mediated heterosynaptic depression can spread may be dynamic. Because the extent of calcium signaling within and between astrocytes is regulated by chemical transmitters (Sul et al., 2004), the range of the second wave of inhibition has the potential to be continuously variable, depending on the state of integration within the astrocyte. Under some conditions it may be highly localized to only a few adjacent synapses, while under others it may be distributed and act on literally hundreds of thousands of synapses (Bushong et al., 2002).

The study presented in this issue of *Neuron* provides insights into a new form of synaptic inhibition. However, in order to understand its role, more selective reagents that can be targeted in a cell-specific manner to this particular pathway will be needed. An understanding of release mechanism is essential to allow the power of molecular genetics to be brought to bear on this fascinating physiological problem. Not addressed in this study is the role of glutamate, another prominent transmitter that can be released from astrocytes (Parpura et al., 1994). Are ATP and glutamate coreleased from the same type of astrocyte through similar or distinct mechanisms? Since astrocyte-derived glutamate has been shown to be capable of facilitating neuronal NMDA receptors and synaptic transmission (Araque et al., 1998; Kang et al., 1998), it will be intriguing to determine how these two gliotransmitters interact on common targets. Moreover, since astrocytes change structure and protein expression in a variety of neurological disorders, including epilepsy, the time is ripe for focusing attention on modifications to gliotransmission and for determining whether this may be a contributing cause of modifications of neuronal excitability associated with such disorders. Perhaps we have been looking under the spotlight while a prominent source of synaptic regulation has been hidden in the shadows. This and other recent studies have turned on a new beam; we should explore further to see what it reveals.

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A Gradient of Single-Channel Conductance in the Cochlea: Tuning the Cochlea's Strings?

Frequency tuning in the vertebrate cochlea requires a continuously varying amplifier in auditory hair cells. In this issue of *Neuron*, Ricci et al. show that the mechanosensitive transduction channel, a possible amplifier component, has a single-channel conductance that increases systematically along the frequency axis of the turtle cochlea.

Hair cells in the mammalian cochlea respond to sound-induced vibration of the basilar membrane on which they ride and convert the mechanical vibration to a neural code. Remarkably, they simultaneously amplify that vibration in a frequency-specific manner, both increasing the sensitivity of the cochlea by 100-fold or more and sharpening its frequency tuning. Because the tuning varies continuously along the length of the cochlea (by three orders of magnitude in humans, from 20 Hz to 20 kHz), there must be molecular features in the amplification mechanism that also vary continuously. Thus far, such systematic molecular changes have not been observed; indeed, the nature and molecular identity of the amplifier is a subject of much debate. In this issue of *Neuron*, Fettiplace and colleagues (Ricci et al., 2003) describe a systematic variation in the single-channel conductance of the mechanosensitive hair cell transduction channel, one possible component of an amplifier complex. In the turtle cochlea, they observed that conductance increases perhaps 5-fold from the low-frequency to high-frequency end. The speed of fast adaptation of hair cells, which occurs on a millisecond time scale and is thought to underlie a type of mechanical feedback, also increases several fold along the cochlea.

The coiled mammalian cochlea is the most highly evolved of vertebrate hearing organs. Spiraling within the cochlea and carrying the mechanosensitive organ of Corti, the basilar membrane has a natural resonance that varies along its length like a xylophone or piano. Hair cells within the organ of Corti are arranged in longitudinal rows: three rows of outer hair cells sense the vibration and amplify it, while one row of inner hair cells senses vibration and sends the information to the brain. While it is clear that outer hair cells amplify, two different mechanisms for the amplifier have been proposed.

The first involves an extraordinary electromotility of outer hair cells, mediated by the transmembrane motor protein prestin (reviewed in Dallos and Fakler, 2002).

Depolarization causes outer hair cells to shorten by as much as several micrometers for large depolarizations, more than enough to cause movement of the basilar membrane. Electromotility is extremely rapid, occurring in tens of microseconds for a fast voltage change, as would be needed for sharp tuning in the kilohertz range. Yet there are difficulties: the capacitance of the hair cell membrane slows the receptor potential, causing a low-pass filtering of the voltage signal needed to drive prestin. Also, electromotility and prestin have only been found in mammalian cochlear outer hair cells, whereas lower vertebrates (including the turtle) appear to have a cochlear amplifier as well (reviewed in Manley, 2001).

An alternate proposal involves a fast, Ca^{2+}-dependent adaptation mechanism found in both mammalian and nonmammalian hair cells (Howard and Hudspeth, 1988). Forward deflection of the mechanosensitive hair bundle (toward the tallest stereocilia) exerts tension on the tip links between stereocilia and opens transduction channels. Ca^{2+} entering through the channels then binds to some internal element that promotes rapid channel closure. As they close, channels pull back on the tip links, exerting a small force that moves the bundle backward ever so slightly. If the timing is right, the backward force can amplify the vibration. Both experiment and theory show that this mechanism is proportionally largest for small bundle deflections, consistent with the experimental observation that very small sounds produce the greatest amplification. Yet this mechanism must have a way to work over a large frequency range.

First seen in frog vestibular hair cells, fast adaptation has also been observed in turtle and mouse (Howard and Hudspeth, 1988; Ricci et al., 1998; Kennedy et al., 2003). In all three, its speed depends on the influx of Ca^{2+} through transduction channels. If fast adaptation drives amplification and tuning, the tuned frequency should increase with transduction current. In turtle cochlea, both the total transduction current and the time constant of fast adaptation vary along its length, with larger current and faster time constants at the higher-frequency end (Ricci and Fettiplace, 1997). But how does the cochlea vary the size of the transduction currents: by changing the number of channels per cell, by increasing maximum open probability, or by changing the channel conductance? Single-channel recording would easily resolve these three alternatives.

There is just one problem: the hair cell transduction channel is opened by an extracellular filament, the tip link, but a patch pipette on the channel would break the tip link, preventing mechanical stimulation and channel activation. Ricci et al. (2003) found a clever but technically challenging solution: they recorded from hair cells with whole-cell patch clamping and broke the tip links by brief exposures to submicromolar Ca^{2+} until only one or a few channels remained mechanically activated. Recording noise was reduced so that single-channel currents were observed even in whole-cell clamp. The transduction channels (clearly demonstrated as such by a standard response to mechanical stimuli) had a large single-channel conductance, about 90 pS, that increased to more than 150 pS from low-frequency toward high-frequency regions of the cochlea. When extracellular Ca^{2+} was reduced from 2.8 mM to 50 μM, like that within the turtle cochlea, conductance at all locations

nearly doubled, consistent with Ca^{2+} acting as a permeant blocker. If extrapolated from their experimental range, the single-channel conductance in low Ca^{2+} changes from ~80 pS to >400 pS along the entire length of the turtle cochlea.

What could explain such a variation in conductance? The transduction channel in zebrafish hair cells contains a member of the TRP channel family (Sidi et al., 2003), which presumably forms tetrameric channels. If the channel is a heteromultimer, subunit composition could vary along the length of the cochlea. Alternative splicing or RNA editing of a single gene product might cause the variation, as might posttranslational modifications like phosphorylation. Variation in the local Ca^{2+} concentration outside the channels, perhaps regulated by the PMCA2 Ca^{2+} pump in stereocilia, is a less likely mechanism because the spatial variation in single-channel conductance was seen in both high and low Ca^{2+}. These experiments do not by themselves settle the issue of the cochlear amplifier, of course, but they do provide at least one molecular component that varies with frequency along a hearing organ.

Finally, the authors touch on the interesting issue of how many channels are on each end of a tip link. From the average number of stereocilia in a hair bundle and the maximum transduction current measured, they calculate one or two channels per tip link on average, consistent with Ca^{2+} imaging of hair bundles suggesting that channels are situated at both ends of the tip link (Denk et al., 1995). However, high-resolution electron microscopy shows that tip links fork and attach to stereocilia at both ends, predicting not two but four or five channels per tip link (Kachar et al., 2000). Perhaps there are many channels but some are inactivated; if so, there may be yet more ways to regulate transduction channels.

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