

# Efference Copies: Hair Cells Are the Link

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**Animals must distinguish external stimuli from self-generated sensory input to guide appropriate behaviors. A recent study elucidates a cellular mechanism by which zebrafish perform this distinction while maintaining sensitivity to external environmental signals.**

Neural circuits receive a wealth of sensory input from both external (exafferent) environmental stimuli and self-generated (reafferent) behaviors. Consequently, to respond appropriately to sensory input, animals must first disambiguate its origin; does an image moving across the retina, for example, originate from a moving external object or from self-generated eye movements? A considerable body of work has illuminated how diverse sensorimotor systems use precise copies of self-generated motor behaviors, or efference copy signals, to predict the extent to which sensory input is self-originating [1–3]. However, the precise cellular details underlying this feat have remained mysterious. A new study by Pichler and Lagnado [4], reported in this issue of *Current Biology*, offers deeper insight into how zebrafish suppress sensory input generated during swimming while maintaining sensitivity to external environmental stimuli.

The lateral line of zebrafish measures changes in water flow, and fish use these measurements to guide behavior. Both exafferent sources, such as predator or prey movement [5], and reafferent signals arising from self-generated swimming motions [6] can elicit changes to water flow. Early work looked to the exquisite neural architecture of the lateral line as a potential cellular substrate that might disambiguate exafferent from reafferent activation and gate motor responses. Changes in water flow deflect mechanosensory hair cells in lateral line neuromasts, and lateral line afferent neuron projections relay encoded information about water flow to sensory processing areas (Figure 1A) [7]. Neuromasts also receive cholinergic efferent innervation (Figure 1A); these

efferent fibers are poised to suppress afferent neuron activity during self-generated movements [8–11]. But while efferent fibers are well-poised to convey an efference copy signal, technological limitations in monitoring activity changes simultaneously at multiple sites (efferent fibers, hair cells, and afferent neurons) have precluded mechanistic insight.

In their work, Pichler and Lagnado [4] combined new high-resolution, *in vivo* imaging techniques with an existing motor behavior paradigm to examine the mechanism by which efference copy signals suppress self-generated activity in the lateral line. What does the efference copy signal quantitatively convey? What is the cellular mechanism by which it suppresses afferent neuron activity? Most importantly, does suppression of self-generated activity occur at the expense of maintaining sensitivity to external perturbations?

To investigate the content of the efference copy signal, the authors [4] performed *in vivo* calcium imaging of efferent fiber activity while simultaneously recording from the motor nerve during fictive swimming. This preparation preserves endogenous motor activity patterns that naturally drive swimming [12]. The authors first replicated early qualitative findings that bursts of efferent fiber activity are synchronous with swimming-derived motor nerve activity [9,10]. They extended this observation by relating the strength of the motor signal to the time-course of efferent fiber activation. This result is particularly timely; recent complementary work found that the efference copy signal most closely translates the swim duration component of motor activity [13]. Together, these findings demonstrate that efferent fibers

are quantitatively and temporally poised to suppress self-generated activity.

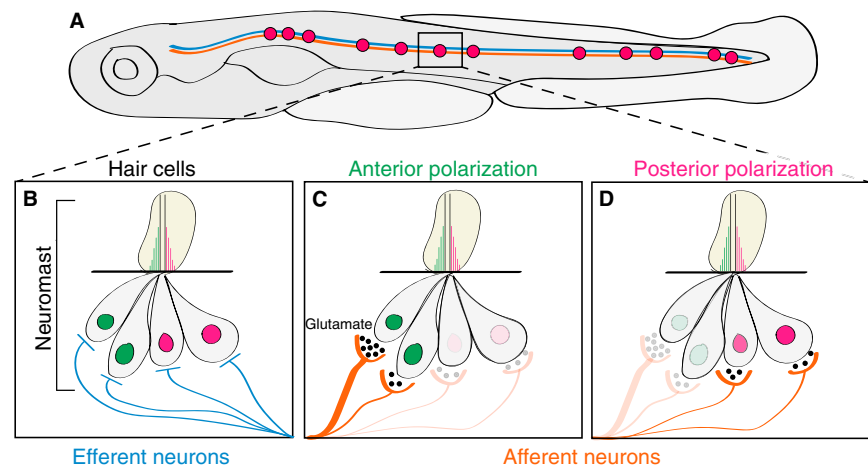
Pichler and Lagnado [4] next sought to elucidate a cellular mechanism by which efferent fibers suppress afferent neuron activity [9–11,13,14]. They postulated that the efference copy signal modulates neuromast output, the first synapse in the lateral line system, with ensuing consequences for downstream afferent neuron activity. To test this hypothesis, the authors leveraged a fluorescent reporter of glutamate concentration, iGluSnFR [15], to measure the spontaneous synaptic output of hair cells and afferent neurons in real-time. Simultaneously, they recorded motor nerve activity during fictive swimming. Each fictive swimming bout was highly correlated with suppression of hair cell glutamate release by 50%, with corresponding reduction of afferent neuron activity. These findings support a role for hair cells as a cellular target by which efference copy signals suppress afferent neuron activity.

Notably, hair cell suppression and recovery followed a behaviorally relevant time-course. Zebrafish locomote in ‘beat-and-glide’ intervals, in which a strong, active burst of swimming is followed by a longer period of either passive translation or motionlessness. Pichler and Lagnado [4] observed maximal glutamate release suppression within 50 ms of a motor nerve spike, and substantial recovery by 100 ms. This time-course closely aligns with previous reports that afferent neuron activity is most strongly attenuated soon after swimming onset [14] and persists shortly into the glide interval [13]. Thus, the lateral line likely regains full sensitivity within a behaviorally-relevant time period.

To investigate the consequences of exafferent activation, the authors [4] repeated these measurements following mechanical neuromast stimulation. Coincidental stimulation and motor nerve activity suppressed 71% of hair cell synapses. Moreover, in some cases, suppression fell below spontaneous baseline levels. This observation has two critical implications. First, exafferent suppression could serve to dampen activation, preventing hair cell saturation and enabling neuromasts to maintain sensitivity to environmental perturbations. Second, the strong suppression of hair cell glutamatergic output below spontaneous baseline levels suggests a strong role for efference copy suppression of reafferent activity. Together, these findings point to a robust mechanism by which the lateral line could discriminate exafferent from reafferent sensory input.

That such a robust efference copy signal suppressed only 71% of hair cells is particularly intriguing in light of a previous report that many, but not all, afferent neurons are inhibited following efferent fiber stimulation [14]. Pichler and Lagnado [4] had observed that all efferent fibers exhibit closely-synchronized activity levels and postulated that all hair cells receive a highly-similar efference copy signal (Figure 1B). Why, then, are not all hair cells and afferent neurons suppressed? The authors speculated that these differential responses arise from the population's considerable heterogeneity [16]. To test this hypothesis, they analysed the distribution of hair cell responses with respect to one primary subtype distinction: polarity. Hair cells are preferentially activated by either posterior deflections, such as during forward swimming, or anterior deflections [17]. Indeed, the authors discovered a salient response bias: suppression occurred in all hair cells polarized to posterior deflections, but in only half of their anterior counterparts (Figure 1C,D).

The functional implications of this polarity bias for lateral line discrimination of exafferent and reafferent signals are striking. The lateral line uses a 'push-pull' system to detect the direction of stimulation by comparing the activity of opposing (posterior/anterior) hair cell populations [18]. Consequently, suppression of one population will block



**Figure 1. Hair cell polarity underscores differential responses to efferent neuron suppression.**

(A) Schematic of the zebrafish lateral line system. Neuromasts (red) containing mechanosensory hair cells send afferent projections (orange) to sensory areas and also receive cholinergic efferent innervation (blue). (B) Efferent fibers transmit a highly-similar, suppressive efference copy signal to all hair cells, regardless of polarity. (C) Glutamatergic output is suppressed in approximately half of hair cells polarized to anterior deflections (green). (D) Glutamatergic output is suppressed in all hair cells polarized to posterior deflections (magenta).

directional signaling, such as reafferent activity during forward swimming. Most importantly, however, that some hair cells remain able to signal stimulation underscores a crucial mechanism by which the lateral line could selectively suppress self-generated activity while maintaining sensitivity to environmental perturbations.

Pichler and Lagnado's [4] focus on hair cells, the ultimate source of lateral line mechanosensation, extends a great body of literature that previously correlated efferent fiber stimulation with afferent neuron attenuation. Nevertheless, their findings remain at odds with a recent report [19] that afferent neuron activity increases during free swimming. Possibly, free swimming could impose additional hydrodynamic stimuli that could modulate the strength of the efference copy signal. Alternatively, hair cells tuned to anterior deflections could become up-regulated to further increase sensitivity to external stimulation with ensuing consequences for afferent neuron responsiveness. Either scenario would certainly explain why only some afferent neurons demonstrate suppression following self-generated behaviors [14]. Thus, it would be interesting in the future to examine the physiological relationship between

anteriorly-polarized hair cells and their corresponding afferent neuron partners.

With the resolution of iGluSnFR and the ability to monitor activity at multiple synaptic sites simultaneously, Pichler and Lagnado's [4] work significantly advances our understanding of how efference copy signals suppress self-generated movement. Moreover, their discovery that hair cell populations differentially employ efference copy signals to disambiguate the origin of sensory input opens several new avenues of study. For example, what molecular distinctions underscore these differential responses? Pichler and Lagnado [4] hypothesize that presynaptic differences in efferent fiber spike efficiency or differences in postsynaptic hair cell specializations could be key. Together with exciting new insights into the molecular origins of lateral line hair cell polarity [20], this work offers the potential to next uncover molecular mechanisms underscoring these cellular distinctions.

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## Chemosensation: Hate Mosquitoes? Peel Beetroots!

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Finding the right lure for trapping pest insects is difficult. The typical smell of rain and humid soil, geosmin, now turns out to be a strong attractant for the yellow fever mosquito *Aedes aegypti*.

Picture a hot summer's day, and then a thunderstorm; now imagine the smell. Chances are, you are recalling the smell of geosmin (together with other compounds). Chemically speaking, this substance is a germacranoid sesquiterpene, produced by mold fungi and bacteria such as *Streptomyces* [1, 2]. It is the typical smell of wet soil. A new study in this issue of *Current Biology* by Nadia Melo, Marcus Stensmyr and colleagues [3] now shows that geosmin is a strong attractant for gravid female yellow fever mosquitoes (*Aedes aegypti*; Figure 1), right when they are searching for wet soil to lay their fertilized eggs. Geosmin, derived from simple sources, such as beetroot peel, might turn out useful to have female mosquitoes lay their eggs in traps, instead of raising more offspring.

Humans are very good at detecting geosmin [4], though the reason for our sensitivity remains unknown. Could it have allowed our ancestors to find a water well in arid landscapes, as has been proposed for camels? As we do not know the specific geosmin-sensitive receptor in mammals, we cannot reconstruct its evolutionary history. But mammals are not the only animals to have evolved a high sensitivity for geosmin: some insects also have highly sensitive receptors! Their olfactory receptors have a separate evolutionary origin from the mammalian ones, and therefore we know that geosmin sensitivity has evolved separately, at least twice: in mammals and insects. Geosmin receptors were first identified in *Drosophila melanogaster* [5]. Interestingly, unlike humans who love the smell, these flies

loathe it: geosmin is one of the best-known repellents of *Drosophila*, leading to an innate avoidance response. One possible explanation for this finding is that moldy fruit might release geosmin. *Drosophila* uses fruit as a substrate for oviposition, and is quite choosy in terms of the fruit's ripeness: moldy fruit are not a good substrate, and therefore fruit flies should avoid laying eggs there [5]. However, mold on fruit is not a strong geosmin source. Thus, an alternative hypothesis would be that *Drosophila* prefer laying eggs on fallen but not on hanging (unripe) fruit, and geosmin would contribute to a spatial odor search push-pull mechanism: avoid the dirt, find the fallen peach! This is analogous to human vision, when seeing a red apple is easiest against a green leafy background, and evolution has shaped

