion. It has been suggested that this gap is caused by a lingering calcium signal in the output synapses of fly optic lobe neurons. However, whether this directly affects the responses of the post-synaptic descending neurons, leading to the observed behavioral output, is not known. We use extracellular electrophysiology to record from optic-flow-sensitive descending neurons in response to prolonged wide-field stimulation. We find that, as opposed to most sensory and visual neurons, and in particular to the motion vision sensitive neurons in the brains of both flies and mammals, the descending neurons show little adaption during stimulus motion. In addition, we find that the optic-flow-sensitive descending neurons display persistent firing, or an after-effect, following the cessation of visual stimulation, consistent with the lingering calcium signal hypothesis. However, if the difference in after-effect is compensated for, subsequent presentation of stimuli in a test-adapt-test paradigm reveals adaptation to visual motion. Our results thus show a combination of adaptation and persistent firing in the neurons that project to the thoracic ganglia and thereby control behavioral output.

INTRODUCTION

Sensory systems typically adapt to prolonged stimulation by reducing their response over time. The dependence on stimulus history takes place across multiple timescales, via evolution, through development to the most recent sensory experience [1]. Indeed, adaptation has been described in different animals for different senses, from peripheral sensory neurons, through neurons in higher-order processing centers, such as the mammalian cortex, to whole-organism sensory perception [2–4]. Sensory adaptation is an active, stimulus specific process, separate from neural fatigue [2, 5, 6]. For example, if rats are repetitively stimulated with an odor, they perceive this odor as having reduced intensity, but other odors are not affected [3].

In contrast to this, neurons in many other cortical and sub-cortical areas show persistent neural activity following a brief sensory stimulus, lasting from hundreds of milliseconds to tens of seconds (for Review, see [7, 8]). For example, post-stimulus persistent firing of pre-motor neurons in the velocity-to-position neural integrator maintains oculomotor fixation [9, 10] and is thus used for sensory short-term memory [11]. Persistent firing can arise from intrinsic persistent currents but also from local network feedback. Indeed, persistent firing is common in areas with local feedback loops, including the superior colliculus [12], posterior parietal cortex [13], and spinal cord [14, 15].

Importantly, areas that display persistent activity often integrate afferent input and have multiple efferent targets, in both vertebrates (e.g., [9, 10, 12]) and invertebrates [16, 17].

In motion vision, widefield optic-flow-sensitive neurons in the fly optic lobes, the wallaby pre-tectum, and the mammalian visual cortex (V1) all adapt strongly [18–20]. For example, fly lobula plate tangential cells (LPTCs) reduce their response amplitude during continuous stimulation [18, 21, 22], whereas behavioral output remains sustained [23]. Following stimulation, many optic-flow-sensitive neurons also show an antagonistic after-effect [18, 24, 25]. However, in flies the behavioral response persists for several seconds after stimulation [23, 26], even though they can adjust their behavior quickly [27–29]. This discrepancy between LPTC adaptation and behavior has been attributed to a lingering calcium signal in the LPTC output synapses, working as a leaky integrator by outlasting the membrane potential change by many seconds [23, 26].

LPTCs connect with optic-flow-sensitive descending neurons. In fruit flies and blowflies, the horizontal system (HS) LPTCs provide input to DNHS1 and the vertical system (VS) cells to DNOVS1 and DNOVS2 [30–33]. Physiologically similar neurons have been identified in the hoverfly [34]. These descending neurons provide input to motor neurons in the halteres, the wings, and the neck motor region [30–36] and thus work as sensorimotor integrators.

Figure 1. Optic-Flow-Sensitive Descending Neurons Do Not Adapt Strongly

(A) We stimulated optic-flow-sensitive descending neurons for 1 s with a full-screen, full-contrast, preferred-direction sinusoidal grating (5 Hz, wavelength 7 degrees). The screen was at mid-luminance before and after stimulation. (B) Example time-aligned raw data from an optic-flow-sensitive type 2 neuron. (C) Spike histograms from optic-flow-sensitive type 1 neurons to preferred-direction, full-contrast sinusoidal gratings moving at different temporal frequencies for 1 s. $N = 5-9$, mean $\pm$ SEM. (D) Spike histograms from optic-flow-sensitive type 2 neurons. $N = 8-10$, mean $\pm$ SEM. (E) Magnification of the mean response onset of type 1 (left) and type 2 (right) neurons, color coded from lowest (darker) to highest temporal frequency (brighter). Data replotted from (C) and (D). Dashed line indicates stimulus onset, and the horizontal line the 20 ms analysis window used for quantification. (F) The response transient divided by the later response from (B–D) (mean $\pm$ SEM, two-way ANOVA). In (B–D), dashed lines indicate peri-stimulus duration, and horizontal lines the early and late 200 ms analysis windows used for statistical comparisons (Wilcoxon signed rank test, or paired t test).

We quantified the effects of visual adaptation in optic-flow-sensitive descending neurons of the hoverfly *Eristalis tenax*. We found that, as opposed to most sensory and visual neurons, descending neurons show little adaption to continuous visual motion and moreover, they display long-lasting persistent firing post stimulation. This is thus more consistent with the lingering calcium signal in the output synapses of the LPTCs [23] and more comparable to neural circuits involved in rhythmic behaviors or working memory (for Review, see e.g., [8, 37]). We used a test-adapt-test protocol to quantify the effect on the contrast sensitivity function and found that when the persistent firing was accounted for, the descending neurons show adaptation. Our results are important, as they show that optic-flow-sensitive descending neurons not only display persistent firing but that they also show adaptation, thus displaying characteristics of both sensory and pre-motor neurons.

RESULTS

Descending Neurons Show Limited Adaptation

When optic-flow-sensitive descending neurons in the hoverfly are stimulated for 1 s with a full-contrast, full-screen, preferred-direction sinusoidal grating (Figure 1A), they respond with vigorous firing, as seen in the raw data from an optic-flow-sensitive type 2 neuron (Figure 1B, dashed lines indicate peri-stimulus duration). Both optic-flow-sensitive type 1 (Figure 1C, $N = 5-9$) and type 2 neurons (Figure 1D, $N = 8-10$) show an onset response transient to a high-contrast grating. This onset transient is similar at lower temporal frequencies but larger at 20 Hz (Figure 1E). If there is no difference between the onset transient and the sustained response, their ratio should be 1, but we found that it was bigger than 1 at frequencies below 20 Hz (dotted line, one sample t test, Figure 1F).

Following the onset transient, there is little or no adaptation to 1 s of continuous motion across temporal frequencies (Figures 1C and 1D), and even an increase in response to 0.5 Hz stimulation in the type 2 neuron (Figure 1D). This is strikingly different to what is seen in the pre-synaptic LPTCs, which adapt strongly to continuous motion stimulation, especially at higher temporal frequencies [22, 38, 39].

Persistent Firing Post Stimulation

Given that the spike rate appeared to increase following stimulation (Figures 1C and 1D), we next quantified this after-effect (Figure 2A). We find post-stimulus persistent firing, or an after-effect, after stimulation with a range of temporal frequencies (open symbols, Figures 2B and 2C, $N = 11$ and $N = 6$, respectively). The

Figure 2. Persistent Firing in Descending Neurons

(A) The response of type 2 neurons to 1 s full-screen, full-contrast, preferred-direction sinusoidal grating (6 Hz, wavelength 7 degrees). Before and after stimulation the screen was left at mid-luminance. The three 200 ms analysis windows are indicated.
(B) The response and after-effect in type 2 neurons as a function of stimulus temporal frequency. The after-effect depended on temporal frequency and was significantly larger than spontaneous rate (two-way ANOVA).
(C) The after-effect in type 1 neurons depended on temporal frequency and was significantly larger than spontaneous rate (two-way ANOVA).
(D) Magnification of the data in (A) around the cessation of motion (dashed line), together with an exponential decay function, where τ is the time constant.
(E) The decay time constant in type 2 neurons as a function of adapting temporal frequency.
(F) The decay time constant in type 1 neurons as a function of the temporal frequency of the adapting stimulus.
The “response” data in (B) and (C) has been shown previously [34]. In (A), (B), (D), and (E), $N = 11$, mean $\pm$ SEM. In (C) and (F), $N = 6$, mean $\pm$ SEM.

after-effect is significantly larger than spontaneous rate and depends on the adapting stimulus’s temporal frequency (Figures 2B and 2C), with smallest after-effect after 3–10 Hz stimulation. This is strikingly different to most sensory systems, including the pre-synaptic LPTCs, which show an antagonistic after-effect [18, 40, 41], but is suggested by the accumulating calcium in the LPTC output synapses [23].

We quantified the time course of the after-effect by fitting an exponential decay function to the response following stimulus offset (Figure 2D). We found that the time constant of the decay depended on the temporal frequency of the preceding stimulus in both type 2 (Figure 2E, $N = 11$) and type 1 neurons (Figure 2F, $N = 6$), and that it is especially long after stimulation at low frequencies.

We next compared the strength of the after-effect following 0.5 s, full-contrast, preferred-direction stimulation after which the screen either switched to mid-luminance levels or the sinusoidal grating simply stopped. We found that the after-effect was significantly larger when the grating stopped (Figure 3A, $N = 10$). We next quantified the after-effect as a function of stimulus direction following 1 s full-contrast adaptation and found that it was significantly larger than the spontaneous rate following 7 out of 8 directions (open symbols, Figure 3B). Interestingly, the after-effect was still significantly larger than spontaneous rate after stimulation in the anti-preferred direction (last data point, Figure 3B).

When using a preferred-direction grating, we found that the after-effect was significantly different from spontaneous rate after

0.5 s or 1 s stimulation but not after 5 s (Figure 3C, $N = 10$). We also found that the after-effect depends on stimulus contrast (open symbols, Figure 3D, $N = 10$), but this is not necessarily correlated with the response during stimulation, which starts at much lower contrasts (filled symbols, Figure 3D).

Contrast-Dependent Effect of Adaptation

To investigate whether the after-effect (Figures 2 and 3) influences subsequent responses, we next used a test-adapt-test protocol [18] to record the contrast-response function before and after adaptation (Figure 4A). In LPTCs, which are likely pre-synaptic to the descending neurons (Figure 4B), the response before adaptation (Test 1) is bigger than the response after adaptation (Test 2, Figure 4A). There is a right-shift of the contrast-response function, referred to as a contrast gain reduction. This is non-directional, because the function is right-shifted following preferred- as well as anti-preferred-direction adaptation [18, 42]. There is a non-directional output range reduction [18, 42], suppressing the response (Figure 4A) to full-contrast stimuli. In addition, there is an antagonistic after-effect, i.e., a down-shift of the contrast response function after preferred-direction adaptation (Figure 4A), and vice versa [18]. The Test 2 response can be “normalized” by subtracting this after-effect (dash-dotted line, Figure 4A).

Here, we adapted optic-flow-sensitive descending neurons (Figure 4B) for 1 s with a full-screen, full-contrast, preferred-direction sinusoidal grating drifting at different temporal frequencies (Figure 4C). In LPTCs, one second adaptation is

Figure 3. The Stimulus Affects the After-Effect

(A) The after-effect following 0.5 s full-screen, full-contrast, preferred-direction stimulation (5 Hz, wavelength 7 degrees), with the screen either left at mid-luminance or the sinusoidal grating stopping. Stars indicate significant difference ($p < 0.01$) between the two after-effects (two-way ANOVA, followed by Sidak's multiple comparison test).

(B) The after-effect following 1 s, full-screen, full-contrast stimulation in different directions (5 Hz, wavelength 7 degrees), with the screen at mid-luminance.

(C) The after-effect as a function of stimulus duration (5 Hz, wavelength 7 degrees). Timer icon by Prepic (<https://www.flaticon.com>).

(D) The after-effect as a function of stimulus contrast (0.5 s, 5 Hz, wavelength 7 degrees).

In (A–D), data from type 2 neurons displayed as mean $\pm$ sem, N = 10. In (B–D), stars indicate significant differences ($p < 0.05$) between the after-effect and spontaneous rate (two-way ANOVA, followed by Sidak's multiple comparison test).

long enough [43] to saturate the three motion adaptation components (Figure 4A). The test stimuli consisted of full-screen sinusoidal gratings with varying contrast but always identical before and after adaption (Figure 4C). The example data show the response of optic-flow-sensitive type 2 neurons to test stimuli with a contrast of 0.02, and an adapting stimulus at 0.5 Hz (Figure 4D, N = 8). The Test 1 response (see analysis window, Figure 4D) is barely larger than the spontaneous rate. However, the Test 2 response to the same contrast (see analysis window, Figure 4D) is increased.

In the full-contrast response function, the Test 2 responses (open symbols, Figures 4E and 4F) are substantially and significantly larger than the Test 1 responses (closed symbols, Figures 4E and 4F) for the lowest test contrasts in both neuron types. Indeed, the Test 2 response to lower test contrasts is similar to the after-effect (test contrast = 0, open symbol, Figures 4E and 4F). At higher contrasts, the Test 2 response is smaller than the Test 1 response in the type 1 neuron (Figure 4E), but not in the Type 2 neuron (Figure 4F). We normalized the data by subtracting the after-effect, i.e., the Test 2 response to a test contrast of 0. The normalized data in the descending neurons (dash-dotted lines, Figures 4E and 4F) is similar to normalized LPTC responses (dash-dotted line, Figure 4A), by showing an output range reduction and a contrast gain reduction. We see qualitatively similar effects after adapting at other temporal frequencies (Figure S1).

Quantification of Adaptation

We quantified three parameters from each contrast-response function (Figure S1). The first was the after-effect, i.e., the Test 2 response to a test contrast of 0 (open symbols, Figures 5A and 5D), with the Test 1 response to a contrast of 0 acting as baseline (closed symbols, Figures 5A and 5D). In type 2 neurons,

the after-effect was smallest after adapting at 5 Hz (open symbols, N = 8–10, Figure 5A), whereas in type 1 neurons there was no dependence on adapting temporal frequency (Figure 5D, N = 5–9). This after-effect (open symbols, Figures 5A and 5D) means that the neuron was still firing above baseline post stimulation. Note that in LPTCs, the after-effect after preferred-direction adaptation is inhibitory [18, 41], whereas it was excitatory in the descending neurons (open symbols, Figures 5A and 5D).

We defined the output range reduction as the Test 2 response to a contrast of 1 divided by the Test 1 response to a contrast of 1, after removing the spontaneous rate (Figures 5B and 5E). If the two responses are identical, this ratio will be 1 (dotted line, one sample t test, Figures 5B and 5E), but if it is below 1, the response to a full-contrast grating is suppressed. We found that the ratio was below 1 after adapting at the higher and lower frequencies. For the normalized data, the ratio was below 1 after adapting at all frequencies (dash-dotted line, Figures 5B and 5E) and of similar amplitude to that seen in LPTCs [18, 43]. However, as opposed to LPTCs [44] the output range reduction depended on adapting temporal frequency (Figures 5B and 5E).

We extracted the contrast that generated 50% of the maximum Test 1 response, which we call C_{50} , from the Test 1 (dotted line, Figures 5C and 5F), the Test 2 (solid line, Figures 5C and 5F), and the normalized response (dash-dotted line, Figures 5C and 5F). C_{50} did not depend on the adapting temporal frequency (Figures 5C and 5F), like in the LPTCs [44]. In both neuron types, the normalized C_{50} (dash-dotted lines, Figures 5C and 5F) was of similar amplitude to that seen in LPTCs [43, 44]. This means that following adaptation, a higher contrast is needed to generate the same normalized response.

In summary, after subtracting the after-effect, which reflects the post-stimulus persistent firing of the optic-flow-sensitive descending neurons, to get the normalized response, the output

Figure 4. The Test-Adapt-Test Stimulus

(A) An LPTC contrast response function before (Test 1) and after (Test 2) preferred direction adaptation, with an antagonistic after-effect, a contrast gain reduction, and an output range reduction [18]. The Test 2 response can be “normalized” (dash-dotted line) by subtracting the after-effect [18].
 (B) LPTCs are pre-synaptic to the optic-flow-sensitive descending neurons [30–32].
 (C) The test-adapt-test stimulus, where test stimuli had varying contrast but were always identical before and after adaptation. The adapting stimulus was shown at full contrast but had different temporal frequencies. All stimuli were preferred direction.
 (D) Time-aligned spike histogram from type 2 neurons to an 0.5 Hz adapting stimulus and test contrasts of 0.02 (N = 8, mean $\pm$ SEM). 200 ms analysis windows used for quantification are indicated. The dashed lines show stimulus durations.
 (E) The contrast response function of type 1 neurons to an 0.5 Hz adapting stimulus (N = 8, mean $\pm$ SEM; also in Figure S1H).
 (F) The contrast response function of type 2 neurons to 0.5 Hz adaptation (N = 8, mean $\pm$ SEM; also in Figure S1C).
 In (E) and (F), stars (*) indicate significant differences between the Test 1 and the Test 2 response (two-way ANOVA, followed by Sidak’s multiple comparisons test).

range reduction and contrast gain reduction are of similar amplitude to normalized LPTC responses.

The Effects of Adaptation Are Non-directional

In LPTCs, the contrast gain reduction and the output range reduction are non-directional, i.e., present after both preferred- and anti-preferred-direction adaptation, whereas the after-effect is directional and antagonistic [18]. To investigate whether direction has an effect in the descending neurons, we changed the direction of either adapting or test stimuli. The control data (both test and adapting stimuli move in the preferred direction) are shown in Figure 6A (N = 10). When the adapting stimulus instead moves in the anti-preferred direction, the resulting output range reduction (as defined in Figures 5B and 5E) is similar (0.85 ± 0.04 and 0.90 ± 0.04 , respectively, Figures 6A and 6B). Furthermore, there is no significant difference in the Test 2 C_{50} after adapting in the anti-preferred direction (0.14 ± 0.02 , Figure 6B) compared with preferred direction (0.10 ± 0.02 , Figure 6A), or in the normalized C_{50} (0.15 ± 0.03 and 0.18 ± 0.02 , respectively, Figures 6A and 6B). This suggests that both the contrast gain reduction and output range

reduction are non-directional, as in the pre-synaptic LPTCs [18]. However, as opposed to LPTCs [18, 41], the after-effect in the descending neurons is similar following adaptation in either the preferred or anti-preferred direction (52 ± 3 and 48 ± 7 spikes/s, respectively, 0 contrast, open symbols, Figures 6A and 6B).

We next performed the inverse experiment, where we tested in the anti-preferred direction (Figure 6C). After 5 Hz preferred-direction adaptation, the Test 2 response to the lowest contrasts (open symbols, Figure 6C) is similar to control (open symbols, Figure 6A), i.e., testing direction does not affect the after-effect (52 ± 3 and 64 ± 9 spikes/s, respectively, Figures 6A and 6C). It was difficult to get reliable curve fits for the individual contrast response functions, but a qualitative inspection suggests that the contrast gain reduction is similar for the Test 2 data (open symbols, Figure 6C). Furthermore, the output range reduction was similar (0.89 ± 1.8) but was again difficult to quantify reliably as the neuron cannot go below 0 spikes. Nevertheless, in summary, in descending neurons, none of the adaptation effects appear to depend on the direction of the adapting stimulus.

Figure 5. Quantification of Adaptation Effects

(A) The type 2 neuron after-effect, i.e., the response to a 0 contrast test, as a function of adapting temporal frequency. Because the contrast was zero, the Test 1 "response" is the spontaneous rate. The Test 2 response depended on temporal frequency and was significantly larger than the Test 1 response (two-way ANOVA).

(B) The output range reduction defined as shown in equation. If there was no effect of adaptation, this would be 1 (dotted line, stars indicate significant reduction, one sample t test). The output range reduction depended significantly on adapting temporal frequency (two-way ANOVA).

(C) Type 2 neuron C_{50} , i.e., the contrast that generated 50% maximum Test 1 response (two-way ANOVA).

(D) The after-effect in type 1 neurons. The Test 2 response did not depend on temporal frequency but was significantly larger than the Test 1 response (two-way ANOVA).

(E) The output range reduction in type 1 neurons. The dotted line indicates no reduction (one-sample t test). The output range reduction depended significantly on adapting temporal frequency (two-way ANOVA).

(F) The contrast gain reduction in type 1 neurons (two-way ANOVA).

In (A–C), $N = 8$ –10. In (D–F), $N = 5$ –9. All data displayed as mean $\pm$ SEM, extracted from contrast response functions in Figure S1.

Global Adaptation

To investigate whether the adaptation effects (Figures 4, 5, and 6) are locally or globally generated we next used non-overlapping strip stimuli, which test and adapt in different parts of the receptive field (modified from [42]). If the adaptation is a global phenomenon, the Test 2 response should look the same as when both stimuli cover the entire screen (Figure 7A, top right). However, if adaptation is local, the Test 2 response should be similar to the Test 1 response (Figure 7A, bottom right).

We placed the test stimulus in the ventral visual field and the adapting stimulus in the dorsal visual field (Figure 7B). This strip stimulus generated a small but clear response (test contrast = 0.2, $N = 7$, Figure 7C). Testing and adapting in different parts of the receptive field generated a much smaller after-effect (25 ± 8 spikes/s, $N = 7$, open symbol, 0 contrast, Figure 7D) compared with full screen stimuli (60 ± 11 spikes/s, $N = 9$, open symbol, 0 contrast, Figure 7E). This is not necessarily caused by the smaller response to the adapting stimulus (gray, Figure 7D), because the after-effect is poorly correlated with the response during adaptation (e.g., Figure 2C). There was no significant difference between the Test 2 C_{50} after adaptation with the strip stimulus (0.37 ± 0.13 , t test, Figure 7D) compared with full-screen adaptation

(0.17 ± 0.10 , Figure 7E). However, the output range reduction was much stronger after adaptation with the strip stimulus (0.38 ± 0.06 , Figure 7D) compared with full-screen adaptation (0.84 ± 0.07 , t test, Figure 7E), with a similar result for the normalized data.

As there are evident differences between the Test 2 and the Test 1 contrast response functions (Figure 7D), we can rule out local mechanisms (bottom right, Figure 7A). However, as the after-effect was smaller, the output range reduction larger, and the C_{50} unaffected, more work using a range of stimulus conditions is clearly needed.

DISCUSSION

We have shown that following an onset transient, hoverfly optic-flow-sensitive descending neurons adapt poorly to continuous widefield motion (Figure 1). In addition, the neurons display persistent firing following the cessation of stimulation (Figures 2 and 3). We used a test-adapt-test protocol to investigate the effect of persistent firing, or after-effect (Figure 4). Like their pre-synaptic LPTCs [18, 42, 43], the descending neurons show non-directional output range reduction (Figures 5B, 5E, and 6) and contrast gain reduction (Figures 5C, 5F, and 6), which are unlikely to be generated locally (Figure 7).

Figure 6. Adaptation Is Non-directional

(A) The contrast response function following preferred direction adaptation when testing in the preferred direction (N = 10; also in Figure S1D).

(B) The contrast response function following anti-preferred direction adaptation when testing in the preferred direction (N = 8).

(C) The contrast response function following preferred direction adaptation when testing in the anti-preferred direction (N = 7).

In (A–C), stars indicate significant difference between Test 1 and Test 2 responses (two-way ANOVA, followed by Sidak's multiple comparisons test). All stimuli were shown at 5 Hz to type 2 neurons. All data displayed as mean ± SEM.

LPTCs and Behavior

We found that descending neurons provide a better match with behavior than LPTCs do. First, LPTCs show an antagonistic after-effect, i.e., they are inhibited following preferred-direction stimulation [18, 22, 40, 41]. However, the behavioral optomotor response continues for several seconds following the cessation of visual motion [23, 45]. The descending neurons displayed persistent firing following stimulation (Figures 2 and 3), thus more consistent with behavior. During preferred-direction stimulation LPTCs accumulate calcium in the input dendrites [46] and the output synapses [23]. The lingering calcium signal in the output synapse outlasts visual stimulation by several seconds [23] and could potentially explain the persistent firing (Figures 1, 2, and 3). Indeed, when we tested and adapted in different parts of the receptive field, thereby driving input from fewer presynaptic LPTCs, the after-effect was reduced (Figure 7D).

Second, the temporal frequency tuning of fly LPTCs peaks at temporal frequencies around 1 Hz in *Drosophila* [47], just under 10 Hz in *Calliphora* [46, 48], and around 10 Hz in *Eristalis* [49]. This is shifted to higher temporal frequencies in physically active animals [47, 50] but not enough to match behavior [23, 51, 52]. Instead, optomotor behavior appears to plateau as temporal frequency is increased [23, 51]. The calcium signal in the LPTC input dendrites is tuned to higher temporal frequencies than the intracellularly recorded membrane potential [46] and if the calcium in the output synapse follows a similar pattern this could explain the response of the descending neurons (Figures 2B and 2C, see also [34]), beyond temporal frequencies expected from intracellular LPTC data [49]. However, in the descending neurons the after-effect did not increase with increasing stimulus duration (Figure 3C), which is different to the LPTC calcium, which works as a temporal integrator [23]. Maybe some of these differences are caused by different behavioral states (flight versus non-flight).

Third, LPTCs adapt strongly during continuous motion, whereas flight behavior does not (e.g., [45, 51, 53]). LPTCs adapt less when the animal is physically active [23, 52], but the difference is not enough to explain behavior. We found that following an onset transient, descending neurons do not adapt strongly

(Figure 1), thus more closely matching behavior. Furthermore, descending neurons adapted more to low temporal frequencies (Figures 1C and 1D), whereas LPTCs adapt more to high frequencies (e.g., [22, 48]).

The onset transient was similar for stimuli below 20 Hz (Figure 1E) and substantially larger than the sustained response (Figure 1F). Many olfactory and visual neurons display a similar initial transient response to strong stimuli, which is often followed by slower response decay (e.g., [22, 24, 54, 55]). The initial response transient is the information rich component with higher contrast gain and response gain compared with the sustained response [56]. Indeed, in cat V1, the initial response transient shows increased contrast gain (i.e., a left shift of the contrast response function), whereas the decay phase shows a decrement in response gain (a combination of an after-effect and output range reduction) [57]. This indicates that the analysis window plays an important role. We used analysis windows beyond this initial transient, to make our results (Figures 2, 3, 4, 5, 6, and 7) comparable with the literature, of both vision [18] and audition [4]. Nevertheless, in future work it could be interesting to investigate how more dynamic visual stimuli (e.g., [58]) affects adaptation.

Adaptation and Persistent Firing

The after-effect was poorly correlated with the response during stimulation. For example, even if the descending neurons responded weakly to low temporal frequencies, the after-effect was strong (Figures 2B and 2C). In addition, we saw an after-effect following inhibition (Figures 3B and 6B), and even after stationary stimuli (Figures 2B, 2C, 5A, and 5D). Stationary stimuli do not lead to an after-effect in LPTCs [18], nor does anti-preferred-direction motion generate calcium accumulation [46], suggesting that additional processes could generate the after-effect we recorded. Given that the after-effect was smaller after low-contrast adaptation (Figure 3D), it is likely driven by the high-contrast pattern, rather than by motion per se. Indeed, the after-effect was smaller after local adaptation (Figure 7D), which is again different to the presynaptic LPTCs [42]. Note, though, that the after-effect was even larger when the pattern simply stopped rather than switched to a mean luminance screen (Figure 3A).

Figure 7. Local Adaptation

(A) The contrast response function when adapting with full-screen stimuli (left). If adaptation mechanisms are global, the resulting contrast response functions after testing and adapting in non-overlapping parts of the receptive field (top) should not change. If adaptation mechanisms are local, the Test 2 contrast response function should look similar to the Test 1 function (bottom).
(B) The non-overlapping test-adapt-test stimulus (modified from [42]), where test stimuli had varying contrast but were identical before and after adaptation. The adapting stimulus was shown at full contrast, at 5 Hz.
(C) The time aligned spike histogram to 5 Hz adaption and test contrasts of 0.2 (N = 7). The boxes indicate the 200 ms analysis windows and the dashed lines peri-stimulus durations.
(D) The contrast response function to non-overlapping stimuli (N = 7).
(E) The contrast response function to full-screen stimuli (N = 9; also in Figure S1).
In (D) and (E) stars indicate significant differences between Test 1 and Test 2 responses (two-way ANOVA, followed by Sidak's multiple comparisons test). All data from type 1 neurons, displayed as mean $\pm$ SEM.

We found that when we normalized the adapted contrast response functions of the descending neurons (dash-dotted lines, Figures 4, 6, and S1), the contrast gain reduction and output range reduction were comparable to LPTCs [18, 43], suggesting that these effects are coming from the LPTCs. However, when using local stimuli, the output range reduction was larger (Figure 7D). In addition, the contrast gain reduction depended on temporal frequency (Figures 5B and 5E), which is not seen in LPTCs [44]. Our results therefore suggest that the descending neurons do adapt themselves.

As the descending neurons connect with pre-motor neurons controlling behavior [30, 35, 36, 59], they work as neural sensori-motor integrators. It is thus interesting that in addition to adaptation, they show persistent firing, like other neurons and networks involved in sensori-motor transformations. Indeed, persistent activity is common in vertebrate and invertebrate central pattern generators to coordinate, e.g., walking and swimming (see e.g., [16, 37, 60, 61]). Persistent firing can arise from intrinsic or extrinsic mechanisms. Intrinsic mechanisms include

intracellular currents from e.g., voltage-dependent sodium channels, in both vertebrates and invertebrates [62] or sodium-activated potassium channels [63]. Extrinsic mechanisms include feedback loops in local networks, such as within the spinal cord [60, 61]. In vertebrate central pattern generators, positive feedback loops [64] and reciprocal inhibition mediating rebound firing [65] is fundamental for left-right coordinated motor activity. Importantly, as we recorded extracellularly, we cannot deduce the mechanisms behind the persistent firing shown here. However, as described above, it is likely that at least a part of the persistent activity come from accumulated calcium in the LPTC output [23]. Future dual recordings between LPTCs and descending neurons could clarify this.

Concluding Remarks

Even if the after-effect was similar in type 1 and type 2 neurons (Figure 2), its effect on responses to subsequent stimuli sometimes differed. For example, the output range reduction was smaller in type 2 neurons (Figures 5B and 5E), and type 1 neurons

adapted more to low temporal frequencies (Figure 1). It would be interesting to look at behavioral adaptation and correlate this with different descending neurons [66]. The type 1 and type 2 neurons are physiologically similar to DNHS1 and DNOVS2 in other flies [34], which both project to the wing and haltere neuropils, but likely have different behavioral roles [30]. Indeed, some phasic flight muscles adapt rapidly whereas the activity of slow tonic muscles decays more slowly [26], suggesting that they could be controlled by different descending neurons.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at <https://doi.org/10.1016/j.cub.2020.05.019>.

ACKNOWLEDGMENTS

This research was funded by the US Air Force Office of Scientific Research (FA9550-19-1-0294), the Australian Research Council (DP170100008, DP180100144, and FT180100289), and the Flinders Foundation. We thank the managers of the Botanic Gardens for their support.

AUTHOR CONTRIBUTIONS

S.N. and K.N. conceived experiments, analyzed data, and wrote the manuscript. S.N. performed experiments. K.N. secured funding.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: March 4, 2020

Revised: April 22, 2020

Accepted: May 6, 2020

Published: May 28, 2020

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and Algorithms		
MATLAB R2015b	Mathworks	https://au.mathworks.com/
FlyFly 3.1.3		https://hoverflyvision.weebly.com/software.html
LabChart 7 Pro	ADInstruments	https://www.adinstruments.com/products/labchart
Prism 7.0d	GraphPad	https://www.graphpad.com
Psychophysics toolbox	[67, 68]	http://psychtoolbox.org/download.html
Electrophysiology Hardware		
DAM50 differential amplifier	World Precision Instruments	https://www.wpiinc.com/sys-dam50-dam50-extracellular-amplifier
HumBug	Quest Scientific	http://www.quest-sci.com/
Tungsten electrodes	Microprobes	https://www.microprobes.com/products/metal-microelectrodes/monopolar-electrodes/tungsten
Powerlab 4/30	ADInstruments	https://www.adinstruments.com/products/labchart
165 Hz LCD screen	Asus, Taipei, Taiwan	https://www.asus.com/Monitors/ROG-SWIFT-PG279QE/
Deposited Data		
All data	This paper	https://doi.org/10.5061/dryad.fbg79c9
Experimental Models: Organisms/Strains		
Male <i>Eristalis tenax</i> hoverflies	Reared from wildtype, [69]	N/A
BugDorm	Australian Entomological Supplies	https://www.entosupplies.com.au/equipment/laboratory/breeding-cages-laboratory/bugdorm-insect-rearing-cages-em42222-245x245x245mm-and-em4030-325x325x325mm-series-lightweight/

RESOURCE AVAILABILITY

Lead Contact

Further information and requests for resources and data should be directed to and will be fulfilled by the Lead Contact, Karin Nordström (Karin.nordstrom@flinders.edu.au).

Materials Availability

This study did not generate new unique reagents.

Data and Code Availability

The datasets generated during this study are available at DataDryad (<https://doi.org/10.5061/dryad.fbg79c9>). Raw electrophysiological data is available upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Eristalis tenax hoverflies were reared from eggs laid by wild-caught females as described previously [69]. Briefly, female *Eristalis tenax* hoverflies were collected in the Wittunga Botanic Garden, Adelaide, South Australia. Larvae were raised in a rabbit dung slurry, kept at room temperature. Adult hoverflies were kept in bugdorms with a 24.5 cm side in a fridge with a mean temperature of 11°C. Twice a week the hoverflies were brought to room temperature for 6–8 h, and given fresh water, honey and pollen. We recorded from 78 neurons in 76 male hoverflies, around 3 months old. We kept data from all neurons that gave a minimum 80% response of the mean published response to a preferred direction full-screen sinusoidal grating [34].

METHOD DETAILS

Electrophysiology

At experimental time, the hoverfly was immobilized ventral side up with a beeswax and resin mixture, and a small hole cut over the cervical connective at the anterior end of the thorax. A sharp polyimide-insulated tungsten electrode (2 MOhm, Microprobes, Gaithersburg, USA) was inserted into the cervical connective, with mechanical support given by a small wire hook. The animal was grounded via a silver wire inserted to the cavity, which also served as the recording reference.

Extracellular signals were amplified at 1000x gain and filtered through a 10 – 3000 Hz bandwidth filter on a DAM50 differential amplifier (World Precision Instruments), with 50 Hz noise removed with a HumBug (Quest Scientific, North Vancouver, Canada). The data were digitized via Powerlab 4/30 and acquired at 40 kHz with LabChart 7 Pro software (ADInstruments, Sydney, Australia).

Visual stimulation

Visual stimuli were displayed on a linearized Asus LCD screen (Asus, Taipei, Taiwan) with a mean illuminance of 200 Lux, a spatial resolution of 2560 × 1440 pixels, running at 165 Hz, using the Psychophysics toolbox [67, 68] in MATLAB (Mathworks, 2017). *Eristalis* males were placed at a distance of 6.5 cm, giving a projected screen size of 155 × 138 degrees. We defined optic-flow-sensitive neurons as type 1 or type 2 based on their receptive field and direction selectivity to full-screen sinusoidal gratings [34].

We used full screen, preferred-direction sinusoidal gratings with a wavelength of 7 degrees, moving at 5 Hz, unless otherwise mentioned. The test-adapt-test protocol (adapted from [18]) used varying contrasts, but these were always identical before and after adaptation. Before and after stimulation the screen was left at mid luminance for 3–10 s, unless otherwise mentioned. After adapting at 20 Hz, this was increased to 60 s. To compare the local and global components of adaptation the test stimuli and adapting stimuli covered the width of the screen, but only 20 degrees of the height (modified from [42]). The test stimulus was placed in the equatorial-ventral visual field, and the adapting stimulus in the dorsal visual field, separated by 76 degrees.

QUANTIFICATION AND STATISTICAL ANALYSIS

Spike sorting was done using LabChart 7 Pro with the Spike Histogram Add-On (ADInstruments, Sydney, Australia), which uses the action potential amplitude and width to identify responses from individual neurons. All further data analysis was done in MATLAB and GraphPad Prism (version 7.0d, GraphPad Software Inc, USA).

Consistent with previous work [18, 44], we quantified the spike rate in 200 ms analysis windows, starting 100 ms after each stimulus' onset, unless otherwise mentioned. The spontaneous rate was quantified 300–100 ms before the onset of the first stimulus. The after-effect was defined as the response to a mid-luminance screen 100–300 ms after stimulation. The onset transient was quantified in a 20 ms analysis window starting 15 ms after stimulus onset. All histograms shown in figures are displayed with 1 ms resolution, after smoothing with a 20 ms square-wave filter. We fitted an exponential decay to this data immediately following the offset of stimulation to extract the time constant.

For test-adapt-test experiments, we normalized the adapted data by subtracting the after-effect, i.e., the Test 2 response to a test contrast of 0. The output range reduction was defined as the ratio between the responses to a full contrast test stimulus before and after adaptation after removing the spontaneous rate, i.e., $(\text{response}_{\text{Test 2 or Normalized}} - \text{spontaneous rate}) / (\text{response}_{\text{Test 1}} - \text{spontaneous rate})$. The contrast gain reduction was defined by finding C_{50} . This was done by fitting a Weibull function [42] to the Test 1, Test 2 and normalized contrast response functions of each neuron and then determining the contrast which generated 50% of the maximum Test 1 response.

Statistical analysis was performed in GraphPad Prism. For each test, we checked if the data were normally distributed, before testing for significance, with details of the tests given in the figure legends. P values below 0.05 were used to refute the null hypothesis, after doing a Bonferroni correction for multiple comparisons. In all figures one star (*) indicates $p < 0.05$ and two stars (**) indicate $p < 0.01$.