Neuron, Vol. 34, 337–340, April 25, 2002, Copyright ©2002 by Cell Press

Protons at the Gate: DEG/ENaC Ion Channels Help Us Feel and Remember

Minireview

Laura Bianchi and Monica Driscoll[1]
Department of Molecular Biology and Biochemistry
Rutgers, The State University of New Jersey
A232 Nelson Biological Laboratories
604 Allison Road
Piscataway, New Jersey 08854

The DEG/ENaC ion channel family contributes to channels of striking functional diversity. Neuronally expressed family members include the *C. elegans* degenerins that mediate touch and are thought to be mechanically gated, and the mammalian ASICs, which are gated by protons. ASICs affect a range of sensory functions that includes perception of gentle touch, harsh touch, heat, sour taste, and pain. Family member ASIC1 is now implicated in long-term potentiation, suggesting that minute fluxes in synaptic pH may activate ASICs to enhance learning.

Introduction

In 1993, studies of touch transduction in *C. elegans* and Na^+ reabsorption in specialized kidney and lung epithelia (Canessa et al., 1993) unexpectedly converged to identify a novel ion channel class now known as the DEG/ENaCs (named for founding members *C. elegans deg*enerins and human *e*pithelial amiloride-sensitive *Na*$^+$ *c*hannel). Recent analyses of neuronally expressed members of this superfamily reveal that these channels play multiple roles in how we perceive mechanical stimuli and now implicate at least one family member in how well we remember such encounters. Here we briefly review recent advances and current understanding of the neurobiology of the DEG/ENaC channel superfamily, highlighting pressing questions regarding a channel class with truly remarkable range of biological functions including touch sensation, proprioception, pain sensation, heat sensitivity, taste, and learning.

DEG/ENaCs 101: Basic Features of the Channel Class

Identified members of the DEG/ENaC family include 21 *C. elegans,* 30 *Drosophila*, 1 snail, and at least 9 mammalian family members grouped into 5 major subfamilies (Figure 1A). DEG/ENaC subunits range from approximately 500–1000 amino acids in length and include two transmembrane domains (TM1 and TM2) (Figure 1B). Channel subunits are situated in the membrane such that relatively short N- and C-terminal domains project into the cell and a single large central loop extends extracellularly. Understanding of structure/function relations within this channel class is still in its infancy, but studies to date have highlighted three conserved regions in DEG/ENaC function. TM2 contributes to the channel pore, as may a short but highly conserved intracellular stretch adjacent to TM1 that influences open probability, ion permeation, and Na^+ selectivity. The extracellular domain harbors a conserved Cys-rich region important to basic function of the channel class. Most (but not all) neuronally expressed family members can be altered to create hyperactivated channels by substitution of a large side chain amino acid for a highly conserved small side chain amino acid situated at the extracellular mouth of the channel pore. Such substitutions were originally identified in unusual *C. elegans* mutants (for example, *mec-4(d)*) that experienced neurodegeneration, the reason the nematode channel class was named the "degenerin" family (Driscoll and Chalfie, 1991). Subunit stoichiometry of DEG/ENaC channels has been somewhat controversial, with models including 4 or 9 subunits supported.

DEG/ENaCs form homo-and heteromeric voltage-insensitive cation channels (most common $Na^+ >>> K^+ > Ca^{2+}$) that can be inhibited by amiloride. DEG/ENaC channels are gated by diverse stimuli including protons (the ASIC acid-sensing channel), neuropeptides (snail FaNaC), and possibly mechanical forces. The ENaCs, which mediate Na^+ reabsorption in lung and kidney epithelia, are constitutively open.

A MEC-4/MEC-10 Channel Mediates Touch Transduction in C. elegans

Mechanical signaling is critical for our ability to feel touch, balance, and hear, yet very little is understood about molecular mechanisms of mechanotransduction. Electrophysiological studies have identified specialized ion channels gated by mechanical force as key mechanotransducers, but these channels long eluded cloning efforts. Analyses of *C. elegans mec* mutants (*mec*hanosensory abnormal) specifically defective in the response to the gentle stroke of an eyelash hair identified two *mec* genes, *mec-4* and *mec-10,* that encode DEG/ENaC family members essential for body touch sensation (Driscoll and Chalfie, 1991; Huang and Chalfie, 1994). MEC-4 and MEC-10 are coexpressed exclusively in the six neurons that sense gentle touch and form a heteromeric channel postulated to constitute the core of the long-sought touch-sensitive mechanosensory ion channel.

Gating tension must be applied for a channel to be mechanically gated. Localized tension is postulated to be administered by tethering the extracellular MEC-4/MEC-10 channel domains to a specialized extracellular matrix that surrounds the touch receptor neurons and anchoring intracellular channel domains to a 15-protofilament microtubule (MT) network exclusively assembled in the touch receptor neurons (reviewed in Tavernarakis and Driscoll, 1997). Other MEC proteins are proposed to provide these tethers (Figure 2A).

One candidate intracellular tether is MEC-2/stomatin (Huang et al., 1995), which has recently been shown to influence MEC-4/MEC-10 channel activity in a *Xenopus* expression system (Goodman et al., 2002). MEC-2 features an N-terminal unique domain, a central region with a high degree of homology to human stomatin (a membrane-associated protein that can influence red blood cell permeability), and a C-terminal region that includes an SH3 binding domain. Coexpression of the mutant hyperactivated channel subunits MEC-4(d) and MEC-10(d) in oocytes induces an amiloride-sensitive Na^+ current that is increased in amplitude up to 40-fold when MEC-2 stomatin is added to the mix. MEC-2 stimulation does not alter channel surface expression, but does influence ion permeation by reducing P_{Li+}/P_{Na^+}. Although an intact MEC-2 protein is required to convey full enhancement of MEC-4/MEC-10 channel activity, either the central MEC-2 stomatin domain alone or human stomatin (which, unlike MEC-2, does not include N- and

[1]Correspondence: driscoll@mbcl.rutgers.edu

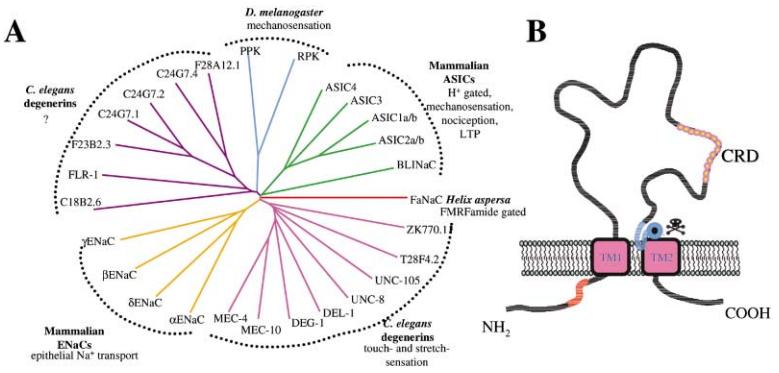

Figure 1. Summary of DEG/ENaC Family Features

(A) Members of the DEG/ENaC family. Phylogenetic tree of DEG/ENaC ion channel family, including documented genes from nematodes, flies, mollusks, and mammals. Summary properties of the group are indicated.

(B) Anatomy of a DEG/ENaC subunit. TM2 (channel pore), a conserved region in the intracellular amino terminus that influences channel gating and ion permeation (red), and a conserved Cys-rich domain are highlighted. A short loop preceding pore domain TM2 is thought to enter the membrane and participate in pore formation (blue). The site of the highly conserved small residue at which large side chain AA substitutions hyperactivate the neuronal channels is indicated by the black circle.

C-terminal domains) partially elevates current amplitude, suggesting that stomatins may have conserved interactions with DEG/ENaC channels. Consistent with this possibility, another *C. elegans* stomatin UNC-1 appears to interact with degenerin channel UNC-8, and mammalian stomatin is coexpressed with DEG/ENaCs in mechanosensory neurons. Clearly, it will be of interest to determine the effects of mammalian (and even nematode) stomatins on ASIC and ENaC channel activities.

How might a stomatin influence MEC-4/MEC-10 function? Since mammalian stomatins are known to oligomerize, are palmitoylated, and are associated with lipid rafts, the MEC-2 stomatin domain may help anchor the channel in a membrane microenvironment critical for function (MEC-2 does coimmunoprecipitate with MEC-4 and MEC-10). Additional MEC-2 functions influencing mechanosensory channel activity, such as the effect on the ion selectivity filter or postulated association with MTs, might be provided by the unique N- and C-terminal domains. Electrophysiological studies of mutant variants should address these possibilities in the future.

The major challenge remaining in the touch transduction field is to demonstrate, and then define the mechanism of, mechanical gating of the MEC-4/MEC-10 channel. Not unexpectedly, attempts to gate the MEC-4(+)/MEC-10(+)/MEC-2 complex in oocytes by osmolarity changes were unsuccessful. Demonstration of mechanical gating will probably need to await development of an in vivo assay system—currently a challenge for the tiny *C. elegans* body touch receptors.

Mammalian DEG/ENaCs ASIC2 and ASIC3 Influence Cutaneous Mechanoreceptor Function, Suggesting Conserved Roles in Mechanosensation for Some DEG/ENaC Family Members

Another important question is whether function in mechanotransduction is a shared feature of other members or of the DEG/ENaC family. Nematode channel subunit UNC-8 has been implicated in body stretch feedback required for nematode proprioception (Tavernarakis et al., 1997), and *Drosophila* Pickpocket is expressed early in dendritic varicosities of the da/md abdominal peripheral neurons that function in the adult as mechanoreceptors (Adams et al., 1998), suggesting potential for mechanotransduction. The most compelling data for a conserved function in touch sensation comes from analysis of skin mechanoreceptor responses in ASIC2 and ASIC3 knockout mice.

The mammalian acid-gated ASIC subfamily includes five known genes, at least two of which produce alternatively spliced products (see Supplemental Table S1 at http://www.neuron.org/cgi/content/full/34/3/337/DC1). The best-characterized ASICs are predominantly neuronal ion channels, expressed both in the central and peripheral nervous systems. ASIC2a is expressed in medium and large diameter mechanosensory neurons of the dorsal root ganglia (DRG) and is localized to several specialized cutaneous mechanosensory nerve termini (de la Rosa et al., 2002; Garcia-Anoveros et al., 2001; Price et al., 2000). Analyses of responses of various mechanoreceptor classes in nerve-skin preparations from an ASIC2 knockout mouse revealed that two types of low threshold (i.e., light touch) mechanosensitive fibers (the rapidly adapting (RA) and, to a lesser extent, the slowly adapting (SA)) are defective in increasing firing frequency in response to stronger displacement force stimuli. This reduced sensitivity of low threshold mechanoreceptors could have considerable biological relevance since in humans, the dynamic sensitivity of RA and SA mechanosensors is thought to play an important role in light touch perception.

ASIC3 is also localized to several types of mechanosensory nerve terminals and influences specific mechanosensory responses (Price et al., 2001) (Figure 2B). In nerve-skin preparations from the ASIC3 knockout mouse, both the threshold sensitivity and the response frequency of AM fiber mechano-nociceptors (which respond to high threshold stimuli—like pinching) are reduced. By contrast, response frequencies of RA mechanoreceptors are actually increased 2-fold in the ASIC3 knockout. Thus, ASIC3 is involved in distinct ways in responding to both cutaneous touch and painful stimuli.

The demonstration that ASIC2 and ASIC3 deletions cause defects in mechanoreceptor function is exciting in that it supports a conserved role for DEG/ENaC family members in touch sensation. On the other hand, known knockout effects are at first glance surprisingly subtle: in the ASIC2 and ASIC3 knockout mice, no mechanoreceptor responses are fully eliminated and, for example, threshold sensitivity is maintained in the ASIC2$^{-/-}$ RA receptors while other mechanoreceptors that do express the ASIC2 have normal responses in the knockout. Three possible explanations can be considered, none of which are mutually exclusive. First, general work on the channel class suggests that within a given heteromeric channel, one subunit may provide the most critical contributions to current with other subunits having more modulatory effects. Possibly, ASIC2 and ASIC3 serve

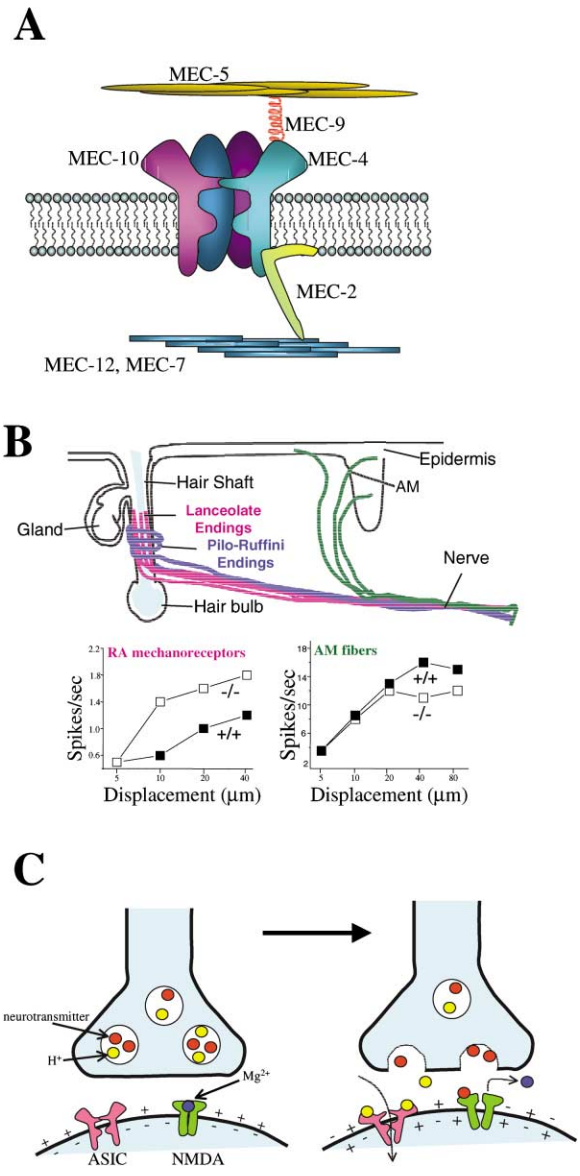

Figure 2. DEG/ENaC Channels as Mechanosensors and Modulators of LTP

(A) Working model for the *C. elegans* touch-transducing complex. The channel is thought to be a MEC-4/MEC-10 tetramer. Extracellular channel domains are postulated to be tethered to the extracellular collagen MEC-5 and/or EGF-rich/Kunitz protease inhibitor-rich MEC-9. Intracellular domains are thought to be tethered to 15-pf microtubules made up of MEC-12 α-tubulin and MEC-7 β-tubulin, perhaps through the stomatin-related protein MEC-2, which might localize the channel in a lipid raft microdomain. (B) Responses to mechanical stimuli are defective in ASIC3 knockouts. Top, some cutaneous mechanoreceptors affected by ASIC3 KO; RA responses are associated with lanceolate terminals, free termini with AM responses. Bottom, example of increases in the response to stimulations of increasing force in RA receptors from ASIC3 KOs; in contrast, the response to increasing force in AM fibers is decreased (from Price et al., 2001). (C) Model for ASIC1 contribution to synaptic plasticity. Protons (yellow) released during neurotrasmitter (red) vesicle fusion could activate ASIC1a on the postsynaptic membrane, inducing depolarization that in turn could release the well-defined Mg^{2+} (blue) block on the NMDA receptor (green).

more modulatory roles in cutaneous mechanosensory channels. Second, coexpressed DEG/ENaCs could form homo-and heteromeric channels that may provide functionally redundant activities. A third possibility is that other channel types may contribute to all or some aspects of mechanotransduction function in the cutaneous mechanoreceptors. Analyses of additional knockouts and double mutant combinations should help distinguish among these possibilities. We note that epithelial ENaC subunits should not be overlooked in the screen for mechanosensory roles: ENaC subunits have been found in termini of peripheral nerve and pressure-sensing baroceptors.

ASIC Channels as Acid-Sensing Channels Involved in Pain Perception

Protons rapidly activate and inactivate ASIC channels, giving rise in most cases to transient currents that display different pH sensitivity (see Supplemental Table S1). A large variability in gating sensitivity has been reported for the proton sensitivity of heterologously expressed single ASIC subunits, with ASIC2a and ASIC3 being activated at more acidic pH and ASIC1a and ASIC1b at pH levels closer to the physiological range (reviewed in Waldmann and Lazdunski, 1998). Coexpression of different ASIC subunits in most cases produces heteromultimeric channels with unique properties. In vivo, ASIC channel properties are expected to vary with subunit composition, type of accessory subunits associated (for example, ASIC3 interacts with multivalent PDZ domain protein CIPP and ASIC2a interacts with PICK1), and the presence of upmodulators such as FMRFamide-like neuropeptides, divalent cations (Ca^{2+}, Zn^{2+}), and lactate. Overall, a diverse spectrum of channel sensitivities and responses are likely to be available in vivo.

What are the physiological conditions under which acid gating has biological relevance? In pathological conditions such as inflammation and ischaemia, acidosis can be severe, with the extracellular pH decrease sometimes more than two pH units, proton concentrations that can activate heterologously expressed ASIC channels. ASIC2 does not appear to significantly influence nociception (Price et al., 2000), but analysis of pain perception in the ASIC3 knockout mouse supports a contribution of this subunit (Price et al., 2001). ASIC3 is expressed in small diameter DRG neurons that coexpress pain-associated substance P and are thought to be primarily nociceptive. About 30% of C-MH fiber neurons, the peripheral projections of the small diameter nocicpetor neurons, respond to low pH. Although the same proportion of acid-sensitive neurons is apparent in the ASIC3 null background, the magnitude of the response is reduced at pH 5. A second nociception defect can be detected in the behavioral response to acid injection into skeletal muscle. Wild-type mice exhibit an enhanced sensitivity to mechanical stimuli outside the area of acid injection that is attributed to acid activation of nociceptors (this is called secondary mechanical hyperalgesia). ASIC3 null mice exhibit lowered sensitivity after acid injection. Interestingly, these phenotypes clearly implicate ASIC3 in some aspects of pain perception, yet several other nociceptive parameters appear unchanged and it seems likely that other nociceptors play the central role in cutaneous nociception. These observations highlight the possibility that acid sensitivity of this channel class may not always be of clear functional relevance in a native environment; protons may be modulatory rather than gating stimuli.

ASIC channels might play more critical roles in the

pain that accompanies pathological crises of the heart such as myocardial ischaemia (angina pectoris). Sensory cardiac afferents innervate cardiac muscle and are activated by protons released by damaged tissue. ASIC3 has been postulated to be the nociceptor of cardiac afferents based on the observations that the prevalent proton-gated current present in these neurons remarkably resembles that of homomeric ASIC3 and that ASIC3 is expressed in these neurons (Sutherland et al., 2001).

CNS ASIC1 Is Required for H^+-Gated Currents and Is Implicated in Synaptic Plasticity

The source, and biological significance, of substantial H^+-activated currents in the CNS has been a long-standing mystery, and acid-gated ASIC channels are obvious candidates for current source. ASIC1a is expressed in cerebral cortex, hippocampus, and cerebellum. Electrophysiological analyses of hippocampal neurons from ASIC1 knockout mice have identified a strict requirement for ASIC1a for proton-gated current in hippocampus (Wemmie et al., 2002). Despite the near elimination of some H^+-gated currents in brain, the ASIC1 KO mouse is basically normal in viability, appearance, behavior, and brain development.

Synaptic vesicle contents are acidic (pH 5.6) (Miesenbock et al., 1998), and a transient drop in extracellular pH is associated with synaptic transmission, suggesting that proton fluctuations at the synapse can influence synaptic transmission. Since ASIC1 appears postsynaptically localized, it could be activated consequent to presynaptic vesicle fusion. Interestingly, neurotransmitter release appears normal in the ASIC1 knockout, and field excitatory postsynaptic potentials (fEPSPs) in Schaffer collateral-CA1 synapses (indicators of induced responses in the postsynaptic neuron) are unchanged under low Mg^{2+} conditions, indicating that baseline synaptic transmission does not depend upon ASIC1 function. However, a closer look at long-term potentiation (LTP) at these synapses implicates ASIC1 in synaptic plasticity.

The Schaffer collateral-CA1 synapse of the hippocampus has been studied as one molecular model for LTP. $ASIC1^{-/-}$ hippocampal neurons are defective in the response to high-frequency stimulation—they do not experience fEPSPs larger than the initial one during the early phase of LTP and they do not exhibit sustained fEPSPs over time. Interestingly, this lack of LTP is correlated with specific learning defects in the ASIC1 null mice—mutants display defects in spatial learning and eyeblink conditioning. Technically, the latter occurs in cerebellum and involves long-term depression (an NMDA-independent form of synaptic plasticity) but, importantly, this phenotype suggests that acid-activated currents may facilitate the induction of long-term synaptic changes through multiple mechanisms.

How might ASIC1 and H^+-gated currents contribute to the development or maintenance of LTP? NMDA receptor activation is strongly implicated in the synaptic plasticity at the Schaffer collateral-CA1 synapse. $ASIC1^{-/-}$ LTP defects can be rescued by conditions that potentiate NMDA activation, including PKC activation and low Mg^{2+} conditions. The mechanism of NMDA potentiation remains to be addressed, with an important question being whether the modest changes in pH anticipated at the synaptic cleft (possibly only 0.2 unit drop in pH, although conceivably larger in microdomains or under conditions of sustained release) would gate ASIC channels in their native contexts (a colossal pH 5 was used in this study to detect the H^+-dependent current in hippocampal slices). One attractive model is that ASIC1 channels may be activated by increases in proton concentration associated with synaptic vesicle release, inducing a depolarization of the postsynaptic membrane that could release the well-characterized NMDA Mg^{2+} block and thus facilitate LTP induction (Figure 2C), but numerous other possibilities for NMDA receptor activation exist. It is intriguing that ASIC1 can associate with PICK1, which itself can associate with synaptic NMDA receptor complexes—perhaps ASIC1a and NMDA receptors cluster at the synapse? Observations of Wemmie et al. (2002) provide a starting point for a number of exciting follow-up investigations into roles of neuronal ASIC channels on synaptic function.

Concluding Remarks

As studies of neuronally expressed members of the DEG/ENaC have emerged, we are left with the impression of a broad range of channel types, gated in response to diverse stimuli, that contribute to our ability to perceive a wide range of sensory stimuli. Future work on this fascinating class of channels will focus on how mechanical and acid-sensitive gating is accomplished, how channel-associated proteins and modulators influence in vivo channel function, and how ASIC channels affect synaptic plasticity. The field has made remarkable progress, but we have a lot to learn—apparently with the help of our ASIC channels.

Selected Reading

Adams, C.M., Anderson, M.G., Motto, D.G., Price, M.P., Johnson, W.A., and Welsh, M.J. (1998). J. Cell Biol. *140*, 143–152.

Canessa, C.M., Horsiberger, J.D., and Rossier, B.C. (1993). Nature *349*, 588–593.

de la Rosa, D.A., Zhang, P., Shao, D., White, F., and Canessa, C.M. (2002). Proc. Natl. Acad. Sci. USA *99*, 2326–2331.

Driscoll, M., and Chalfie, M. (1991). Nature *349*, 588–593.

Garcia-Anoveros, J., Samad, T.A., Zuvela-Jelaska, L., Woolf, C.J., and Corey, D.P. (2001). J. Neurosci. *21*, 2678–2686.

Goodman, M.B., Ernstrom, G.G., Chelur, D.S., O'Hagan, R., Yao, C.A., and Chalfie, M. (2002). Nature *415*, 1039–1042.

Huang, M., and Chalfie, M. (1994). Nature *367*, 467–470.

Huang, M., Gu, G., Ferguson, E.L., and Chalfie, M. (1995). Nature *378*, 292–295.

Miesenbock, G., De Angelis, D.A., and Rothman, J.E. (1998). Nature *394*, 192–195.

Price, M.P., Lewin, G.R., McIlwrath, S.L., Cheng, C., Xie, J., Heppenstall, P.A., Stucky, C.L., Mannsfeldt, A.G., Brennan, T.J., Drummond, H.A., et al. (2000). Nature *407*, 1007–1011.

Price, M.P., McIlwrath, S.L., Xie, J., Cheng, C., Qiao, J., Tarr, D.E., Sluka, K.A., Brennan, T.J., Lewin, G.R., and Welsh, M.J. (2001). Neuron *32*, 1071–1083.

Sutherland, S.P., Benson, C.J., Adelman, J.P., and McCleskey, E.W. (2001). Proc. Natl. Acad. Sci. USA *98*, 711–716.

Tavernarakis, N., and Driscoll, M. (1997). Annu. Rev. Physiol. *59*, 659–689.

Tavernarakis, N., Shreffler, W., Wang, S., and Driscoll, M. (1997). Neuron *18*, 107–119.

Waldmann, R., and Lazdunski, M. (1998). Curr. Opin. Neurobiol. *8*, 418–424.

Wemmie, J.A., Chen, J., Askwith, C.C., Hruska-Hageman, A.M., Price, M.P., Nolan, B.C., Yoder, P.G., Lamani, E., Hoshi, T., John, H., Freeman, J., and Welsh, M.J. (2002). Neuron *34*, this issue, 463–477.