

“Knocking out” a Neural Circuit

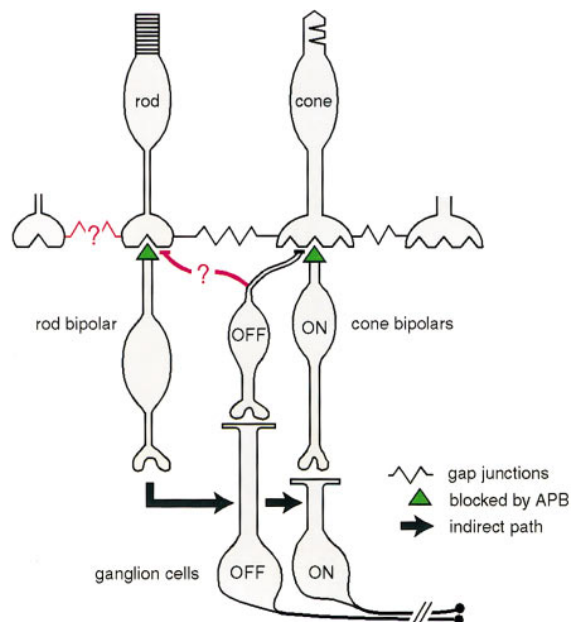
Although the visual system occupies nearly half of the mammalian brain, we still do not completely understand its first synaptic stage. One reason is that the dendrites postsynaptic to photoreceptors comprise such a maze of fine processes that doubt remains whether all the second order circuits have been identified—even after 4 decades of electron microscopy. Now advanced functional methods applied to a mammalian rod pathway suggest a circuit previously unsuspected from anatomy.

The standard model for two rod circuits is shown in the diagram. The rod terminal contacts a single type of dedicated bipolar cell (left) and also forms an electrical junction with the cone terminal. The rod-to-bipolar circuit is thought to serve starlight, which is so dim that over minutes no rod transduces more than one photon. This single-photon signal requires huge amplification, which renders the circuit vulnerable to saturation in brighter light. The rod-to-cone circuit is thought to serve twilight, which is bright enough that over one second a rod transduces tens to hundreds of photons. This multi-photon signal can thus “piggyback” onto the cone bipolar circuit (Smith et al., 1986).

Now, Soucy and colleagues (1998) in the September issue of *Neuron* have used diphtheria toxin controlled by a cone-selective promoter to produce a coneless mouse. The retina, except for the absence of cones and cone-driven responses, looks essentially normal. Bipolar cells that normally collect from cones remain intact, which is somewhat surprising, because neurons commonly degenerate when deprived of their primary input. But most surprising is that the multi-photon rod signal still reaches ganglion cells. Since this signal cannot take the expected route, from rod via gap junctions to cone, the rod must excite an unknown type of bipolar cell.

The simplest experiment conceptually would have been to monitor the output of many bipolar cells, one at a time, by intracellular recording. This is technically difficult and extremely tedious. But bipolar cells excite ganglion cells, many of whose individual spike trains can be recorded simultaneously in a multi-electrode dish (Meister et al., 1994). So, the experiment actually monitored ganglion cells and blocked the single-photon circuit through the rod bipolar cell with the agonist APB, which also blocks ON cone bipolar cells (Slaughter and Miller, 1981; Muller et al., 1988). With these circuits blocked, the multi-photon rod stimulus excited OFF ganglion cells. In a coneless retina, this implies a synapse from rod to OFF cone bipolar cells (see figure, indicated in red).

In a normal retina, similarly blocked, the multi-photon rod response was also present in OFF ganglion cells. In this case, the electrical pathway could not be ruled out. However, the responses in coneless and normal were virtually identical in amplitude and time course, which would be surprising if their circuits differed. This functional evidence that a normal mouse retina transmits a multi-photon rod signal via an OFF bipolar cell



Two Rod Pathways to Ganglion Cells

will certainly trigger a search for the anatomical substrate. The diagram suggests a cone bipolar cell that also collects from rods, as reported briefly for gray squirrel (West, 1978). Such a connection might explain how a human “achromat,” born essentially coneless, nevertheless possesses a functional multi-photon rod pathway (Stockman et al., 1995).

The present results do not contradict the standard version of the “twilight circuit” that routes the multi-photon rod signal via an electrical synapse to the cone. This signal, identified by its rod action spectrum and time course, is recorded in cat and monkey directly from cones (Nelson, 1977; Schneeweis and Schnapf, 1995). Furthermore, since 20 rods couple to each cone, this twilight signal would represent thousands of photons, comparable to what the cone itself supplies in daylight (Smith et al., 1986). Finally, where a synaptic terminal transmits a multi-photon response via a chemical synapse, it tends first to average the signal by coupling to neighbors (Sterling, 1998). This coupling is true for cones in mammals, but in cat and primate it is not the case for rods (Raviola and Gilula, 1973; Kolb, 1977; Smith et al., 1986). Thus, if rods in normal mouse do transmit a multi-photon signal to the OFF bipolar cell, they should also form gap junctions with other rods.

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Hot Peppers and Pain

Capsaicin, the main pungent ingredient in hot chili peppers, has been used for centuries as a spice. Aside for its expanding culinary use, in the past 2 decades capsaicin has also provided remarkable insight not only into the neurobiology of primary afferent nociceptors but also into new treatments for chronic pain.

The major reason that capsaicin has been so useful is its remarkable cellular specificity. Nearly all of the actions of capsaicin can be attributed to a single mechanism—activation of a nonspecific cation channel in a population of primary afferent sensory neurons known as C fibers, 80% of which are polymodal nociceptors. Polymodal nociceptors are activated by noxious thermal, mechanical, or chemical stimuli and as such are thought to be intimately involved in the generation and maintenance of chronic pain. The belief among many pain researchers is that insight into the cellular and molecular mechanisms that underlie transducer functions in nociceptors will lead to an increased understanding of the peripheral events involved in the generation and/or maintenance of chronic pain (Levine, 1998). This belief is buttressed by the finding that repeated application of capsaicin induces desensitization of nociceptors and that this treatment can ameliorate several chronic pain states, including those arising from herpes zoster and oral cancer (Maggi, 1992).

To understand the action and cellular specificity of capsaicin, a key question was to define whether capsaicin interacted with one or more receptors and, if so, to determine the structure and normal function of this receptor. Numerous studies demonstrated that capsaicin and capsaicin analogs had a well-defined structure–activity relationship. On the basis of these studies, the competitive antagonist of capsaicin known as capsaizepine was developed (Bevan et al., 1992), and resiniferatoxin, a naturally occurring ultrapotent structural analog of capsaicin, was used to define a ligand binding site in sensory neurons (Szallasi, 1994). While this data suggested that capsaicin did exert its actions via a receptor, additional data suggested that there was more

than one type of capsaicin receptor. To emphasize the possible heterogeneity of receptors that interact with capsaicin, the term vallinoid receptor was coined (Szallasi, 1994).

Despite the enormous interest surrounding capsaicin, it was not until 1997 that a capsaicin receptor was cloned. In this groundbreaking work, Julius and his colleagues expression cloned the capsaicin receptor (Caterina et al., 1997), which they named the vallinoid receptor 1 (VR1), predicting as the name implies several more as yet undescribed receptor genes. This receptor was a nonselective cation channel that is structurally related to members of the transient receptor potential family of ion channels. Capsaicin and heat in the noxious range activated the VR1, although it was not clear whether heat was activating the VR1 directly or through other thermally sensitive molecules. This study also generated a host of other questions regarding the endogenous ligand for the VR1: its physiological function, which neurons express the VR1 protein, and whether there are other VRs in sensory neurons or other areas of the brain.

In a recent paper (Tominaga et al., 1998) in the September issue of *Neuron*, the combined efforts of the Julius and Basbaum labs elegantly address several of these important questions. Using excised membrane patches, they show that heat gates VR1 directly and that an increase in protons, at levels that occur at sites of inflammation, infection, or ischemia, activates VR1 at room temperature. In light of this data, they propose a model that highlights the notion that vallinoids, heat, and protons act in concert to regulate VR1 activity and that the effects of any one stimulus cannot be considered in isolation. Thus, whether thermal or chemical stimuli will be important in activating VR1 in vivo will likely vary with the site and degree of the injury. As an example, they suggest that whereas both temperature and pH would be expected to play a role in VR1 activation in skin, in areas such as the viscera where temperature is more constant, pH and not temperature will play a more important role in VR1 activation. The authors then go on to suggest that VR1 functions as an integrator of multiple pain-producing stimuli. What is particularly exciting and intellectually satisfying about this work is that it begins to unite the often disparate findings regarding the actions of capsaicin and its analogs into a comprehensible whole.

This paper also addresses the types of sensory neurons that express the VR1 protein. In the past several years, a hypothesis has emerged that suggests that polymodal nociceptors can be divided into at least two large groups (Hunt et al., 1993; Snider and McMahon, 1998). One population contains peptides such as substance P and calcitonin gene-related peptide, expresses the trkA receptor, and is responsive to nerve growth factor (NGF); a second population is trkA negative and does not contain peptides but can be identified from their IB4 lectin binding sites and the expression of the P2X3 receptor. The latter population of neurons loses its trkA receptors 3 weeks after birth but expresses ret receptor components and responds to glial cell line-derived neurotrophic factor (GDNF). It has also been shown that these two populations of sensory neurons preferentially terminate in different parts of the spinal cord. Whereas the peptide/trkA fibers terminate in superficial laminae of the dorsal horn, particularly laminae

I and II outer, the IB4/ret population terminates deeper in a narrow band within lamina II inner in association with a layer of neurons expressing high levels of protein kinase C γ (PKC γ). A functional correlation has also been made between the two types of sensory neurons. Loss of the PKC γ gene using homologous recombination in mice prevents the development of neuropathic pain following partial nerve section (Malmberg et al., 1997), while experimental inflammation of the hind paw in rats and mice results in an increased expression of substance P in the peptide/trkA neurons. This has led to the suggestion that chronic inflammatory pain is mediated largely by the peptide/trkA-containing sensory neurons that terminate in superficial laminae, while neuropathic pain resulting from peripheral nerve damage is mediated by the IB4/ret population that terminates in the deeper regions of lamina II (Snider and McMahon, 1998).

To determine which sensory neurons express the VR1 protein, antibodies were generated to the predicted carboxyl terminus of VR1, and an immunohistochemical analysis was performed. The results are surprising. Staining of the sensory ganglion revealed that about 80% of both IB4/ret- and peptide/trkA-containing sensory neurons express VR1 protein-like immunoreactivity. In other words, there appears to be a small but substantial population of sensory neurons that do not express the VR1 but which previously have been shown to be sensitive to capsaicin. This data, along with binding studies with tritiated resiniferatoxin, suggests that there are additional vanilloid receptors.

The immunohistochemical analysis also revealed an unexpected heterogeneity in the IB4/ret population of sensory neurons that terminates in the inner portion of lamina II. It has previously been shown that the medial and lateral regions of the dorsal horn of the spinal cord represent distal and proximal parts of the hindlimb, respectively (Devor and Claman, 1980). What is unique in the present report is that whereas the IB4/ret population that terminates in the medial aspect of lamina II inner shows colocalization with VR1, the IB4/ret population that terminates in the lateral aspect of lamina II inner shows virtually no colocalization with VR1. This data suggests that either the IB4/ret populations which innervate the distal aspects of a limb express the VR1, whereas those that express the proximal aspect of the limb do not, or that there is differential transport of the VR1 receptor protein to the spinal cord in these two populations of sensory neurons. There is a precedent for differential transport of a receptor in primary afferents: the neuropeptide Y Y1 receptor that is expressed in sensory neurons is found in the cell bodies but rarely in the axons that terminate in the spinal cord (Zhang et al., 1994). Also, Robert Elde's lab has suggested that the synthesis and trafficking of a receptor and neurotransmitter can be coregulated in sensory neurons. Thus, disruption of the preprotachykinin gene inhibits the translation of δ opiate receptor—a protein normally coexisting in the same synaptic vesicles as substance P (Dray and Rang, 1998). Thus, the expression of VR1 could potentially be regulated at the transcriptional, translational, or axoplasmic transport level. Differential regulation of transport would result in modified terminal sensitivity within discrete populations of sensory neurons.

Why do pain researchers find this work so interesting? The majority of therapies used today for the treatment of chronic pain (opiates, aspirin, and codeine) have been utilized for over a century and have significant side effects, especially with long-term use. One of the most promising avenues for discovering new molecules to treat chronic pain is to understand the molecular and cellular mechanisms that underlie transducer function in primary afferent nociceptors. The beauty of the present work is that it begins to provide a cellular and molecular framework for understanding the vast and often provocative literature on the biological actions of capsaicin. It would be highly surprising if future work on capsaicin and VR1 does not contribute significantly to our knowledge of sensory transducer function and ultimately to the development of new therapies for treating persistent pain.

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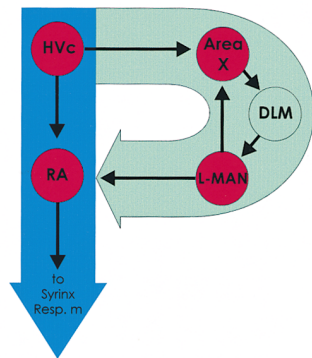
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Modulation by Social Context Sheds New Light on Mechanisms of Vocal Production

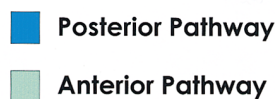
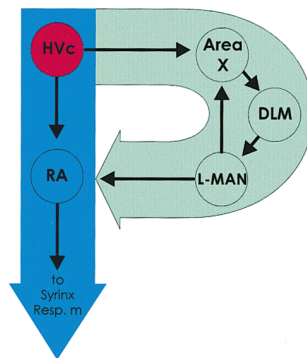
Molecular tools are used increasingly to probe aspects of nervous system function. In many cases, such studies

ZENK expression

“Undirected” song



“Directed” song



Schematic of ZENK Expression Patterns during Production of the Same Song in Two Different Social Contexts

High levels of ZENK expression (shown in red) are observed in RA as well as area X and MAN of the “indirect” anterior pathway when male zebra finches sing alone or in the presence of other males (“undirected” song). Singing in the presence of a female (“directed” song) does not induce ZENK expression in these nuclei. The forebrain nucleus Hvc, also known as high vocal center, shows high levels of ZENK expression during both directed and undirected song. Expression patterns in the thalamic nucleus DLM do not appear to be correlated with singing.

are performed without a solid understanding of the behavioral relevance of the specific molecules, synapses, or circuits under investigation. The potential danger of ignoring behavioral context is that the significance of certain results may go unnoticed. Given the sometimes subtle variations of an animal’s behavioral repertoire, relating specific molecular or physiological events to the correct behavioral context may be difficult unless a detailed understanding and analysis of the animal’s behavior is performed. This point is nicely illustrated by Jarvis et al. (1998) in this issue of *Neuron*. Studying song production in zebra finches, they combine careful behavioral analysis with gene expression to show that a remarkably subtle variation in behavior (whether the bird sings alone or toward a female) can lead to striking differences in gene expression.

The male zebra finch only produces one song, which consists of a variable number of introductory notes followed by several repetitions of a stereotyped sequence of syllables. The song is produced when the bird is alone (“undirected” song) or in the presence of a female (“directed” song), and, although containing the same song elements, directed song is often delivered slightly faster, contains a few more introductory notes, and is accompanied by a courtship dance. The structure of each song type, however, is nearly indistinguishable, even when viewed on a sound spectrograph. The brain structures responsible for the production of these different song types are organized as an interconnected network of nuclei known collectively as the song system. This system can roughly be divided into a “direct” vocal motor pathway, referred to by Jarvis et al. (1998) as the posterior pathway, and an “indirect” vocal pathway known as the anterior pathway (see figure). Whereas the posterior pathway is known to be hierarchically organized and conveys song motor commands to the syrinx

(avian vocal organ) and various respiratory muscles (Margoliash, 1997; Suthers, 1997), the functional significance of the anterior pathway is less well understood. Previously thought to be involved exclusively during the phase of song acquisition in juvenile birds, recent studies now implicate the anterior pathway in song maintenance in adult birds as well (Doupe and Solis, 1997). The recent findings by Jarvis et al. (1998) suggest that this pathway may also play an important role in modulating song motor output within the behavioral, or social, context in which song is produced.

By carefully monitoring different song types and controlling for the overall number of songs produced within a given experimental period, expression of the immediate early-gene ZENK, as shown by in situ hybridization, is shown to increase 10- to 40-fold in most nuclei of the song system during undirected song. Remarkably, with the notable exception of nucleus Hvc, these nuclei show little or no expression during directed song (see figure). Because ZENK is thought to be an indicator of neural activation (Chaudhuri, 1997), these results suggest that activity patterns in RA and the anterior pathway differ considerably depending on the context in which singing occurs. The authors conclude that the differences in ZENK expression are likely caused by singing-related motor activity, since expression patterns are similar to those observed in deafened birds. Because of the recent findings, however, that auditory flow into the song system may be dependent on behavioral context (Schmidt and Konishi, 1998), the possibility nevertheless remains that differential “gating” of auditory feedback signals may contribute to the different patterns of ZENK expression.

While the differential expression of ZENK during directed and undirected singing provides new insight into the control of vocal production, the behavioral significance of these different context-dependent song types,

as well as why brain areas are differentially activated during these behaviors, is not clear. One possibility is that the differential activation of the anterior pathway during undirected and directed song may serve to coordinate other motor behaviors that are generally associated with these different song types. For example, when the male directs his song to a female, the anterior pathway may well be optimized to coordinate, or bind, song motor activity with other motor behaviors such as the courtship dance, which is only observed during directed singing.

The use of immediate-early genes, such as ZENK, as markers for neural activity, provides a powerful tool that can be used in identifying new ways in which neural circuits may be organized. As the authors point out, however, interpreting the relationship between ZENK expression and neural activity should be done cautiously. A case in point is the pattern of ZENK expression observed in nucleus RA. This structure, which forms part of the direct motor pathway, is known to be active during singing (Margoliash et al., 1997), exhibits high levels of *c-fos* expression (Kimpö and Doupe, 1997), and yet expresses relatively low levels of ZENK. Clearly, the findings by Jarvis et al. (1998) set the stage for a detailed characterization of this phenomenon at the electrophysiological level. Although technically challenging, recording neural activity in birds singing under different behavioral contexts is now an active area of research and promises to yield exciting new findings.

Increasing anatomical and neurochemical evidence (Bottjer and Johnson, 1997; Luo and Perkel, 1998; Jarvis et al., 1998) suggests that there are significant parallels between the anterior song control pathway (HVC → area X → DLM → MAN) and the mammalian cortical-basal ganglia pathway (cerebral cortex → basal ganglia → thalamus → cortex). There are functional parallels as well: the basal ganglia, in addition to being involved in motor execution, are also thought to be involved in motor learning, in sensorimotor integration, and in the control of motor acts that require motivational or cognitive drive (Graybiel et al., 1994). Of specific interest in the context of the results obtained by Jarvis et al. (1998) is the finding that "motor" neural activity in striatum (to which area X may be homologous) can be modulated by behavioral context. A striking example of such context-dependent neural activation can be shown in striatal input neurons during a memory-guided saccade task (Kawagoe et al., 1998). By combining saccade tasks with a differential reward schedule, the authors show that firing patterns that are normally predictive for a given spatial location can become significantly depressed depending on whether that saccade target is, or is not, paired with a reward. Thus, although the motor act, the saccade, is identical in both cases, pairing a motor act with a reward (a motivational, or emotional, drive) can completely alter neural responses within the striatum.

As in the mammalian striatum, area X is strongly innervated by midbrain dopaminergic areas (Bottjer and Johnson, 1995). These inputs in mammals are thought to convey reward-related and behavioral context-dependent information to the striatum (Graybiel et al., 1994). Given the similarities between the two systems, a detailed characterization of dopaminergic inputs into the

song system is likely to yield important insights into the mechanisms underlying the differences observed between directed and undirected singing. More generally, important mechanistic insight into the functioning of the song system is likely to emerge from a deeper understanding of the mammalian cortical-basal ganglia pathway and its role in motor control and function, while, conversely, work on the mammalian basal ganglia will likely benefit from a deeper understanding of the song system.

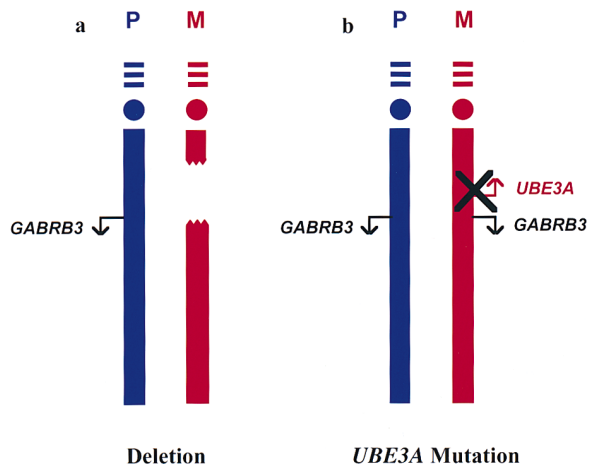
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Strange Bedfellows? Protein Degradation and Neurological Dysfunction

Angelman syndrome (AS) is a severe neurological disorder characterized by seizures, ataxia, and cognitive dysfunction. AS occurs in ~1 of every 15,000 births and appears to arise from the functional loss of a maternally expressed imprinted gene or genes. The majority of cases are attributable to a chromosomal deletion on the maternally inherited chromosome 15 (see figure). Loss of the functional maternal allele in AS also occurs by paternal uniparental disomy (UPD), in which two copies of chromosome 15 are inherited from the father, by defects in the imprinting process (Nicholls et al., 1998), and it recently has been shown to arise from gene mutation of the maternally inherited allele of *UBE3A*, an E3 ubiquitin ligase (see figure; Malzac et al., 1998). The paper by Jiang et al. (1998) in this issue of *Neuron* confirms the importance of *UBE3A* in AS, by demonstrating in a mouse model that a mutation in the maternal allele of *Ube3a* is sufficient for an AS-like phenotype. This result raises the interesting question of why a mutation in a gene involved in a general cellular process such



Molecular Basis of Angelman Syndrome

A 4 Mb deletion of the maternally inherited (M, red) chromosome 15, at cytogenetic bands 15q11–q13, occurs in ~70% of AS patients (a), whereas inactivating mutations (X) of the maternal *UBE3A* allele occur in ~5% of cases (b). The latter are equivalent to the *Ube3a*-deficient mice ($m-/p+$) generated by Jiang et al. (1998 [this issue of *Neuron*]). Allelic expression of key genes discussed in the text are shown by arrows. P, paternally inherited chromosome (blue).

as ubiquitin-mediated protein degradation should have such specific neurological consequences; these mutant mice may in fact be just the tools to address this question.

Many aspects of cellular function require regulatory protein turnover, and the process of ubiquitin-mediated protein degradation by the 26S proteasome is a key component of this regulation (Hochstrasser, 1995; Johnson and Hochstrasser, 1997). The ubiquitin ligases are thought to present ubiquitinated substrate proteins bound for degradation to the proteasome complex. The cellular function of UBE3A is unknown, despite its initial identification as E6-associated protein (E6-AP), a cellular protein required for the degradation of p53. p53 has major roles in control of the cell cycle and apoptosis and turns over rapidly in normal cells. With the generation of mice deficient in *Ube3a*, Jiang et al. (1998) now provide critical results and reagents for the definition of the molecular role of UBE3A, and perhaps p53, in neuronal function, particularly in the cerebellum and hippocampus.

Human genetics of AS predicts that *UBE3A* is imprinted and only the maternal allele is expressed (Nicholls et al., 1998). Indeed, in the mice, paternal transmission ($p-/m+$) of the mutant *Ube3a* allele is phenotypically silent, whereas maternal deficiency ($m-/p+$) represents a genetic model of AS and generates mice that, while viable and fertile, have a characteristic phenotype consistent with AS. This includes fine motor skill and coordination deficits, abnormal electroencephalogram (EEG) recordings and inducible seizures, an impairment of hippocampal long-term synaptic potentiation (LTP), and abnormally high cytoplasmic p53 levels in postmitotic neurons (Jiang et al., 1998). The LTP results correlate with a defect in context-dependent learning in these mice, which is suggested to be analogous to the severe cognitive impairment in AS (Jiang et al., 1988). Interestingly, the defects in *Ube3a* ($m-/p+$) mice occur despite

apparently normal neuroanatomy, even in cerebellar Purkinje cells and hippocampal neurons, the cell types showing brain-specific imprinting of *Ube3a* (Jiang et al., 1998).

Is AS caused solely by *UBE3A* deficiency? From human (Williams et al., 1995; Malzac et al., 1998) and mouse (Jiang et al., 1998) studies, it is clear that mutations in this gene lead to most of the typical neurobehavioral features of AS, including ataxia, seizures, and severe mental retardation, albeit to a lesser degree in the mouse than in humans. However, several human studies suggest more severe and frequent seizures, ataxia, and lower cognitive function in AS patients with maternal deletions than in AS patients with UPD, imprinting defects, or *UBE3A* mutations (Williams et al., 1995; Minasian et al., 1998). Consequently, it has been suggested that nonimprinted genes deleted in AS may contribute to the phenotype, and that the GABA_A receptor $\beta 3$ subunit (*GABRB3*) gene (see figure), in particular, may contribute to the seizure phenotype in AS (DeLorey et al., 1998; Minassian et al., 1998). Since the same chromosomal region is deleted on the paternal chromosome in Prader-Willi syndrome, and these patients do not have seizures or other AS features (Nicholls et al., 1998), simple deletion of one *GABRB3* allele is unlikely to explain seizures in AS. However, homozygous null *Gabrb3* mice that survive cleft palate in the newborn period show phenotypic similarities to AS (DeLorey et al., 1998), including EEG abnormalities, seizures, learning and memory deficits, hyperactivity, and a disturbed rest-activity cycle (the latter not yet tested in *Ube3a*-deficient mice). Therefore, if there is a role for anomalous GABA_A neurotransmission in AS, this would have to involve a threshold effect with a concomitant reduction in *GABRB3* gene expression accompanying loss of maternally inherited *UBE3A* function. Studies of *Ube3a* ($m-/p+$) (Jiang et al., 1998) and hemizygous ($-/+$) *Gabrb3* (DeLorey et al., 1998) mutant mice, as well as comparison to AS mouse models with large chromosomal deletions, may allow determination of whether severe seizures or other phenotypic features in AS require *Gabrb3* or any other *Ube3a*-linked gene.

UBE3A's involvement in protein degradation raises a number of questions. For instance, although *UBE3A* clearly targets specific ubiquitinated ligands to the proteasome protein degradation pathway, might it also play a role in ubiquitin-like protein trafficking pathways (Johnson and Hochstrasser, 1997)? Does stabilization of cytoplasmic p53 play a role in the cellular basis of AS? Although p53 is required for some but not all neuronal apoptosis pathways (Sadoul et al., 1996), it is not clear what function, if any, p53 may have in postmitotic neurons. It does appear to play a role in seizure-induced brain injury, and loss of p53 protects neurons from excitotoxin treatment (Sakhi et al., 1994; Xiang et al., 1996). Therefore, analysis of seizure susceptibility and neuronal phenotype of mice deficient in both *Ube3a* (Jiang et al., 1998) and *p53* function (Williams et al., 1994) may be particularly instructive as to the role of p53 in the nervous system and as to whether overproduction of the p53 protein plays a role in the neuropathogenesis of AS.

In conclusion, the development of specific mouse models of AS, particularly the *Ube3a* ($m-/p+$) knockout

mutation (Jiang et al., 1998), will be critical for determining the biochemical and physiological pathways within the cerebellum and hippocampus for protein turnover mediated by the *Ube3a* E3 ubiquitin ligase. Ultimately, the hope is that AS animal models will allow investigation of potential therapeutic approaches toward amelioration of neurological and behavioral deficits in Angelman syndrome.

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More than Just Another Modifiable Synapse

“What’s the point, aren’t all synapses in the brain plastic?”

This quote paraphrases the response of a graduate school colleague, when I explained that my work involved trying to find the synapses in the brain that change during a particular form of learning. He argued that even if learning modified certain synapses, these changes might cascade through the network to other synapses. My colleague’s point of view was atypical for

the mid-1980s. At that time, plasticity had been characterized at only a handful of mammalian synapses—most notably, long-term potentiation (LTP) at synapses in the hippocampus and long-term depression (LTD) at the granule to Purkinje synapses in the cerebellar cortex (see Bliss and Collingridge, 1993; Linden and Conner, 1995). As such, it seemed most thinking was influenced by the tacit assumption that learning is mediated by activity-dependent plasticity at select excitatory synapses.

The article by Aizenman et al. (1998 [this issue of *Neuron*]) represents another groundbreaking step away from this relatively narrow point of view. This group has used a cerebellar slice preparation to demonstrate, with clear and careful experiments, both LTP and LTD at an *inhibitory* synapse—namely, the GABAergic Purkinje cell synapses onto cells in a cerebellar nucleus (see also Morishita and Sastry, 1996). This finding is exciting, even simply from the standpoint of adding to the list of synapses that we know can undergo activity-dependent plasticity. However, there are features of this plasticity that make it especially interesting and thought provoking.

One of the most important things to understand about a form of plasticity is the rule that governs the induction of changes. For example, interest in LTP at synapses in the CA1 region of the hippocampus seemed to accelerate with the discovery of the involvement of NMDA receptors (Bliss and Collingridge, 1993). The voltage and ligand dependence of NMDA receptors provided a possible detector of coincident postsynaptic activity (depolarization) and synapse activity (ligand). Subsequent studies showed that both LTP and LTD at these synapses are triggered by increases in intracellular free calcium, via NMDA receptor activation (Bear and Malenka, 1994). Although others have argued to the contrary (Neveu and Zucker, 1996), it appears that small increases in calcium lead to the induction of LTD at active synapses, whereas larger increases induce LTP. Thus, through the work of many labs, a Hebbian synapse was characterized—that is, a synapse where postsynaptic activity is the signal that controls the induction of plasticity and its polarity (i.e., LTP versus LTD). The significance of this accomplishment is considerable. Knowing something about the signals that control plasticity has broad implications that reach all the way to models of learning in networks.

Amazingly, Aizenman et al. (1998) have characterized the signals that govern bidirectional plasticity at Purkinje-to-nucleus synapses (Pkj→nuc) in a single set of studies. Like virtually all known (perhaps all) forms of plasticity at excitatory synapses in mammals, plasticity at the inhibitory Pkj→nuc synapses appears to depend on levels of intracellular free calcium. The evidence suggests that, like certain synapses in the hippocampus, large increases in postsynaptic calcium promote the induction of LTP at these synapses, whereas smaller increases promote the induction of LTD.

What makes this story really interesting is the source of the calcium influx into the postsynaptic cells in the cerebellar nuclei. How can there be an activity-dependent signal related to inhibitory inputs? Inhibitory synapses increase chloride conductances and generally

hyperpolarize cells. Moreover, chloride is too abundant inside of neurons for further influxes to serve as a signal, and hyperpolarization (or inhibitory depolarization) seems to preclude the activation of standard voltage-sensitive calcium channels.

The answer, it seems, is a collection of conductances in the nucleus cells that produce an increase in calcium during transient decreases in Purkinje input. Cerebellar nucleus cells show a characteristic rebound depolarization, apparently mediated by low-threshold, voltage-sensitive calcium channels, the activation of which requires release from hyperpolarization (Llinas and Muhlethaler, 1988). Depending on conditions, this rebound depolarization can be accompanied by bursts of action potentials. Aizenman et al. (1998) show that these bursts are associated with increases in intracellular free calcium, apparently mediated by high-threshold, voltage-sensitive calcium channels. Moreover, strong bursts of spikes, and the associated large increase in calcium, lead to the induction of LTP at the Pkj→nuc synapses. In contrast, activating the rebound depolarization under conditions that produce a relatively weaker burst of spikes leads to the induction of LTD at the same synapses. The authors hypothesize that LTP may occur when a rebound depolarization is accompanied by a burst of spikes, which back-propagate to the dendrites and activate high-threshold, voltage-sensitive calcium channels. In contrast, a rebound depolarization accompanied by just a few spikes leads to only a small increase in intracellular calcium, mostly via the low-threshold calcium channels, and to the induction of LTD.

Combined with what is known about the behavior of Purkinje cells, these observations provide interesting hints about the signals that control plasticity at cerebellar nucleus synapses. Recordings *in vivo* have shown that Purkinje cells fire at fairly high ongoing rates. A transient pause in this ongoing activity would be the kind of input that could evoke a rebound depolarization and thereby potentially induce plasticity at nucleus synapses. The work by Aizenman et al. (1998) suggests that when these pauses produce strong bursts of action potentials in the postsynaptic nucleus cells, LTP can be induced at the Pkj→nuc synapses. Thus, there seems to be little doubt that the signals that control plasticity at Pkj→nuc synapses actually occur *in vivo*, an issue that remains unresolved for many other forms of plasticity. The questions are, when do these signals occur and what is the function of the corresponding plasticity at Pkj→nuc synapses?

Aizenman et al. (1998) address two possible general roles for bidirectional plasticity at Pkj→nuc synapses. One is a contribution to cerebellar-mediated motor learning. The second is a homeostatic mechanism that helps maintain proper excitability of nucleus cells to keep their activity within a useful dynamic range. These roles are not mutually exclusive and neither is supported nor contradicted by current data. So, although any guesses are strictly speculative, the clear characterization of the signals that control plasticity at Pkj→nuc synapses at least makes speculation possible.

Aizenman et al. (1998) point out that plasticity at Pkj→nuc synapses will be of great importance for refining models of cerebellar motor learning. This will be true

whether the induction of this plasticity is related directly to learning or plays a more homeostatic role. At first glance, however, it is not immediately clear how plasticity at Pkj→nuc synapses, as controlled by postsynaptic spiking, can contribute directly to motor learning. Evidence suggests that transient decreases in Purkinje activity would contribute to the expression of a conditioned eyelid response or to a large gain vestibulo-ocular reflex—two cerebellar-dependent forms of motor learning (Raymond et al., 1996; Mauk and Donegan, 1997). However, if these decreases in Purkinje activity induce LTP at Pkj→nuc synapses, it would seem that the corresponding increase in inhibitory input to the nucleus cells would work against the expression of the eyelid response or vestibulo-ocular reflex. Of course, this may not make sense only because there is more to learn about how the cerebellum learns and expresses these responses. If so, then indeed the characterization of plasticity at Pkj→nuc synapses will aid in the refining models of cerebellar motor learning.

It is, however, this intrinsic negative feedback property of plasticity at Pkj→nuc synapses that suggests on first principles a possible role in homeostatic regulation of spiking activity in nucleus cells. Essentially, the signals that control this plasticity dictate that robust spiking in nucleus cells leads to increased inhibition and then presumably a decrease in spiking. This self-correcting property, which is in stark contrast to LTP and LTD at excitatory synapses, is ideally suited to keep the average activity of the nucleus cell in a useful place in its dynamic range. Indeed, Marder, Turrigiano, Abbott, and their colleagues have demonstrated, both empirically and computationally, the existence and utility of such mechanisms (see LeMasson et al., 1993; Turrigiano et al., 1994).

Regardless of the outcomes for these interesting issues, the discovery and characterization of bidirectional plasticity at Pkj→nuc synapses will improve our understanding of the neural basis of behavior in general and of the cerebellar mechanisms of motor learning in particular. Although it remains to be seen whether all synapses in the brain are plastic, as my colleague suggested, Aizenman et al. (1998) have added a particularly interesting example to the list.

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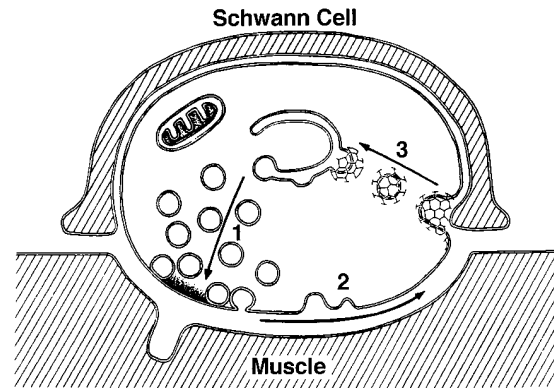
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The Schwann Song of the Glia-less Synapse

Although synapses throughout the brain are ensheathed by glial cells, the possibility that glia play an active role in synaptic function has received remarkably little attention. Glia passively help to maintain synaptic function by buffering ion concentrations, clearing released neurotransmitters, and providing metabolic substrates to synapses. But glia can also sense nearby neuronal activity. They depolarize in response to neuronal activity (Orkand et al., 1966; Kelly and Van Essen, 1974), and in hippocampal slices glia not only depolarize (Bergles and Jahr, 1997; Lüscher et al., 1998) but also elevate their intracellular calcium levels in response to synaptic activity (Dani et al., 1992). These studies show that glia are listening attentively to nearby synaptic conversations.

But do glia talk back to the neurons? An indication that they might comes from recent culture experiments. When purified CNS neurons were cultured in serum-free medium together with trophic peptides that promoted their survival and growth, they formed ultrastructurally normal synapses upon each other but displayed little synaptic activity (Pfrieger and Barres, 1997). Addition of purified astrocytes, which normally ensheath these synapses *in vivo*, increased spontaneous synaptic activity more than 70-fold and increased action potential-independent quantal release more than 10-fold. These studies showed that, at least in culture, developing neurons form inefficient synapses that require glial signals to become fully functional. Evidence that glia modulate synaptic transmission in the animal, however, has been more difficult to come by.

In simple and elegant experiments, Robitaille (1998) in this issue of *Neuron* has now provided convincing evidence that glia really do modulate synaptic transmission in a relatively intact tissue preparation. By taking advantage of a classical synaptic preparation, the frog neuromuscular junction, Robitaille was able to study the role of glia in synaptic function at a single synapse. Perisynaptic Schwann cells (PSCs) ensheath the neuromuscular junction (see figure) and, just as astrocytes do in the brain, sense and respond to high frequency nerve stimulation by increasing their intracellular calcium levels (Jahromi et al., 1992). Because previous studies have shown that such high frequency stimulation also progressively diminishes the evoked synaptic response, Robitaille wondered whether the Schwann cells might actively mediate this form of synaptic depression. To test this possibility, Robitaille pharmacologically manipulated the G proteins in the PSCs that mediate the



Schwann Cells Ensheath the Synaptic Terminal at the Frog Neuromuscular Junction

Upon stimulation, synaptic vesicles fuse with the presynaptic membrane (1), move laterally along the presynaptic face (2), and are retrieved by endocytosis at the junction between the synaptic terminal and the Schwann cell (3) (figure modified from Heuser and Reese, 1973).

Schwann cell calcium response to synaptic activity. He simultaneously recorded the nerve-evoked responses from the postsynaptic muscle fiber in the intact nerve-muscle preparation. Remarkably, activation of PSC G proteins with GTP γ S reduced the evoked muscle response by nearly 60%, a reduction almost identical to the synaptic depression induced by high frequency stimulation. The decreased muscle response appeared to be largely presynaptic. It was neither associated with a change in amplitude and kinetics of miniature events nor affected by postsynaptic manipulations. Rather, the decreased muscle response resulted from a large decrease in quantal content, the average number of vesicles released in an evoked response. Consistent with these results, previous studies have suggested that synaptic depression at the neuromuscular junction results from a depletion of synaptic vesicles in the presynaptic nerve terminal, leaving fewer vesicles available for subsequent release (Del Castillo and Katz, 1954; Zucker, 1989).

These data were consistent with the possibility that Schwann cells mediate synaptic depression. To test this possibility directly, Robitaille examined the effects of inhibition of PSC G proteins by injecting GDP β S into the PSCs. This led to a 50% diminution of synaptic depression induced by high frequency stimulation. Moreover, when synapses were first depressed by injecting GTP γ S, high frequency stimulation could induce little further depression, as expected if the two effects were mediated by the same mechanism. Taken together, Robitaille's findings demonstrate that Schwann cells play a crucial role in either modulating or mediating synaptic depression in response to high frequency stimulation at the neuromuscular junction, showing for the first time that Schwann cells actively participate in synaptic function.

So how could Schwann cells reduce the number of synaptic vesicles released? An intriguing possibility is suggested by the work of Heuser and Reese (1973), who observed that at the frog neuromuscular junction, retrieval of fused synaptic vesicles occurs at sites just

adjacent to contacting Schwann cells (see figure). Could stimulated Schwann cells signal the underlying terminal to slow the endocytotic recovery of synaptic vesicles, thereby regulating the size of the releasable vesicle pool? Such a model would fit in nicely with the slow endocytotic recovery rate of synaptic vesicles induced by high frequency stimulation at the frog neuromuscular junction (Wu and Betz, 1996). Whatever the mechanism, the Robitaille paper makes it clear that glia are not just listening to synaptic transmission but participating in the conversation as well.

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